FACULTY OF NATURAL SCIENCES,
Ibrahim Badamasi Babangida University,
Lapai, Niger State.

2nd FACULTY OF NATURAL SCIENCES ANNUAL CONFERENCE (FONSAC 2021)

Theme:

CATALYZING NATIONAL ECONOMIC RECOVERY IN POST-COVID-19 ERA THROUGH INNOVATIVE RESEARCH

Book of Proceedings

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Monday 30th August – Thursday 2nd September, 2021

Editors

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WELCOME ADDRESS BY THE CHIEF HOST

Welcome Address by Prof. Abu Kasim Adamu, Vice Chancellor, Ibrahim Badamasi Babangida University, Lapai at the Second Faculty of Natural Sciences Annual Conference (FONSAC 2021) On 31st August, 2021

PROTOCOLS, GREETINGS……

On behalf of the IBBUL community – a partnership of students, teachers, researchers, supporting staff, alumni and linkage partners – I welcome you to the 2nd Faculty of Natural Sciences Annual Conference (FONSAC 2021). As you may already know, Ibrahim Badamasi Babangida University is a great university, with a global reputation for its teaching and its scholarship. Our researchers have also pushed back the frontiers of knowledge with several cutting-edge research output. We took a lead in Africa as our researchers were the first to provide empirical seroprevalence data for COVID-19 at the height of the pandemic in June 2020. This feat was acknowledged by several national and international agencies. It is thus fitting that we organize a conference to curate research ideas that can be used to revitalize our economy in a post-COVID-19 era.

Nigeria today continues to be challenged by the twin issues of security and economic recovery. This provides an opportunity for world-class universities and other higher institutions of learning that are involved in knowledge creation and their researchers to confronts these challenges through innovative research. When we at IBB University Lapai started thinking about these issues, it became clear to us that it was our duty to use our strengths – our academic excellence and cutting-edge research – to be of service to society. It is therefore essential that fountain of new ideas, technologies and innovations flow from our institutions of higher learning and research institutes to the market place with national and international impact.

The theme of this year's Faculty of Natural Sciences Annual conference is Catalyzing National Economic Recovery in Post COVID-19 Era Through Innovative Research. Indeed, the COVID-19 pandemic has negatively impacted the global economy, and the most pressing challenge now is how economies world over can recover. Innovative scientific research has a great role in engendering economic recovery because the competitiveness of economies around the world now increasingly depends on their capability to create, use and diffuse knowledge. At IBB University Lapai, we have challenged ourselves with the task of converting our research outputs to tangible assets that can aid in economic recovery for Nigeria.

This conference provides a very unique opportunity for all of us because of its scope; it has attracted researchers from all over Nigeria and other African countries; people with wide-ranging knowledge on the issue of innovative scientific research that can be commercialized to aid national economic recovery: From vulnerability to resilience, innovation and bloom.

IBB University Lapai continues to place a high value on faculty and student scholarship and involvement in research and creative activities. Over the past few years, we have grown our support for scholars and grantsmanship, which has translated to a steady increase in proposal submissions and more members of our faculties winning national and international fellowships, grants and awards. The University Management has increased its level of support for researchers, and is prepared to take calculated risks to push at the edges of knowledge creation. However, one critical challenge that serves as a limiting factor

to all our efforts is unreliable power supply. No meaningful research endeavor can flourish under an atmosphere of darkness and intense heat. In fact, If Universities and our institutions of higher learning and research institutes must play the leading role in revitalizing our economy through innovative researches, then provision of uninterrupted power supply must be prioritized.

The Federal Ministry of Science and Technology has been positioned by the Government of Nigeria to be the primary driving force for development and deployment of science and technology apparatus which will enhance the pace of socio-economic development of the country. The ministry through her agencies and departments has been at the forefront of promoting innovative research in partnership with universities. Such partnership should be encouraged, enhanced and tailored towards societal problem solving so as to ensure maximum benefit of research outputs to the states, local government areas and even individual homes and lives, and our economy at large.

It is in response to this desire of government and the need to revitalize our economy after the downturn occasioned by the COVID-19 pandemic that the Faculty of Natural Sciences, IBB University Lapai, have put together this conference to curate research ideas and outputs that can catalyze the creation of knowledge-intensive economic activities in this country. Indeed, IBB University is well placed to drive this initiative as our research profile is rising to enviable heights, with our research efforts which continues to attract funding to the University.

The First Faculty of Natural Science Annual Lecture (FONSAC 2019) held on 6th to 9th May 2019. The keynote lecture titled Commercialization of Research Outputs for National Development was delivered by Prof. Sunday Asuquo Thomas, the former DG/CEO Sheda Science and Technology Complex, Abuja. The lecture was a thought provoking one and it set the tone for the deliberations that was held during the technical sessions of the Conference. The Second Faculty of Natural Science Annual Conference was expected to hold between 31st May and 3rd June 2020, but was truncated by the COVID-19 pandemic and the attendant lockdown. We thank God that the Second Faculty of Natural Science Annual Conference would now hold, and we are privileged to have the esteemed presence of the Honourable Minister of Science and Technology, Dr Ogbonnaya Onu, a distinguished scholar, who has also graciously accepted to deliver the keynote lecture for this conference.

As I conclude, allow me to appreciate the support we have continued to receive from the Ministry of Tertiary Education of Niger State and the Niger State government. Allow me to also thank the keynote speaker and the plenary speakers and our distinguished invited guests for honouring us with your presence in the opening ceremony of the conference. May I also thank the Conference Organizing Committee of the Faculty of Natural Sciences IBB University for putting together this beautiful conference.

For our dear participants at this conference, I wish you impactful presentations and fruitful deliberations at the technical session. To those of you who might be visiting Lapai for the first time, I invite you to feel at home. While here, please take time to look around and enjoy the beauty of our serene campus and let it inspire you to more groundbreaking research ideas.

I wish you all a happy and successful conference. May God bless you all and grant you journey mercies at the end of the conference. Thank you for coming.

WELCOME SPEECH BY THE CHAIRMAN OF THE CONFERENCE ORGANIZING COMMITTEE

Being a speech delivered by Musa Achimugu Dickson, PhD at The Second Faculty Of Natural Sciences Annual Conference (FONSAC 2021) on 31st August, 2021

- GREETINGS, PROTOCOLS,….
    - The executive Governor of Niger State, Dr. Alh. Abubakar Sani Bello represented by…
    - The Honorable Minister for Science and Technology, ably represented by a deputy director in a parastatal under the ministry of Science and Technology, Dr. Andrew Iloh
    - The Commissioner for Tertiary Prof Baba Aliyu, ably represented by,
    - The Commissioner for Health of Niger State, Dr Mohammed Mohammed Makusidi
    - The Emir of Lapai, Engr. Alh. Umaru Bago III (bagadozi)
    - The Chairman of Lapai Local Government
    - The Vice Chancellor IBBUL, our amiable Professor Abu Kasim Adamu
    - The Deputy Vice Chancellor Academics, Prof. Hassan
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    - The Registrar, IBBUL, Alh. Ango Abdullahi
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    - The University Librarian, Dr Abubakar
    - The Dean FNS IBBUL, Dr. Aliyu Yahaya Badeggi
    - The Coordinating Director of Researches IBBUL, Prof. Nuhu Obaje
    - Other Dean's, Directors and Members of Senate here present
    - Distinguished members of Faculty and Students of IBBUL
    - Our esteemed participants at FONSAC 2021
    - Gentlemen of the Press
    - Ladies and gentle men

It gives me great pleasure to welcome you most cordially at the second Faculty of Natural Sciences Annual Conference of the Faculty of Natural Sciences, IBBUL, tagged FONSAC 2021.

The second Faculty of Natural Sciences Annual Conference was supposed to take place in 2020, but it was unfortunately truncated by the COVID-19 Pandemic and the attendant lockdown that ensued. The pandemic did not only truncate our conference, it also destabilized the global economy leading to recession in several countries. It is therefore instructive that our theme for this year's conference is **Catalyzing National Economic Recovery in Post COVID-19 Era Through Innovative Research**.

A scientist once called a world press conference to announce his ground breaking finding. He has been working with spiders and he discovered he could give them instructions and they will follow. So he brought out a spider and asked it to take 10 steps forward, the spider did exactly that and stopped. He asked the spider to take 6 steps backwards, again the spider did just that. His audience was wowed and there was applause everywhere in the hall. The scientist then removed all the legs on the spider, and then repeated the first instructions, take 10 steps forward, the spider remained static, he told the spider to take 6 steps backwards, again the spider did not move. The scientist then proudly turned to the crowd and announced that I HAVE DISCOVERED THAT SPIDERS ONLY HEAR INSTRUCTIONS THROUGH THEIR LEGS.

Well, I want to assure you Mr Chairman sir that we would not be having such presentation during this conference. This is because the conference organizing Committee has painstakingly reviewed all abstract submitted and rejected those that did not meet with our standard. We also went ahead to review the full length manuscripts of our participants before the conference, and so we are sure that the technical sessions would be a highly engaging one.

On behalf big the Conference Organizing Committee of FONSAC 2021 I welcome you all to the historic city of Lapai and to the serene campus if IBBUL.

Enjoy your stay here, and try to use the opportunity of the conference to network with one another.

God bless you.

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Key Note Speech Presented by the Honorable Minister for Science, Technology and Innovation, Federal Ministry of Science, Technology and Innovation (FMSTI) Abuja on the Occasion of the 2nd Faculty of natural Sciences Annual Conference (FONSAC, 2021) with the Theme Catalyzing National Economic Recovery in Post Covid 19 Era through Innovative Research.

Protocols

Your Excellency, The Executive Governor of Niger State, Dr. Abubakar Sani Bello
The Honorable Commissioner for Tertiary Education, Niger State, Prof. baba Aliyu
The Honorable Commissioner for Health, Niger State, Dr. Mohammed Mohammed Makusidi
His Royal Highness the ETSU of Lapai: Alh. Umaru Bago II
Our Chief Host, The Vice Chancellor IBB University, Lapai.
Members of the University Community
Ladies and Gentlemen

It is my pleasure to be here today to wittiness this year's conference which has been tagged catalyzing National Economic Recovery in Post Covid 19 Era through Innovative Research. I am very pleased with the theme as it comes at no better time with the world and indeed our Country is trying to recover from the Monster Covid 19. Unfortunately, I am not here physically to present this key note, As I am away to promote the Presidential Executive Order Number 5.

In recognition of the fact that entrenching science, technology and innovation in everyday life is key to achieving the nation's development goals across all sectors of the economy, the federal government has committed itself to the promotion of domestic and foreign investments, creation of employment and the circulation of national economy.

Accordingly, President Muhammadu Buhari signed the Presidential Executive Order Number 5 (For Planning and Execution of Projects, Promotion of Nigerian Content in Contracts, Science, Engineering and Technology) of 2018 on Monday, February 6, 2018 with a view to improving local content in public procurement with science, engineering and technology components.

Executive Order 5 is a demonstration of the Federal government's efforts to promote the application of science, technology and innovation within Nigeria. Although it is a step towards achieving the nation's developmental goal of improving all sectors of the economy it is hoped that it will be faithfully and efficiently implemented by the federal government and all stakeholders.

Strategically, the main objectives of the Executive Order 5 are the harnessing of domestic talent and the development of indigenous capacity in science and engineering for the promotion of technological innovation needed to drive national competitiveness, productivity and economic activities which will invariably enhance the achievement of the nation's development goals across all sectors of the economy. Beyond recognizing the need to promote made –in- Nigeria goods and services the Order lays the foundation for the promotion of local expertise in the manufacturing value chain. Indeed, for any nation to grow and reach its full potential it must make judicious use of its human and material resources.

The Implementation of this Order is a welcome development and is bound to come with a number of implications on the economy of Nigeria. Some of them are:

1. It would promote the application of local content in Science, Technology and Innovation within Nigeria.
2. It would encourage indigenous experts in diaspora to return to their home for public procurements purposes. This may result to improved standards and healthy competition in all sectors.
3. Preference would be given to Nigerian companies and firms by procuring authorities in the award of contracts.
4. Immigration agencies may create a special immigration class for experts in African countries to work and reside in Nigeria so as to share their knowledge with Nigerian experts. This will aid the capacity of the companies and foster inter-Africa relations.
5. Increased production of local materials for building and construction.
6. Ministries, Departments and Agencies, MDAs would begin to engage indigenous professionals in the planning, design and execution of national security projects.

This is a policy in the right direction and has laid a proper foundation for an industrial, diversified and self-sustaining economy. It is a step towards a sustainable path and to a future in which wholesale importation and consumption of foreign technology is reduced. While Executive Order 5 may be said to have laid the foundation for the gradual take-off of the envisioned standardization and innovative technologies which engender capacity utilization and development. It has equally laid the foundation for the adequate provision for the development of science and technology in Nigeria. Apart from encouraging the teaching of science and technology in all schools the federal government has ensured promotion and support research via creating the enabling STI policies to promote them. I will in the course of this speech discuss some aspects of the STI policy.

The transfer of knowledge, skills and solutions in the science and technology fields can have a profound and lasting impact on the development trajectories of nations and peoples. However, we have seen in the last one year how COVID-19 pandemic has underscored the pressing need for countries to focus more on elevating science, technology and innovation (STI) in both policy and practical terms. This I would say might be a blessing in disguise as it also drives leaders to ensure that the development benefits of STI translate directly into the daily lives of people all over the world. We therefore need more research, collaboration, data and knowledge sharing to cope with the immediate impacts of the coronavirus crisis and go beyond it. More than ever, we need a multilateral approach to ensuring that STI serves both strategic and development ends.

Furthermore, the pandemic has also accelerated digitalization hence promoting digital literacy and skills, including media and information literacy, through life-long learning. On the other hand, the crisis has fast-tracked innovation in medicines, vaccines, biotechnology, digital technologies and artificial intelligence and emphasized the important role of frontier technologies in Scientific discovery and collaborations hence boosting open science.

Science, Technology and Innovation have long been important drivers of economic growth and human development. Growth relies on the integration of basic and applied research, at both public and private levels. The challenges are to ensure that even during phases of economic slowdown (i.e. brought about by the pandemic), science and technology continue to support the objectives of sustainability and improved living standards in the country. Innovation as a key driver of economic growth and a prime

source of competition in the global marketplace becomes pertinent to national development, Notable in this regard are the levels of adoption and creation of technological innovation and technological learning in creating this expansion. In view of this, technological innovation is measured using science and technology (S&T) indicators. These indicators include resources devoted to R&D, patents, technology balance of payments, and international trade in R&D-intensive industries. The importance given to S&T indicators increased with the call for a comprehensive analysis of the economy that not only incorporates economic indicators, but also those that represent knowledge. For S&T to translate to improved economic development (represented by improved quality of life, as well as wealth and employment creation), it needs to be geared towards bringing new products/processes into the marketplace- that is, towards innovation. Attaining national development goals requires evidence-based and informed policy-making. Incorporating S&T indicators offers the scientific evidence needed to effectively design, formulate, and implement national innovation policies that contribute to economic development.

How can we catalyze national Economic recovery through innovative research?

R&D is an important input to national innovation. It includes creative work undertaken systematically to increase the stock and the use of knowledge to devise new applications—both of which have the potential to influence innovation. That is why I am particularly pleased with the Conference Theme where we can catalyze national Economic recovery through innovative R&D. R&D expenditure is often used to encourage innovation and provide a stimulus to national competitiveness. Research has used R&D expenditure as a percentage of GDP (referred to as R&D intensity) to explain the relationship between firm size and innovative effort. As a ministry we are working on a National Research fund to take care of innovative research which can catalyze national economic growth.

Business enterprise expenditure on R&D (BERD) is an important indicator of business commitment to innovation. Although not all business investments yield positive results, efforts towards R&D signal a commitment to the generation and application of new ideas that lead to new or improved products/services for innovation. Research suggests that R&D spending is associated with productivity and GDP growth. An increase of 0.1% point in a nation's BERD to GDP ratio could eventually translate to a 1.2% increase in GDP per capita (Expert Panel on Business Innovation 2009).

Additionally, government funding can stimulate corporate R&D activities. The Federal Government having this as policy has equally increased capital expenditure in Science, Technology and Innovative research in her Agencies and Research Institutes; as well as in higher education institutions where R&D is equally an important stimulus to economic growth.

Knowledge creation and absorption via patents.

In the innovation framework, knowledge creation is the process of coming up with new ideas through formal R&D while Knowledge absorption is the process of acquiring and utilizing knowledge from entities such as universities, public research organizations, or domestic and international firms. Therefore, for knowledge absorption to happen, factors which include human capital, R&D, and linkages with external knowledge sources will have to be in place. On a national level, the creation and absorption of knowledge is manifested through the evolution of intellectual property (IP). FMSTI as a Federal Ministry under my

watch, has galvanized this by encouraging innovative research that can be commercialized to have IP protections. NOTAP under the FMSTI has been saddled with this responsibility. Your Excellency, Ladies and Gentlemen, in the last 5 years the Ministry of Science, Technology and Innovation has embarked on a National Science Fair, to showcase our innovative researches and more so create avenues for their commercialization leading to economic recovery and growth.

Investing in Infrastructures.

The provision of tangible and intangible infrastructure is a pre-requisite for inclusive and sustainable growth. New technology based growth requires new classes of infrastructure, digital connectivity, broadband communication networks, smart renewable energy grids, sustainable transportation system requires the development of new or renovated infrastructure.

Innovation diffusion: matching institutions and markets

If innovation is to advance shared growth and sustainability, a number of conditions need to be met. There is a need for adequate long-term public funding for research and development in order to expand knowledge as a public good. There is also a need for adequate demand from the private and public sectors for the goods and services that are associated with the spread of the new technologies. The Federal government is determined to continue to play that important role in stimulating new demand through targeted public research programmes, procurement for public services and public investment in infrastructure that will promote innovative research hence catalyzing the nation towards economic recovery and growth. Appropriate institutions, Policies (the STI policy) and frameworks for the emergence of new technology-based economic initiative, markets and social activities are in place to galvanize this.

The STI Policy

The new policy on Science, technology and Innovation (STI) has its core mission; the evolution of a new Nigeria that harnesses, develops and utilizes STI to build a large, stronger, diversified, sustainable and competitive economy that guarantees a high standard of living and quality of life for its citizens. Specifically, the STI policy is designed to provide a strong platform for science and innovation engagement with economic transformation that is citizen centered. To effectively foster a seamless engagement of STI with the desired transformation, the policy has recognized also the weakness of the nation's system of innovation and has set out strengthened structures for the co-ordination, promotion and management of interactions within the system as well as reduce time to market of research activities.

Conclusively, as it has become clear that STI will play a pivotal role in the catalyzing the Economy, it is our collective responsibility to make the policy work by ensuring its promotion at all levels. I will therefore recommend the following towards the successful implementation of STI at all levels

1. Facilitate the acquisition of knowledge to adapt, utilize, replicate and diffuse technologies for the growth of SMEs, agricultural development, food Security, power generation and poverty reduction.

2. Support the establishment and strengthening of organizations, institutions and structures for effective co-ordination and management of STI activities within a virile national innovation system.
3. Encourage and promote the creation of Innovative enterprises utilizing Nigeria's indigenous knowledge and technology to produce marketable goods and services
4. Support mechanisms that will harness, promote, commercialize and diffuse locally developed technologies for the production of global competitive goods and services that intensively utilize Nigerian's raw materials
5. Facilitate and support the creation and maintenance of an up to date, reliable and accessible database on Nigeria's STI resources and activities.
6. Initiate, support and strengthen strategic bilateral and multilateral co-operations in scientific, technological and innovative activities across all sectors of the economy

Finally, the Federal Executive Council (FEC) approved the National Science, Technology and Innovation Roadmap (NSTIR 2017-2030), which is aimed at using "Science, Technology and Innovation (STI) to catalyze Nigeria's economic growth and competitiveness." Hence Mr President's approval to rename the Federal Ministry of Science and Technology to be the Federal Ministry of Science, Technology and Innovation, a core policy for driving positive socio-economic outcomes through science, technology and innovation in all activities of the economy.

Thank you for listening.

Anti-Arthrithic Potentials of Ethanol Extract of *Ocimum gratissimum* Leaf

***[a]Gara T. Y, [a]Afolabi T. O., [a]Innalegwu D. A. & [b]Ndayako H. H.**

[a]Department of Biochemistry, School of Life Sciences, Federal University of Technology Minna, Nigeria.
[b]Department of Biological Sciences, Faculty of Natural Sciences, Ibrahim Badamasi Babangida University Lapai, Nigeria.

*Correspondence: Email: yebogana@yahoo.com

ABSTRACT

Arthritis is an inflammatory and autoimmune disease characterized by chronic, symmetric and erosive synovitis associated with combination of a genetic background and environmental triggers leading to defects in immuno regulation. The aim of the study was to investigate the anti-arthritic potential of ethanol extract of *O. gratissimum* leaf. The fresh leaves of *Ocimum gratissimum* were collected and extracted with 70% ethanol. Qualitative and quantitative phytochemical analysis, 2,2-Diphenyl-1picrylhydazyl (DPPH) radical scavenging activity, *in-vitro* protein denaturation (bovine serum albumin (BSA) and egg albumin) activity of *Ocimum gratissimum* leaf were carried out using standard procedures. The results of the qualitative analysis revealed the presences of alkaloids, saponins, phenols, flavonoids and tannins. The quantitative analysis showed that saponins had the highest result followed by phenolics, flavonoids, tannins and alkaloids. DPPH free radical scavenging activity of ascorbic acid displayed a higher percentage (%) inhibition compared to the extract. The IC_{50} value of ascorbic acid was 0.55 mg/mL while that of the extract was found to be 0.77 mg/mL. The *Ocimum gratissimum* leaf extract had a dose dependent response in the protein denaturation activity by BSA and egg albumin assay with the highest activity showed by the extract at 500µg/mL. In conclusion, the phytochemicals may have contributed to the anti-arthritic activity of the extract by controlling the production of auto-antigens and inhibiting protein denaturation.

Keywords: Arthritis, Phytochemicals, Protein denaturation, *Ocimum gratissimum*.

INTRODUCTION

Arthritis is a painful inflammation of the joints caused by either tissue injury or microbial infection. The larger population affected are usually between the ages of 40 – 60 years, with rheumatoid arthritis being one of the most common type affecting women three times more than men (Haris, 2016). The prevalence of rheumatoid arthritis is around 0.3 - 1% of the world population, with the incidence tending to increase with age and limits movement (walking) of people (Mbiantcha *et al.,* 2017).

Rheumatoid arthritis is an autoimmune disorder arising from articular and systemic inflammation leading to progressive persistent destruction of the synovial joint evident with increased

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secretions of cytokines (IL-1β and TNF-α), proteases, and transcription factors (Saleem *et al.,* 2020). Cytokines are signal molecules that promote inflammation and trigger the release of metalloproteases which prompt the bone breakdown, chronic proliferation of synoviocytes cells and destruction of cartilage tissues (Elisha *et al.,* 2016). Reactive oxygen species (ROS) are also produced by chondrocytes and neutrophils in the joints leading to chronic inflammatory disorders and tissue destruction acting by oxidising polyunsaturated fatty acids in the cell membrane triggering cell membrane damage (Mbiantcha *et al.,* 2017).

Plants have tremendously contributed to the growth of herbal medicines and their effects on the immune response of the body have gained acceptance over the observed harmful consequences of drugs and the reoccurrence (Jayaprakash *et al.,* 2013). *Ocimum gratissimum* L. is an herbaceous plant belonging to the family Labiatea, found in the tropics and subtropics with variability in tropical Africa (Nweze and Eze, 2009). It is a home grown shrub commonly called scent leaf in Nigeria. *Ocimum gratissimum has been reported to possess certain properties such as; antioxidant and anti-inflammatory (Ajayi et al., 2017), anti-anaemic and inhibitory potentials on some enzymes associated with erectile dysfunction (Ojo et al., 2014; Ojo et al., 2019),* improved growth performance in broiler chicks (Anugom and Ofongo, 2019), inhibitory effect on postprandial increase in blood glucose (Shimada, *et al.,* 2019) and antimicrobial activities (Shama et al., 2019) among others.

METHODS

Plant collection, preparation and extraction

The plant was collected by plucking from Bosso in Minna, Niger state and washed to remove sand. The fresh leaves were grinded and 200g was weighed and extracted with 600 mL of 80% ethanol on a reflux extractor. The plant extract was concentrated to dryness using water bath at 40˚C.

Qualitative phytochemical analysis of *O. gratissimum* leaf

Qualitative phytochemical analysis was carried out using the standard procedures as described by Sofowora (2006).

Quantitative phytochemical analysis of *O. gratissimum* leaf

The method described by Keay *et al*., (1964) was used to determine the phenolic content of ethanol extract of *O. gratissimum* leaf.

The procedures outlined by Harborne (1973) were used to determine the alkaloid content of ethanol extract of *O. gratissimum* leaf.

The procedures outlined by Ejikeme *et al.*, (2014) were used to determine the flavonoid, saponin and tannin content of ethanol extract of *O. gratissimum* leaf respectively.

Protein bovine serum albumin (BSA) denaturation inhibition assay of *O. gratissimum* leaf

The procedures outlined by Chandra *et al.*, (2015) were used to determine the BSA denaturation inhibition activity.

Protein egg albumin denaturation inhibition assay of *O. gratissimum* leaf

The procedures outlined by Pavithra *et al*., (2015) were used to determine the egg albumin denaturation inhibition activity.

In-vitro antioxidant activity

The DPPH free radical scavenging activity was carried out by the method described by Chan *et al.,* (2007).

RESULT

Qualitative and quantitative phytochemical of *O. gratissimum* leaf

Qualitative phytochemical screening of *O. gratissimum* leaf indicated the presence of alkaloids, saponins, phenols, flavonoids tannins (Table 1).

Table 1. Qualitative phytochemicals screening of ethanol extract of *O. gratissimum* leaf

Phytochemicals	Inference
Saponin	+
Phenolic	+
Flavonoid	+
Alkaloid	+
Tannin	+

+ = present

In the quantitative phytochemical analysis, It was found that saponin (679.99 ± 3.41) mg/100g, contained the highest phytochemicals in the ethanol extract of *O. gratissimum* leaf followed by phenolic (311.05 ± 0.16) mg/100g, flavonoid (148.87 ± 0.89) mg/100g, tannin (44.86 ± 0.05) mg/100g and alkaloid (26.74 ± 0.09) mg/100g (Table 2).

Table 2. Quantitative phytochemical screening of ethanol extract of *O. gratissimum* leaf

Phytochemicals	Quantity (mg/100g)
Saponin	679.99 ± 3.41
Phenolic	311.05 ± 0.16
Flavonoid	148.87 ± 0.89
Tannin	44.86 ± 0.05
Alkaloid	26.74 ± 0.09

Values are expressed as mean ± standard deviation of triplicate determination

DPPH scavenging activity

Figure 1 shows the variation in % DPPH inhibition of the ethanol extract of *O. gratissimum* leaf compared to ascorbic acid (standard). The % inhibition for ascorbic acid were found to be (30, 40, 56, 68, 75.11) while that of ethanol extract of *O. gratissimum* leaf were (17, 27, 39, 50, 63.17). Figure 2 shows the IC_{50} values of ascorbic acid and the ethanol extract of *O. gratissimum* leaf to be 0.55 mg/ml and 0.78 mg/mL.

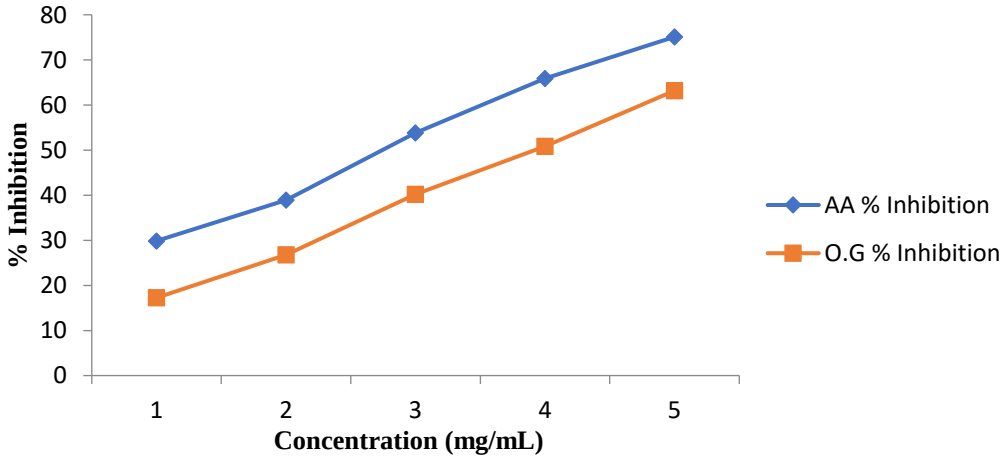

Figure 1: Variations in % inhibition of DPPH against concentrations of ascorbic acid (AA) and ethanol extract of *Ocimum gratissimum* leaf. AA; ascorbic acid, O.G; *Ocimum gratissimum*

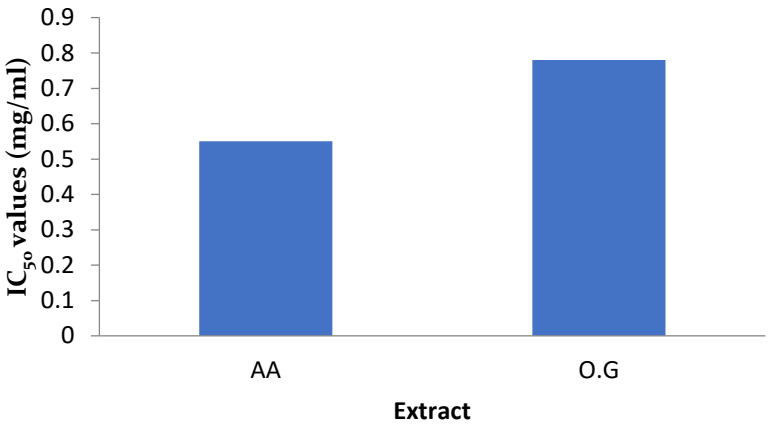

Figure 2. IC_{50} values of ascorbic acid (AA) and ethanol extract of *O. gratissimum* leaf. AA; ascorbic acid, O.G; *Ocimum gratissimum.*

***In-vitro* anti-arthritic activity**

The effect of ethanol extract of *O. gratissimum* leaf on protein denaturation by BSA and egg albumin method is displayed in Table 3 and Table 4. The extract had a dose dependent response in the BSA and egg albumin assay with the highest activity showed by the extract at 500µg/ml. The standard drug used (diclofenac sodium) had a better activity compared to the extract.

Table 3. Anti-arthritic activity of ethanol extract of *Ocimum gratissimum* leaf by bovine serum albumin (BSA) method

Extract concentration (µg/mL)	% Inhibition by BSA method
100 Diclofenac	72.58
100 *O. gratissimum*	25.02
200 *O. gratissimum*	31.32
300 *O. gratissimum*	49.90
400 *O. gratissimum*	53.00
500 *O. gratissimum*	57.76

Table 4. Anti-arthritic activity of ethanol extract of *Ocimum gratissimum* leaf by egg albumin method

	Inhibition by egg albumin method (%)	
Concentration (µg/mL)	**Diclofenac**	**O.G Extract**
100 *O. gratissimum*	31.98	24.16
200 *O. gratissimum*	50.38	41.75
300 *O. gratissimum*	53.32	44.12
400 *O. gratissimum*	55.21	46.12
500 *O. gratissimum*	58.69	51.35

O.G: *Ocimum gratissimum* leaf

DISCUSSION

Protein denaturation involved the loss of protein structures either by hydrogen, electrostatic, hydrophobic or disulphide bonding caused by exposure to heat, stress or high level of salts (Zhou and Pang, 2019). Studies have revealed that the application of heat on BSA causes denaturation and antigen expression associated with type III hypersensitivity reactions such as rheumatoid arthritis (Elisha *et al.,* 2016). Free radicals lead to the generation of oxidative stress and with phenols and flavonoids acting as antioxidants the effects of oxidative stress associated with arthritis will be reduced (Saleem *et al*., 2020). The anti-arthritic activity may have been triggered by the saponin content of the extract as Karthik *et al.,* (2016), had reported that saponin and alkaloids regulates interleukin 1 beta (IL-IB) and tumour necrosis factor alpha (TNFα) contents, reduces arthritic index, inhibit swelling in inflammatory tissues of arthrithic rats.

Phytochemicals such as saponins, phenols and flavonoids exhibit antioxidant effects against free radicals. The IC_{50} value of a compound is inversely related to its antioxidant capacity whereby lower values for from extract indicate a better antioxidant activity. Ascorbic acid had a stronger DPPH scavenging activity compared to the ethanol extract of *O. gratissimum* leaf, this is because ascorbic acid is a known potent antioxidant (Elisha *et al.,* 2016) while the combined effects of saponin, phenolics and flavonoid, may have contributed to the antioxidant balance in ethanol extract of *O. gratissimum* leaf (Shilpa *et al.,* 2018) hence the observed *in-vitro* antioxidant activity . Some phenolics and flavonoids have been reported to possess therapeutic effect on rheumatoid arthritis (Wang *et al.,* 20212). Saponins have the therapeutic potentials for inflammatory disorders and studies has suggested an existence of a strong relationship between antioxidant activity of plants extract and their phenolic content probably due to their redox properties which allows them to act as reducing agents and singlet oxygen quenchers (Sharma *et al.,* 2016).

Protein denaturation is one of the identified causes of inflammation and it has been described as a pathological process involving the loss of configuration which results into loss of functionality (Bailey-Shaw et al., 2017). The increase of the % inhibition with respect to the reference drug indicated the stabilization of proteins in a dose-dependent manner and thus a genuine anti-inflammatory action. This indicated that *O. gratissimum* leaf extracts at higher concentration inhibited the denaturation of proteins. Saponins are reported to possess antioxidant properties which are able to inhibit the mediators of inflammation and the formation of reactive oxygen species that play a major role in inflammation (Tatiya *et al.,* 2007).

CONCLUSION

The ethanol extract of *O. gratissimum* leaf contained significant amount of saponins, phenols and flavonoids which may have improved its antioxidant properties. Thus, the observed anti-arthritic activity of ethanol extract of *O. gratissimum* leaf suggest to be likely due to thecombined action of phenols, flavonoids and saponins which were found to be the major constituents of the extract. These phytochemicals at higher concentrations may help in controlling the production of auto-antigens and inhibiting protein denaturation.

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Spatio-Temporal Variations and Apparent Density of Tsetse Flies (*Glossina* Species) in Selected Old Focal Areas of Niger State, Nigeria

[*1]Musa U. B., [2]Salihu I. M., [3]Ukubuiwe A. C., [4]Adeniyi K. A., [5]Yusuf A. B. & [3]Olayemi I. K.

*Correspondence: usmanbabazago@gmail.com, +234 8034522016.

[1]Vector and Parasitology Research Department, Nigerian Institute for Trypanosomiasis Research, Kaduna, Nigeria.
[2]Department Biological Sciences, Ibrahim Badamasi Babangida University, Lapai, Nigeria.
[3]Department of Animal Biology, Federal University of Technology, Minna, Nigeria.
[4]Department of Biological Sciences, Federal University Dutse, Nigeria.
[5]Biotechnology Research Laboratory Unit, Nigerian Institute for Trypanosomiasis Research, Kaduna, Nigeria.

ABSTRACT

Spatio-Temporal variations and apparent density of tsetse flies (*Glossina* Species) in selected old focal Areas of Niger State was studied. Niger state is an old endemic focus of African trypanosomiasis and the vector tsetse fly (Genus: *Glossina*). Despite the economic and public health impact of the disease in Nigeria, there is no prevailing tsetse record in the various ecological areas of Niger State. Three (3) sites were selected; Bida (Bida LGA), Gbangban (Edati LGA) and Ijah Gwari (Tafa LGA) being old foci of tsetse and trypanosomiasis. Temperature, relative humidity and geo-reference of each sampling point were taken using thermometer, whirling hygrometer and GPS machine while Biconical and Vouvoa tsetse traps were used in catching tsetse flies for 3 days (72 hrs) in each selected site. Catches were removed daily (24 hrs) and identified using standard morphological features and relative abundance recorded. The results were considered significant at P≥0.05. The mean temperature range was 28.48 ± 0.27°C – 32.04 ± 0.42°C while relative humidity was 66.86 ± 1.22% – 74.84 ± 2.19%. The altitude in Ijah Gwari was 494 ± 23.5 m (above sea level), Bida was 109 ± 6.80 m and Gbangban was 93.17 ± 5.18 m. More so, the latitude recorded for Bida was 9.12 ± 0.01°N, Gbangban was 9.14 ± 0.00°N and Ijah Gwari 9.18 ± 0.01°N. A total of 2,251 tsetse flies were caught during the study period. Bida recoded 227 (10.1%), Gbangban 437 (19.4%) and Ijah gwari 1,587 (70.5%). The flies were *Glossina palpalis palpalis* 2,046 (90.9%) and *Glossina tachinoides* 205 (9.1%) both species belong to subgenus *Nemorhina.* A *Glossina* abundance of 2.09 Tsetse/Trap/Day (T/T/D), 4.04 T/T/D and 14.69 T/T/D was recorded for Bida, Gbangban and Ijah Gwari respectively. The difference is attributable to diverse level of vegetation exploitation in study areas.

Key words: Spatio-Temporal, Trypanosomiasis, *Glossina palpalis palpalis* and *Glossina tachinoides, Nemorhina*.

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INTRODUCTION

Tsetse flies (*Glossina* species) are the vectors of trypanosomes which cause trypanosomiasis (sleeping sickness in man and Nagana/Sammore in animals) disease which can be devastating to humans and livestock. This disease occurs mostly in rural areas disturbing agro-pastoral activities in communities (Zeleke, 2011). In 2001, the Pan Africa Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) estimated that tsetse fly infests about 10 million km^2 of the 37 sub-Saharan African countries. Reports indicate a decline in the cases of Human African trypanosomiasis (HAT) to below 10,000 cases per year in the last decade (PATTEC, 2001). However, sleeping sickness remains endemic in several countries, including Nigeria. Animal African trypanosomiasis (AAT) does result to annual losses of about 5 billion US Dollars due to agricultural production inhibitions. The disease poses a big socio-economic burden on sub-Saharan African countries (Shaida *et al*, 2018).

African trypanosomiasis is endemic in Nigeria, and reports indicate an increasing trend of Animal African Trypanosomiasis in Niger State (Adama *et al*, 2010). Records at Niger State Veterinary clinic and pharmacy indicate increase in number of animal trypanosomiasis in the state. There are no concurrent reports of the vectors of the disease in communities of the state where the outbreaks are recorded. During vector control programmes, knowledge of the *Glossina* species is required. Little information is available regarding the spatial variation of the tsetse biotopes and the apparent density of infestation in Niger state. Total eradication has been difficult because of local peculiarities. Though a lot has been done in the past but there are gaps in the available information. This study could, therefore, fill the information gaps to further consolidate the effectiveness of control interventions. The research was aimed at determining spatio-temporal variations and apparent density of tsetse flies in selected old focal areas of Niger State.

METHODOLOGY

Study Area

This study was carried out in three local government areas of Niger State. The State is located in the Southern Guinea Savannah agro-ecological zone in the North central of Nigeria, it lies between latitude 6° 8' E and longitude 8° 44' N of the equator. The State experiences distinct dry and wet seasons with an annual rainfall ranging from 1100 mm in the northern part to 1600 mm in the south with a mean of 1350 mm. The rainfall which peaks in September normally begins in April and ends in October. The temperature ranges between 35 and 37.5°C with relative humidity between 40 and 80% in January. The state has 25 LGAs and is bounded with Kwara, Kogi, Kebbi, Zamfara, Kaduna States and FCT and International boundary with republic of Benin along its Western Border. The State covers a land area of about 76,363 km^2 constituting 9% of Nigeria's total land area (Ahmed *et al*, 2019).

Study Site

Three study sites were selected Ijah Gwari, Bida and Gbangban old foci of tsetse and trypanosomiasis (Nigeria Institute for Trypanosomiasis Research NITR, 1976). Visual inspection revealed suitable habitats along small rivers and stream, Wuye (Ijah Gwari), Muser (Bida) and Etan (Gbangban) in Tafa, Bida and Edati Local Government Areas (LGAs) respectively. The temperature, relative humidity

and geo-reference of each sampling point were taken using thermometers, whirling hygrometer and Garmin e-trex, Global Position System (GPS) machine (Shaida *et al*, 2018). See figure 1.

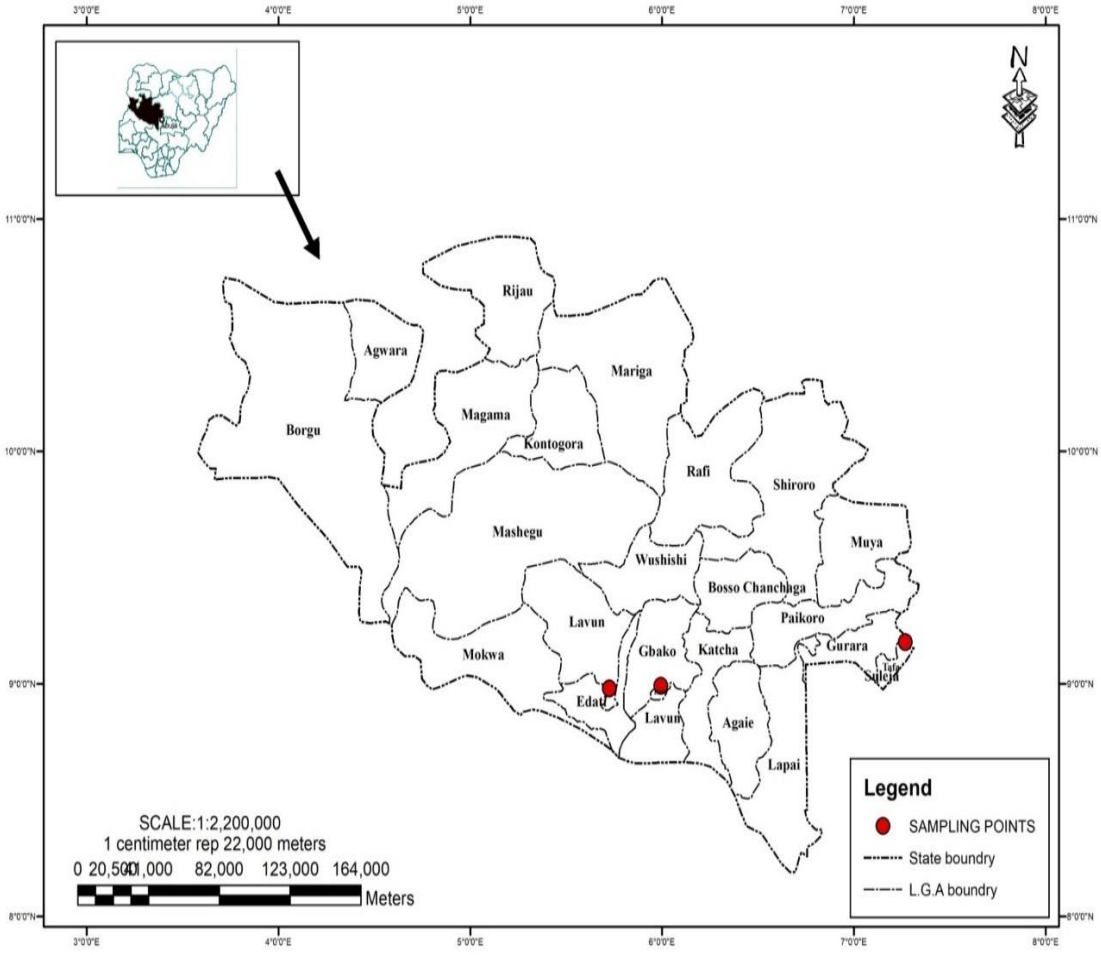

Figure 1: Map of Niger State showing the LGAs and selected study areas.

Entomological Sampling

In each study site, six tsetse traps (Biconical and Vouvoa) were set for three consecutive days (72 hours) in favourable tsetse biotopes (Melachio *et al*, 2011). Flies caught were removed every (24 hours) after which the species, sex and teneral status were identified according to routine morphological criteria (Gibson, 2003). The traps were placed around grazing areas, routes and watering points for animals and suspected larviposition sites (Bocoum *et al*, 2012). The number of tsetse flies caught in each trap was recorded daily and the total for each site was used to estimate the index of apparent abundance (T/T/D) (Adam *et al*, 2012).

Data Analysis

Data collected from all the sites and sampling period were processed as means ± standard deviation (S.D) using excel programme. Mean values of the variables were compared for significance of statistical differences at P≤0.05 level of significance.

RESULT

Physical and Climatic characteristics of *Glossina* habitat in selected areas of Niger State.

The results of physical and climatic parameters of study areas in Niger State are contained in Table 1. The results indicated that there was significant difference (P≤0.05) in the recorded physical and climatic parameters in the three study areas. The temperature ranges from 32.04±0.42°C in Gbangban to 28.48±0.27°C in Ijah Gwari, Relative Humidity ranges from 74.84±2.19 in Ijah Gwari to 66.86±1.22 in Gbangban, respectively. Altitude was significantly highest (P≤0.05) in Ijah Gwari (494±23.5) when compared to other studied areas; Bida (109±6.80 m) and Gbangban (93.17±5.18 m). There was no significant difference (P≥0.05) in Latitude recorded for Bida (9.12±0.01) and Gbangban (9.14±0.00) however, Ijah Gwari recorded a significantly high value of longitude and latitude.

Table 1: Physical and climatic characteristics of *Glossina* habitat in selected areas of Niger State

Study Areas	Temp. (°C) Mean±S.D	R.H Mean±S.D	Altitude (m) Mean±S.D	Latitude (°N) Mean±S.D	Longitude (°E) Mean±S.D
Ijah Gwari	28.48±0.27^{a}	74.84±2.19^{c}	494±23.15^{c}	9.1844±0.01^{b}	7.27±0.00^{c}
Bida	30.57±0.46^{b}	70.56±2.37^{b}	109±6.80^{b}	9.1221±0.01^{a}	6.01±0.00^{b}
Gbangban	32.04±0.42^{c}	66.86±1.22^{a}	93.17±5.19^{a}	9.1389±0.00^{a}	5.80±0.00^{a}

The mean ± standard deviation (S.D) of the temperature (°C), relative humidity (%), altitude Mean value followed by the same super script alphabet in the same column are not significantly different at P=0.05.

***Glossina* abundance in selected areas of Niger State**

The index of apparent abundance (IAA) of *Glossina* is Tsetse per Trap per Day (T/T/D) is presented in Table 3. Ijah Gwari (river Wuye) recorded an overall *Glossina* abundance of 14.69 T/T/D with Bida (river Muser) having 2.09 T/T/D while Gbangban (river Etan) had 4.04 T/T/D. Ijah Gwari (river Wuye) experiences more *Glossina* abundance (22.83 T/T/D and 20.88 T/T/D) during the study in February and March (hot dry season) than any other time in the study area. Moreover, Ijah Gwari (river Wuye) experiences more *Glossina* abundance (22.83 T/T/D in February, 20.88 T/T/D in March, 6.16 T/T/D in June, 14.88T/T/D in July, 12.66T/T/D in August, and 10.72T/T/D in September) than Bida (river Muser) (2.83 T/T/D in February, 1.55 T/T/D in March, 1.55 T/T/D in June, 2.16T/T/D in July, 2.38 T/T/D in August, and 2.11 T/T/D in September) and Gbangban (river Etan) (4.61 T/T/D in February, 3.72 T/T/D in March, 3.38 T/T/D in June, 5.77 T/T/D in July, 3.66 T/T/D in August, and 3.11 T/T/D in September), demonstrating a higher T/T/D values in Ijah Gwari (river Wuye).

Table 3: *Glossina* Relative abundance in selected study areas in Niger State

Month	Location	No. of Traps	No. of Days	No. of *Glossina* caught	Apparent Density (TTD)
February	Ijah Gwari	6	3	411	22.83
	Bida	6	3	51	2.83
	Gbangban	6	3	83	4.61
March	Ijah Gwari	6	3	376	20.88
	Bida	6	3	28	1.55
	Gbangban	6	3	67	3.72
June	Ijah Gwari	6	3	111	6.16
	Bida	6	3	28	1.55
	Gbangban	6	3	61	3.38
July	Ijah Gwari	6	3	268	14.88
	Bida	6	3	39	2.16
	Gbangban	6	3	104	5.77
August	Ijah Gwari	6	3	228	12.66
	Bida	6	3	43	2.38
	Gbangban	6	3	66	3.66
September	Ijah Gwari	6	3	193	10.72
	Bida	6	3	38	2.11
	Gbangban	6	3	56	3.11

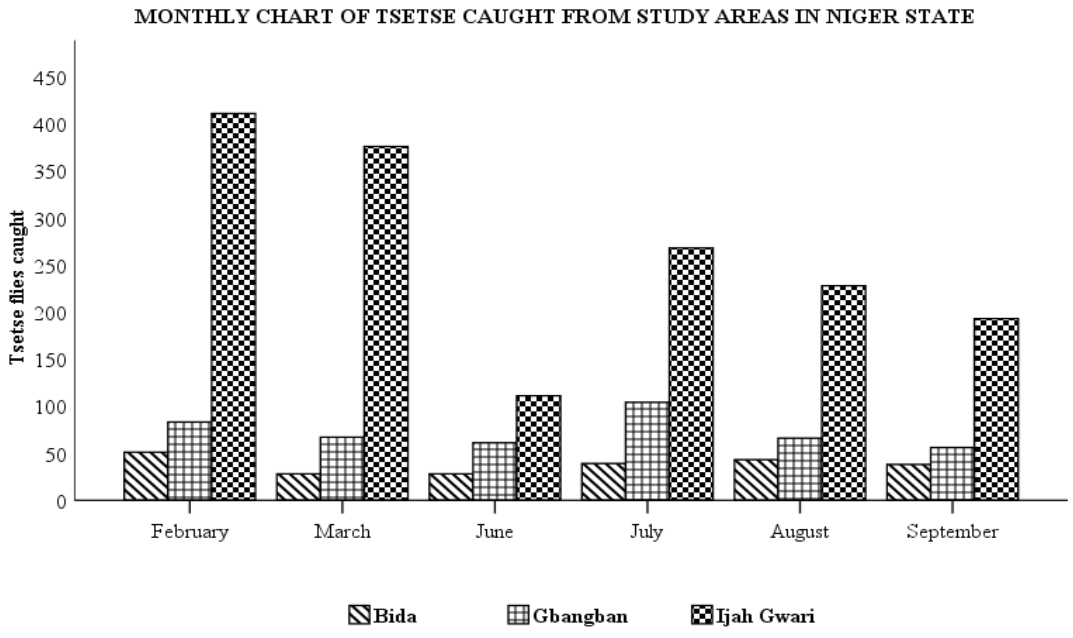

Figure 2: Monthly Chart of Tsetse Fly Caught from Study Areas in Niger State

DISCUSSION

The rivers and type of vegetation encountered in the study areas include Wuye with riverine and fringing forest (Ijah Gwari, Tafa, LGA), Muser with a riverine vegetation (Bida, Bida LGA) and Etan with riverine and woodland Savannah (Gbangban, Edati LGA). All the three rivers flow throughout the year thereby supporting various human and animal activities. The vegetation provides shade and resting site for tsetse fly which is important for survival and larviposition (Leak, 1999).

Human activities were observed to be more at Bida than Gbangban while Ijah Gwari had the least. This may be due to the poor road network to rivers Etan and Wuye which lack vehicle routes, making exploitation difficult (including agriculture practices). This agrees with the findings of Tekle (2010) who reported in a study that, "Man is adjudged the most important animal in the environment of Tsetse fly, people kill them by crushing, modify their environment by various agricultural practices, deliberately destroy the environment, eliminate wild animals and spread poison (insecticide) to kill tsetse directly.

The most exploited of the study areas is Bida where the human settlement and crop farming has interfered with the natural environment. The level of exploitation at Gbangban can be said to be moderate with human settlement 3km away, removal of trees for domestic purposes is quite significant. Ijah-Gwari presents more luxuriant vegetation with two storey forest out layer and human settlement far away, provides ideal tsetse resting and breeding site. The activities of the chain saw operators could lead to reduced insulation due to the removal of big trees which will lead to more sunlight penetration thereby increasing the temperature and reducing the relative humidity as observed and reported by Leak (1999).

Although, the means of temperature and relative humidity recorded in the study areas differed significantly ($P \leq 0.05$), they were found to be within the Tsetse fly tolerable limit. This finding is in agreement with the report of Pagabeleguem *et al* (2016). Generally, under controlled conditions (Laboratory) tsetse does well at temperature near 25^{o}C, departures from this favourable temperature has progressive deleterious effects, culminating in rapid death at 46^{o}C and at sub-zero temperatures (Terblanche *et al*., 2008). The result indicates temperatures at Ijah Gwari more ideal compared to Bida and Gbangban. The relative humidity recorded revealed an inverse relationship with temperature (Lower temperature higher relative humidity) throughout the study period which agrees with the report of Wint and Rodgers (2000). The physical characteristics (Altitude, Longitude and Latitude) of all the study areas were within tsetse distribution limits given by FAO (2004) . Although there is significant difference ($P \leq 0.05$) in the physical characteristics of the study area they all fall in the tsetse distribution limits in Nigeria.

Glossina density recorded from study areas differed significantly ($P \leq 0.05$) and may be attributed to difference in vegetation which is influenced by human activities. Ijah Gwari (river Wuye) with a more dense riverine vegetation and low human activity had a high density. Bida (river Muser) with a sparse riverine vegetation and high human activity while Gbangban (river Etan) with Savannah woodland vegetation and moderate human activity having low densities. This is in agreement with the findings of Bouyer *et al*., (2005), who reported the relationship between plant and human activity and the

distribution of riverine species of *Glossina* which are influenced by the land use land cover.

The study revealed the abundance of 2 species of *Glossina* which belong to the riverine group of flies (*Palpalis* group). This is contrary to the historical records of Glover (1961) who reported abundance of Savannah flies (*Morsitans* group) in the Bida area. Ahmed (2010) reported the abundance of *G. palpalis palplis* and *G. tachinoides* in 3 streams in Agaie LGA of Niger State which agree with the findings of this study. Tsetse flies have specific ecological requirements that differ between species (Reid *et al.,* 2000). *Palpalis* group flies lives in forests where optimum atmospheric conditions can be maintained. The disturbances of riverine forest at Bida and Gbangban and beyond significantly reduce the suitable habitat of *Glossina* and may lead to the complete disappearance of the species. This may be the reason for the low abundance recorded in Bida and Gbangban. Adam *et al*., (2012) stated that *G. tachinoides* can cope with more open vegetation and is less affected by anthropogenic changes of the vegetation. This could be the reason for recording more *G. tachinoides* in the two areas (Bida and Gbangban) than Ijah Gwari which is a highland riverine forest reported by Reid *et al.,* (2000) not favourable for *G. tachinoides* survival which is known to prefer lowland riverine forest which was encountered at Bida and Gbangban. The abundance of *Glossina* was higher in the dry season in all the study areas compared to the rainy season. Okoh *et al*., (2011) reported lower abundance in the rainy season compared to dry season. Low density of tsetse in wet season correlates with low emergence and vice versa in the dry season.

CONCLUSION

The study revealed spatial and temporal diversity in the tsetse habitat. The temperature and relative humidity obtained were found to be within the tolerable limit of the genus *Glossina* and the variation observed in the study areas could be the effect of difference in vegetation cover. Furthermore, the two species of tsetse fly recorded are important in the transmission of trypanosomiasis in Nigeria.

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Competing Interests

The authors declared they have no competing interests and objection to the publication.

Authors Contributions

OIK conceived and supervised the study. MUB designed, executed the fieldwork, morphologically identified the tsetse flies and drafted the manuscript. SIM, UAC, AKA and YAB carried out data collection, statistical analysis, participated in study design and helped in drafting the manuscript. All authors read and approved the final manuscript

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Investigating the Effects of Microbial Infested Feedstuffs on Performance of *Clarias* gariepinus Fingerlings

*Musa G. & Abdullahi M. O.

Department of Water Resources, Aquaculture and Fisheries Technology, Federal University of Technology P.M.B, 65 Minna, Niger State.

*Corresponding author, E-mail: musageorge30@gmail.com, Phone number: 08035165266

ABSTRACT

The research work was carried out to investigate the bacteria and fungi infested feedstuffs on the growth performance of *Clarias gariepinus.* Feedstuffs used were maize, guinea corn, millet, soybean, soybean cake, fishmeal, blood meal, groundnut cake and maize bran. There were grouped into A, B and C grades in quality.Group A was good quality (the control diet), group B was incubated with microbes for 2 months while group C was the combination of group A and B in equal proportion. Three diets were formulated using pearson square method at 45% crude protein.Two hundred and fourty *Clarias gariepinus* fingerlings (3.98±2.5g) were sourced from the Water Resources Aquaculture and Fisheries Departmental fish farm Gidan Kwanu campus (Federal University of Technology Minna) and acclimatized for three (3) days prior to feeding trial. The fishes were randomly distributed in 20 fishes per tank (47cm x 17cm) in triplicate of complete randomized design. Fingerlings were fed twice daily at 3% body weight while the water quality parameters were taken. The results obtained indicated presence of fungi (*Aspergillus niger, yeast, penicillium, rhizobus and aspergillus flavus*) and bacteria (*Escherichia coli, Staphylococcin aureus.and Pseudomona aeruginosa*) in feedstuffs. There were showed significant difference ($p<0.05$) on the growth parameters, where diet B (infested diet) exhibited a significantly ($p<0.05$) higher mean weight gain (1.11g), high protein efficiency ratio (PER) and low feed conversion ratio (FCR) while diet A (good quality diet) exhibited significantly ($p<0.05$) low value (0.48g). However, fish fed good quality diet had higher survival percentage (83.33%) while diet B had the least survival value (43.33%). The haematological parameters also indicated significant differences ($p<0.05$). Pack cell value (PCV) was highest in diet C and A with no significant difference ($p>0.05$) between them (25.49%) but significantly different ($p<0.05$) to diet B (24.70%). White blood cell (WBC) was significantly ($p<0.05$) highest for diet B (2.42) and least in diet A (1.86). Red blood cell (RBC) was significantly ($p<0.05$) highest for diet A and B (3.78 and 3.77) and least in C with no significant difference ($p>0.05$) between them (3.66) respectively. Haemoglobin (HB) was highest for diet C (8.73) and least for diets B and A with no significant difference ($p>0.05$) between them (8.68 and 8.60) respectively. The Mean corpuscular haemoglobin concentration (MCHC) was highest for diets B (3.52) and C (3.42) with no significant difference between them and least in diet A (3.38) similar trend were obtained for Mean corpuscular haemoglobin (MCH), Mean corpuscular volume (MCV) and Neutrophils (NTP) while the Monocytes(MC) Eosinphils(ESP) was highest for diet C and least for diets A and B. The biochemical parameters indicated significant differences ($p<0.05$) where Total bihrubin, Conjugate bihrubin, Pottassium ion (K^+), Creatinine Total protein and Aspartate Aminotransferase (AST)

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but difference in Alkaline phosphate (ALP), Alanine Aminotransferase (ALT) and Sodium ion (Na^+). Microbial infested diets were not significantly different ($p>0.05$) from the initial values to some degree. The findings revealed that, growth of *Clarias gariepinus* fingerlings were better under contaminated diet but with high mortality compared to good quality diet. Thus, fish farmers should ensure that, feedstuffs of high quality are used for fish feed to guarantee high survival of the fish during culturing period.

Key words: *Clarias gariepinus*, feedstuffs, microbial, proximate, haematology, biochemical.

INTRODUCTION

To sustain fish under culture, supplementation diet must be provided to complement natural feeds supply (Karapan, 2002). Generally, nutrition of fish is an important aspect of any viable aquaculture enterprise accounting for at least 60% of the total cost of fish production (Jamu and Ayinla,2003; Madu *et al.,* 2003). The nutrient composition of feed influences feed utilization and ultimately the growth of fish (Adaga, 2014). The prevailing climatic conditions in the tropics experience an increase in temperature and relative humidity of over 25^0C and 70%, respectively (Adaga, 2014). Such conditions accelerate mould growth and lipid oxidation (Berger, 1989; Coppen, 1989; Van den Bergh, 1990). According to Bautista *et al.* (1992) and Ramezandeh *et al.* (1999), feed storage at high temperature results in an increase in both oxidative and hydrolytic rancidity with loss in feed quality. Studies by Van den Bergh *et al.* (1990) and Ruiz *et al.* (2000) indicate that fats are intrinsically unstable when subjected to high temperature above 30^0C. Under such conditions, fats are hydrolysed to release ketonic acids, which further undergo auto-oxidation with degeneration of free radical products (Hamilton, 1989). According to FAO (2001), these environmental factors also predispose fish feeds to microbial spoilage, hence causing feeds to decompose and fed fish to become diseased. Toxin producing fungi are dangerous, most of them producing *aflatoxins, patulins* and *trichotecens* which are strongly carcinogenic and mutagenic (Ciceron *et al.,* 2008; Brown, 2001). Aflatoxins are chemicals produced by fungi such as *Aspergilus flavus* and *A. parasiticus* (mould) (Russo and Yanong, 2006). Mould infested fish feeds have been reported to impact negatively the growth of vundu *Heterobranchus bidorsalis* (Effiong and Alatise, 2009). Manufactured feeds are stored under different storage conditions by commercial fish feed sellers and farmers without respect to the effect of these conditions on the nutrient profiles of the feeds (Adaga, 2014). Since the nutrient profile of a feed determines the growth performance of fish, this study was designed to investigate effect of microbial growth on feed nutrient quality and performance of African catfish *Clarias gariepinus*.

METHODOLOGY

Location of the Study

The experiment was carried out at the laboratory of the Department of Water Resource, Aquaculture and Fisheries Technology of the School of Agriculture and Agricultural Technology Niger State, Federal University of Technology Minna. It is located between latitude 9 39 24.98 and longitude 6 31 42.12.

Procurement and Acclimatization of Experimental Fish

Live Clarias gariepinus fingerlings of 3.98±25 average weight were purchased from Department of Water Resources, Aquaculture and Fisheries Technology of the School of Agriculture and Agricultural Technology Minna, Gidan Kwanu Campus Departmental fish farm, Niger State. The Clarias gariepinus fingerlings were transported to the Department laboratory where the experiment was conducted. The fishes were randomly distributed in 20 fishes per tank (47cm x 17cm) in triplicate of complete randomized design. Flow through system was used.

Water Quality Analysis

Temperature of water was measured using digital thermometer Model NO: BL-T990 by dipping the thermometer in the water for five minutes and record was taken. pH of water was measured using a pH meter (REX), Model PHS-25 by inserting the robe of the meter into the sample water and reading was taken. Dissolved oxygen was determined using samples of water collected in Dissolved oxygen bottles without allowing bubbles of water into it. 2 ml of reagent I (Magnesium sulphate) was added. The bottle corked to allow precipitation to settle, after then 2 ml of concentrated sulphuric acid added to dissolve the precipitate while a yellow dissolution formed. 10ml of solution was measured in a cornical flask and 2.3 drops of starch solution added as an indicator and the mixture turned to blue-black colour and was titrated with 0.025 sodium thiosulphate until it turn colourless.

Ammonia was determined using kjeldahl distillation apparatus. To determined ammonia, 10ml of water sample was discharge into the reaction chamber using a pipette, 5 mls of 40% NaOH was then added into the chamber which will liberate Ammonia. The flask was connected to the condenser and the cooling water turn on. 10ml of boric Acid solution and 3 drops of mixed indicator was discharge into the 250mls receiving flask ensuring that the tip of the condenser is immersed in the receiving solution. Distillation confine slowly until 50ml of distillation was collected in the receiving flask; the distillation was then titrated with 0.0545M of standard HCl Acid until the colour at the end point changes from blue to pink and calculated thus

$$MgNH_3 = \frac{TVXMX17X100}{\text{Vol. of sample}}$$

Where

TV= Titre value

M= Molarity of HCL

Experimental Diet

The feedstuffs were sourced and grouped into good grade feedstuffs (A), microbial contaminated feedstuffs (B) and mixture of A and B as diet (C). The microbial contaminated feedstuffs (B) were incubated at room temperature for 2 months in 10L plastic containers for the contamination to be achieved, mould growth was seen on the feedstuffs as a sign of contamination. Three experimental diets were formulated at (45% crude protein using Pearson square method). Feedstuffs used were Maize, Guinea corn, Millet, Soybean, Soybean cake, Fishmeal, Blood meal, Groundnut cake, Maize bran. All feedstuffs used in the experimental diets were purchased from Kasuwan Gwari, Minna local market Niger State.

Table 1: Formulated Experimental Diets (Dry basis)

Feed Ingredients	Grade A (Good grade) Control Diet	Grade B (Contaminated Diet)	Grade C (Mixture of Diet A & B)
Soybean	26.04	21.00	20.40
Soybean cake	13.02	10.50	10.20
Groundnut cake	13.02	10.50	10.20
Fishmeal	13.02	10.50	10.20
Blood meal	6.51	5.25	5.10
Maize	4.09	7.56	7.49
Guinea corn	4.09	7.56	7.49
Millet	4.09	7.56	7.49
Maize bran	4.09	7.56	7.49
Vegetable oil	7.00	7.00	7.00
Mineral premix	5.00	5.00	5.00
Total	**100**	**100**	**100**
Proximate Composition of Experimental Diets			
Parameter (%)			
Moisture	4.51	4.50	4.50
Ash	4.79	3.97	4.83
Lipid	6.90	6.74	6.82
Fibre	2.20	2.98	2.66
Protein	45.06	45.77	45.06
NFE	36.54	36.04	36.14

Chemical Analysis of Diets

The test diets from each formulated diets were analysed according to the standard methods of AOAC (2000) for proximate composition, moisture, protein, Fat, Ashe, and Fibre.

Microbial Analysis (Microbial count)

39g of Sabouroud Dextrose Agar (SDA) and 28g of Nutrient Agar were weighed into two different 1000ml cornical flask and corked after the addition of 1000ml of distilled water. 9ml of distilled water was also pipette into various test-tubes and corked as well. The tubes and the media were all autoclaved at a temperature of 121^0C for 15min using an autoclave. Serial dilution was carried out on the samples by taken 1ml of the sample into the first test-tube using a micropipette and mixed. Sample aliquot was taken from the first test-tube again this was repeated until the last tube was achieved. 1ml of the diluted sample was taken and dispensed into the sterile Petri-dish and about 20ml of the molten agar was poured into the Petri-dish and rocked/swifted gently for homoginity. The culture plate were allowed to solidify and then transferred into the incubator. The culture plate containing the Nutrient agar was cultured at 37^0C for 24hrs while that containing the Sabouroud Dextrose Agar (SDA) was cultured at 28^0 C between 48-72 hrs. The resulting growths of the cultures were counted to the colony forming unit per mill (CFU/ml) analysed as described by Onwuka (2005).

Biochemical test (Bacteria identification)

Gram's staining

A smear was made on the slide using wire loop and heat fixed by passing it through the flame three times and allowed to dry. The slides were stained with 0.5% of crystal violet (which is the primary stain) for 60 seconds, then washed with tap water. Iodine solution was used to flood the slide for 30 seconds, and then washed off with water. 95% alcohol (ethanol) was used to decolorize the slide and rinsed up immediately with water. The slides were flooded with safranin (which is the secondary stain) for 60 seconds. The slides were drained and allowed to dry. The slide was examined under microscope using the oil immersion objective(x100) analysed according to the standard methods of AOAC (2000).

Biochemical test (bacteria isolation)

Bacteria isolation test were analysed according to the standard method of AOAC (2000)

Catalase Test.

This test is carried out to differentiate those bacteria that produce the enzyme catalase such as *staphylococci* from non-catalase producing bacteria such as *streptococci*. A smear of the bacterium was made on the slide using sterilized wire loop. About 2 drops of 3% hydrogen peroxide was added on the suspension slide. The production of gas bubbles indicates a positive reaction.

Coagulase Test.

This test is use to identify *staphylococcus aureus* which produces the enzyme coagulase. It is the same procedure with that of catalase test except that human plasma was used. A small portion of the culture was emulsified on a clean slide with the aid of a wire loop. Three drops of undiluted human plasma was added to it and observed for clumping. Coagulation indicated clumping as positive result while negative results show no clumping.

Citrate Utilization Test.

This test was carried out by preparing a citrate agar on a petri dish, making a streak on the isolates with sterile wire loop and incubate at 37^0C for 48 hours. A bright blue colour in the medium indicate a positive result.

Oxidase Test.

A piece of filter paper is placed in a clean petri dish and 2-3 drops of freshly prepared 1% oxidase Reagent (Tetramethyl-p-phenylenediamine dihydichloride) was added using a glass rod, the test organism was removed and smeared on the filter paper. The development of purple blue colour within 30 seconds was read as positive result.

Fungal Identification

The identification of fungi was done by observation of the morphological appearance or characteristics of the culture for colour shape and mycelium formation as analysed according to the standard methods of AOAC (2000)**.**

Haematological and Biochemical Test

At the beginning and end of the experiment fish blood samples were collected. Fish was bled (using dissecting blade) from caudal artery by severing the caudal fin 5ml blood samples were collected from each of the treatments. 3ml of the blood samples collected were deposited into labelled sample bottles containing Ethylene Diamine-Tetra-Acetic Acid (EDTA) as an anticoagulant, the bottles were immediately covered and the content mixed gently by repeated inversion to find out the haematological parameters of the fish while the remaining 2ml blood samples were collected into labelled sterilized bottles that does not contain EDTA for the serum biochemical analysis of the fish as described by Ajagbonna, et al, (1999) and Uko et al. (2000).The blood samples were taken immediately to Falal-Rabi Medical Laboratory Services, STD 125 B Paiko Road, Opposite Choice Super Market Tunga, Minna.

The collected blood samples (containing EDTA) was used for the determination of haematological parameters such as the pack cell volume (PCV) were determined using microhaematocrit method, Haemoglobin (HB) was calculated as cyanmethemoglobin. The total red blood cell (TRBC) was determined using wintrobes (Improved NewbauerHaemocytometer). Micro-haematocrit and the platelet (PLT) count were determined as described by Lamb (1981), ciesla (2007) and Okomoda et al. (2013). The mean corpuscular haemoglobin concentrate (MCHC), mean corpuscular haemoglobin (MCH), and mean corpuscular volume (MCV) were calculated using the formulae described by Erhunmwunse & Ainerua (2013).

(i) MCHC = HB x 10/PCV

(ii) MCH = HB x 10/RBC

(iii) MCV = PCV x 10/RBC

Neutrophils**,** Monocytes, Eosinphils and Basophils were determined as described by Rusia and Sood (1992).

Total blood plasma protein was measured by the use of Biuret method as described by Doumas (1975) by using commercial preparation (Sigma Chemical Co.) as protein standard. Urea was determined using diacetylmonoxime method as described by Varley and Bell (1980). Creatinine was determined using Jaffe reaction according to the method described by Henry et al. (1974). Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Bihrubin Total (BT),

Bihrubin Conjugate (BC) and Alkaline phosphatase (ALP) were determined using the method described by Bergmeyer and Bernt (1974). Sodium and Potassium was measure using a flame photometer (Corning model 400; corning scientific limited, England).

Experimental Design and Experimental Procedure.

Complete Randomised Design was used for the experiment. All treatments were assigned randomly and in triplicate. The experimental fishes were randomly assigned into three (3) treatments and three (3) replicates at 20 fish per treatment. The fishes were fed diets containing varying inclusion levels of microbial formulated diets A, B and C respectively for Eight (8) weeks. The fishes were fed twice daily at 3% of their body weight 8am and 4pm. Initial weight of the fishes were taken using digital weighing scale (Electronic Balance, Golden Mettler USA, Model: RC-D) prior to feeding trial. Bulk weight was taken every two weeks of the feeding trial.

Growth Performance and Nutrient Utilization

The growth performance and nutrient utilization parameters were calculated according to the body weight of each fish and measured before and at the end of the trial.

i. Average weight gain = Average Final Weight (g) – Average Initial Weight (g)

ii. Protein efficiency ratio (PER) = $\frac{\text{Mean Weight Gain of fish (g)}}{\text{Mean Weight of protein fed (g)}}$

iii. Feed conversion ratio (FCR) = $\frac{\text{Mean Dry Weight of food fed (g)}}{\text{Mean Weight of Fish (g)}}$ i.e. $\frac{\text{Wt. of food fed}}{\text{Wt. gain of fish}}$

iv. Energy retention (ER%) = $\frac{100\ (\text{Retained Energy (Kcal)}}{\text{Energy intake (Kcal)}^{-}}$

v. Protein intake = $\frac{\text{Final Protein – Initial Protein}}{\text{Weight gain}}$

vi. Specific growth rate (SGR) = $\frac{100\ (\ln W_2 - \ln W_1)}{T}$

vii. Apparent Net Protein Utilization (ANPU %) = $\frac{100\ (\text{Protein gain in fish (g)}}{\text{Protein gain in diet (g)}}$

Statistical Analysis

The obtained data of fish growth, nutrient utilization and survival were subjected to a one way ANOVA using SPSS version 23. Significant mean differences were separated at 5% probability level in a Duncan Multiple Range test.

RESULTS

Microbial Counts of feedstuffs Ingredients used for the study

The microbial counts analysis results for some feedstuffs ingredients used in the formulation of the experimental diets is shown in table 2, grade A (Good feedstuffs), which served as control diet, indicated that guinea corn and blood meal have the least of bacteria and fungi count of

$2.0X10^3$ cfu/g and $2.0X10^5$ cfu/g while maize bran and maize had the highest bacteria and fungi count of $18.4X10^4$ cfu/g and $1.5X10^6$ cfu/g.

Microbial contaminated feedstuffs grade B (Contaminated feedstuffs), indicate that maize and guinea corn have the least bacteria and fungi count of $98.6X10^3$ cfu/g and $48.2X10^4$ cfu/g while soybean cake and groundnut cake had the highest bacteria and fungi count of $138X10^6$ cfu/g and $48X10^6$ cfu/g.

Microbial contaminated feedstuffs grade C (Mixture of A and B feedstuffs), indicated that blood meal and maize bran have the least bacteria and fungi count of $4.2X10^4$ cfu/g and $8.0X10^5$ cfu/g while maize bran and millet had the highest bacteria and fungi count of $8.9X10^8$ cfu/g and $1.8X10^7$ cfu/g.

Table 2: Microbial Count of Feedstuffs Ingredients

	Grade A (Good Feedstuffs)		Grade B (Contaminated feedstuffs)		Grade C (Mixture of A & B)	
Ingredients	**Bact=cfu/g**	**Fung=cfu/g**	**Bact=cfu/g**	**Fung=cfu/g**	**Bact=cfu/g**	**Fung=cfu/g**
Maize	$6.0X10^3$	$1.5X10^6$	$98.6X10^3$	$61.X10^4$	$7.2X10^5$	$3.2X10^6$
Guinea corn	$2.0X10^3$	$2.0X10^5$	$78.4X10^4$	$48.2X10^4$	$5.2X10^4$	$1.4X10^7$
Millet	$6.0X10^3$	$5.0X10^5$	$104.2X10^6$	$71.2X10^5$	$9.2X10^5$	$1.8X10^7$
Soybean	$3.3X10^4$	$7.0X10^5$	$129X10^5$	$101X10^5$	$8.6X10^6$	$2.5X10^6$
Soybean cake	$12.2X10^3$	$7.0X10^5$	$138X10^6$	$21.4X10^6$	$4.1X10^5$	$8.3X10^6$
Fishmeal	$1.1X10^4$	$4.0X10^5$	$107.2X10^4$	$78.1X10^4$	$2.3X10^5$	$8.6X10^6$
Blood meal	$2.1X10^4$	$2.0X10^5$	$132.6X10^4$	$89.2X10^5$	$4.2X10^4$	$5.4X10^6$
Groundnut cake	$5.0X10^3$	$7.0X10^5$	$121X10^6$	$48X10^6$	$8.2X10^5$	$5.0X10^6$
Maize bran	$18.4X10^4$	$16.6X10^5$	$119X10^5$	$98.2X10^4$	$8.9X10^8$	$8.0X10^5$

Microbial Counts of some feed formulated diets used for the Experiment

Microbial count analysis of formulated diets is shown in table 3 and indicated that diets A have the least bacteria and fungi count of $2.75X10^5$ cfu/g and $2.43X10^5$ cfu/g while diet B had the highest bacteria and fungi count of $4.76X10^5$ cfu/g and $4.78X10^5$ cfu/g.

Table 3: Microbial Count Analysis of Experimental diet fed Experimental fish

Microbial Load	Grade A (Good Feedstuffs)	Grade B (Contaminated feedstuffs)	Grade C (Mixture of A & B)
Bacteria (x 10^5cfu/g)	2.75 ± 0.13^a	4.76 ± 0.14^c	2.97 ± 0.05^a
Fungi (x 10^5cfu/g)	2.43 ± 0.53^a	4.78 ± 0.15^c	3.43 ± 0.30^b

Values on the same row with different alphabetic superscripts are statistically significant at (P≤0.05) test level.

Fungal and Bacteria Organisms found in microbial contaminated Feedstuffs

The fungi and bacteria organisms found in microbial contaminated feedstuffs are shown in Table 4 below.

Table 4: Fungal and Bacteria Organisms found in Microbial Contaminated Feedstuffs

Feedstuffs	Fungal Identified	Bacteria Identified
Maize	*Aspergillus niger*	*Escherichia coli, Staphylococcin aureus.and Pseudomona aeruginosa.*
Guinea corn	*Rhizobus, penicillium*	*Escherichia coli, Staphylococcin aureus.and Pseudomona aeruginosa.*
Millet	*Penicillium, aspergillusniger*	*Escherichia coli, Staphylococcin aureus.and Pseudomona aeruginosa.*
Soybean	*Penicillium, rhizobus*	*Escherichia coli, Staphylococcin aureus.and Pseudomona aeruginosa.*
Soybean cake	*Penicillium, rhizhobus*	*Escherichia coli, Staphylococcin aureus.and Pseudomona aeruginosa.*
Fishmeal	*Yeast, penicillium aspergillus flavus, rhizobus*	*Escherichia coli, Staphylococcin aureus.and Pseudomona aeruginosa.*
Blood meal	*Penicillium, rhizobus*	*Escherichia coli, Staphylococcin aureus.and Pseudomona aeruginosa.*
Groundnut cake	*Yeast, rhizobus*	*Escherichia coli, Staphylococcin aureus.and Pseudomona aeruginosa.*
Maize bran	*Penicillium, rhizobus*	*Escherichia coli, Staphylococcin aureus.and Pseudomona aeruginosa.*

Growth Performance and Nutrients Utilization of *Clarias gariepinus* fed Experimental Diets

There was no significant difference (p> 0.05) in the initial mean weight of the fish used for the experiment. However, there was significant difference (p< 0.05) in final mean weight. The final mean weight varied between 4.48g-5.10g. Fish fed diet (A) had the least final mean weight (4.48g), while that fed diet (B) had the highest final mean weight (5.10g). Similarly, there was significant difference (p< 0.05) in mean weight gain. Fish fed diet (B) had the highest mean weight gain (1.11g) and the least was diet (A) 0.48g). There was no significant difference (p> 0.05) in specific growth rate. Where fish fed diet B (0.45%) was highest and diet A (0.20%) was least.

There was significant difference (p< 0.05) in feed conversion ratio. Where fish fed diet A (8.58) had the highest and diet B (3.75) had the least. There was also significant difference (p< 0.05) in protein efficiency ratio with diet B (0.59) had the highest and diet A (0.26) had the least respectively. There was significant difference (p< 0.05) in apparent net protein utilization, were diet C (100.78) had the highest and diet B (76.93) had the least. There was significant difference (p< 0.05) in survival rate, where diet A (83.33%) had the highest and diet B (43.33%) had the least.

Table 5: Growth Performance of *Clarias gariepinus* Fed Microbial Infested Diets

Parameters	A	B	C
IMW	4.00±0.04^{a}	3.99±0.03^{a}	3.97±0.00^{a}
FMW	4.48±0.02^{a}	5.10±0.05^{c}	4.71±0.09^{ab}
MWG	0.48±0.04^{a}	1.11±0.04^{c}	0.74±0.09^{ab}
SGR	0.20±0.01^{a}	0.45±0.03^{d}	0.30±0.03^{c}
FCR	8.58±0.78^{b}	3.75±0.17^{a}	5.65±0.82^{a}
PER	0.26±0.02^{a}	0.59±0.03^{c}	0.40±0.05^{ab}
ANPU	91.50±1.35^{b}	76.93±1.43^{a}	100.78±0.73^{c}
%SR	83.33±1.67^{d}	43.33±1.67^{a}	55.00±2.89^{b}

Values on the same row with different superscripts are statistically significant at (p≤0.05) test level.

IMW :- Initial Mean Weight**, FMW :-** Final Mean Weight, **MWG :-** Mean Weight Gain, **SGR :-** Specific Growth Rate, **FCR :-** Food Conversion Ratio, **PER :-** Protein Efficiency Ratio, **ANPU :-** Apparent Net Protein Utilization, **SR :-** Survival Rate.

Haematological Analysis

There was no significant difference (p> 0.05) in the haematological parameters of *Clarias gariepinus* fed experimental diets, but were significant difference (p<0.05) from the initial, where PCV was highest in diet C (25.49%) and least in diet B (24.70%). WBC was highest in diet

B (2.42 (10^3 mm^{-3}) and least in diet A (1.86 (10^3mm^{-3}) respectively. There was no significant difference (p>0.05) in RBC among treatments but were significant difference (p<0.05) form the initial, where diet A (3.78 (10^6mm^{-3}) had the highest and diet C (3.66 (10^6mm^{-3}) had the least. HB was highest in diet C (8.73g/100ml) and least in diet A (8.60g/100ml). There was significant difference (p< 0.05) in LYMPH. However, diet B (67.44%) was the highest and diet A (60.52%) had the least. There was no significant difference (p> 0.05) in MCHC and MCH among treatments but were significant difference (p<0.05) from the initial, where MCHC was highest in diet B (3.52%) and least in diet A (3.38%). MCH was highest in diet C (23.94pg) and least in diet A (22.74pg).There was significant difference (p<0.05) in MCV and NTP, where MCV was highest in diet C (70.14fl) and least in diet B (65,98fl). NTP was highest in diet C (38.67%) and least in B (30.91%). There was no significant different (p> 0.05) in MC, ESP and BSP, where MC was highest in diet A (2.13%) and least in diet B (1.70%). ESP was highest in diet C (2.44%) and least in diet B (2.40%).

Table 6: Haematological Parameters of *Clarias gariepinus* fed Experimental Diets for 56 days

Parameter	Initial	A	B	C
PCV (%)	21.50±0.01^a	25.42±0.37^b	24.70±0.38^b	25.49±0.26^b
WBC (10^3mm^{-3})	1.92±0.01^a	1.86±0.13^a	2.42±0.30^b	1.95±0.03^{ab}
RBC (10^6mm^{-3})	2.90±0.01^a	3.78±0.13^b	3.77±0.23^b	3.66±0.21^b
HB (g/100ml)	5.24±0.01^a	8.60±0.36^b	8.68±0.24^b	8.73±0.18^b
LYMPH (%)	60.07±0.01^a	60.52±0.36^a	67.44±0.39^c	61.53±0.27^b
MCHC (%)	2.44±0.01^a	3.38±0.09^b	3.52±0.13^b	3.42±0.09^b
MCH (pg)	18.07±0.00^a	22.74±0.18^b	23.13±0.72^b	23.94±0.86^b
MCV (fl)	74.13±0.01^a	67.34±1.35^a	65.98±4.23^a	70.14±4.29^a
NTP (%)	34.07±0.00^b	34.91±0.05^c	30.91±0.17^a	38.67±0.23^d
MC (%)	2.02±0.00^a	2.13±0.07^a	1.70±0.16^a	1.73±0.18^a
ESP (%)	2.01±0.01^a	2.43±0.39^a	2.41±0.36^a	2.44±0.39^a
BSP (%)	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a

All values in the results in the Table 5 above were reported as mean ± standard error of mean. Values on the same row with different alphabetic superscripts are statistically significant at (P≤0.05) test levels

PCV:- Packed Cell Volume**, WBC:-** White Blood Cell, **RBC:-** Red Blood Cell, **HB:-**Haemoglobin, **LYMPH:-** Lymphocytes, **MCHC:-** Mean Corpuscular Haemoglobin Concentration, **MCH:-** Mean Corpuscular Haemoglobin, **MCV:-** Mean Corpuscular Volume, **NTP:-** Neutrophils**, MC:-**Moncytes, **ESP:-**Eosinphils, **BSP:-**Basophils.

Biochemical Analysis

There are significant differences ($p< 0.05$) in the biochemical parameters of *Clarias gariepinus* fed experimental diet to some extent. However, Total bihrubin was highest in diet B (3.91 (umol/l) and least in diet A (3.64 (umol/l), while there was no significant difference ($p>0.05$) between diet A and diet C. Conjugate bihrubin was highest in diet C (3.95 (umol/l) and least in diet B (3.73 (umol/l). There was significant difference ($p< 0.05$) in Alkaline phosphate, where diet A (501.50 (u/l) had the highest and diet B (414.14 (u/l) had the least. ALT was highest in diet A (21.69 (u/l) and least in diet B (18.98 (u/l). There was no significant difference ($p> 0.05$) in AST, where diet C (10.18 (u/l) had the highest and diet B (9.91 (u/l) had the least. There was significant difference ($p< 0.05$) in Na^+, where diet B (32.92 (mmol/l) had the highest and diet A (30.53(mmol/l) had the least. There was no significant difference ($p> 0.05$) in K^+, where diet C (1.85 (u/l) had the highest and diet B (1.70 (u/l) had the least. Urea was highest in diet C (2.77 (u/l) and least in diet A (1.77 (u/l). Creatinine was highest in diet A (1.66 (mg/l) and least in diet C (1.63 (mg/l). There was significant difference ($p<0.05$) in total protein, where diet B (6.55 (g/dl) had the highest and diet A (5.63 (g/dl) had the least.

Table 7: Biochemical Parameters of *Clarias gariepinus* fed contaminated experimental Diets for 56 Days

Parameter	Initial	A	B	C
Total Bihrubin (umol/l)	3.37 ± 0.01^{a}	3.64 ± 0.19^{ab}	3.91 ± 0.07^{b}	3.77 ± 0.13^{ab}
Conj. Bihrubin (umol/l)	3.67 ± 0.00^{a}	3.81 ± 0.13^{a}	3.73 ± 0.15^{a}	3.95 ± 0.10^{a}
ALP(u/l)	418.69 ± 0.01^{c}	501.50 ± 0.26^{e}	414.14 ± 0.20^{a}	419.64 ± 0.21^{d}
ALT (u/l)	21.17 ± 0.01^{b}	21.69 ± 0.23^{b}	18.98 ± 0.44^{a}	21.67 ± 0.22^{b}
AST(u/l)	10.47 ± 0.01^{c}	10.15 ± 0.10^{b}	9.91 ± 0.07^{a}	10.18 ± 0.07^{b}
Na^+	31.74 ± 0.01^{b}	30.53 ± 0.27^{a}	32.92 ± 0.14^{c}	30.65 ± 0.29^{a}
K^+ (u/l)	1.70 ± 0.01^{a}	1.77 ± 0.14^{a}	1.70 ± 0.21^{a}	1.85 ± 0.11^{a}
Urea (u/l)	2.48 ± 0.01^{b}	1.77 ± 23^{a}	2.51 ± 0.36^{b}	2.77 ± 0.13^{b}
Creatinine (mg/l)	1.14 ± 0.01^{a}	1.66 ± 0.31^{a}	1.65 ± 0.20^{a}	1.63 ± 0.25^{a}
Tot. Protein (g/dl)	5.72 ± 0.01^{a}	5.63 ± 0.19^{a}	6.55 ± 0.25^{b}	5.79 ± 0.13^{a}

All values in the results in the Table 6 above were reported as mean ± standard error of mean. Values on the same row with different alphabetic superscripts are statistically significant at ($P\leq0.05$) test

ALP:- Alkaline Phosphate**, ALT:-** Alanine Aminotransferase, **AST:-** Aspartate Aminotransferase, **Na^+:-** Sodium ion, **K^+:-** Potassium ion.

Body Composition

There was no significant difference (p> 0.05) in moisture content and crude protein of *Clarias gariepinus* carcass fed experimental diet but were significant difference (p<0.05) from the initial carcass,where diet B (6.05%) had the highest and diet C (5.95%) had the least in moisture content. While crude protein was highest in diet C (56.64%) and least in diet B (56.24%). There was no significant different (p> 0.05) in Ash content. However, diet A (4.48%) had the highest Ash and diet C (3.92%) had the least. There was no significant difference (P> 0.05) in Lipid content. However, diet C (30.72%) was the highest and diet B (29.83%) was the least. There was no significant difference (p>0.05) in nitrogen free extract but significant difference from initial, where diet B (3.48%) had the highest and diet A (2.56%) had the least.

Table 8: Proximate Composition analysis of whole body *Clarias gariepinus* (dry basis) fed experimental diet for 56 days

Parameter	Initial	A	B	C
Moisture (%)	5.16±0.01^{a}	6.01±0.08^{b}	6.05±0.11^{b}	5.95±0.30^{b}
Ash (%)	4.14±0.01^{a}	4.48±0.30^{a}	4.40±0.30^{a}	3.92±0.27^{a}
Lipid (%)	30.15±0.01^{a}	30.45±0.29^{a}	29.83±0.26^{a}	30.72±0.27^{a}
Protein (%)	54.81±0.01^{a}	56.50±0.28^{b}	56.24±0.30^{b}	56.64±0.31^{b}
Nfe (%)	5.74±0.01^{b}	2.56±0.45^{a}	3.48±0.11^{a}	2.77±0.74^{a}

All values in the results in the Table 7 above were reported as mean ± standard error of mean. Values on the same row with different alphabetic superscripts are statistically significant at (P≤0.05) test level.

Water Quality

There was no significant difference (p> 0.05) in Temperature, dissolved oxygen, pH and Ammonia in the experimental set-up. However, temperature ranges from 30.56^{0} C-30.59^{0} C with diet B (30.59^{0} C) as the highest and diet A (30.56^{0} C) as the least. The value of dissolved oxygen ranged from 6.11mg/l-6.13mg/l, where diet C (6.13mg/l) was highest and least in diet A and B (6.11mg/l). pH value ranged from 7.33-7.34, where diet B and C (7.34) was highest and diet A (7.33) was least. There was no significant difference (p> 0.05) in the value of Ammonia.

Table 9: Mean Water Quality Parameters of *Clarias gariepinus* fed Experimental Diets for 56 days

Parameter	A	B	C
Temp(^{0}C)	30.56±0.31^a	30.59±0.25^a	30.57±0.33^a
DO(mg/l)	6.11±0.06^a	6.11±0.07^a	6.13±0.09^a
pH	7.33±0.04^a	7.34±0.05^a	7.34±0.05^a
Amm(mg/l)	0.01±0.00^a	0.01±0.00^a	0.01±0.00^a

All values in the results in the Table 8 above were reported as mean ± standard error of mean. Values on the same row with different alphabetic superscripts are statistically significant at (P≤0.05) test level.

Discussion

The mean initial weight of fish used were not significantly different (p>0.05) indicating a homogenous group distribution of fish stocked. This study revealed that, the microbial infested feedstuffs diets fed *Clarias gariepinus* fingerlings showed that the fingerlings fed with diet (B) microbial infested diets had best growth response, followed by diet (C) mixture of grade A (Good graded feedstuffs) and grade B (Contaminated feedstuffs), while diet (A) good graded diet exhibited least growth.

This disagree with the finding of Effiong and Alatise (2009), who reported that, mold infested fish feeds have been reported to have negatively impact growth in vundu fish. It also disagree with the finding of Jantratrotai and Lovell (1990), who reported that, channel catfish fed mold infested feed for 10 weeks, exhibited decreased growth rates and moderate internal lesions. Although no visible lesions were observed on the experimental fish fed infested feed diets during the experiment.

Diet (A) good graded diet exhibited high survival rate of 83.33%, followed by diet (C) (mixture of grade A and grade B feedstuffs) with 55%, while diet (B) microbial infested diet had least survival rate of 43.33%, as reported by Ciceron *et al.,* 2008 that deaths of fingerlings were likely due to feeding feeds containing toxin-producing fungi which are known to be strongly carcinogenic and mutagenic in nature.

The proximate composition of *Clarias gariepinus* fed different diets at different condition no variation in carcass proximate composition among treatments. The body composition of the fish carcass shows an increase in the value of crude protein and moisture over the initial fish samples. This agrees with reports of Alegbeleye *et al*. (2001), Ochang *et al*. (2007) and Ajani *et al*. (2011) who reported increase in the protein content of experimental fish carcass above initial. The proximate composition of *C. gariepinus* fed different feed diets under different conditions revealed considerable variation in carcass crude protein.The protein content from fish fed with diets A, B and C could be an indication that there are variations in the nutritional value of contaminated feedstuffs and non contaminated feedstuffs. This is in line with the observation of Adewale and Omotosho (1997). The variations in the mean proximate composition among the fish fed different diets formulated from different feedstuffs might be

attributed to the effect of holding condition and environmental factors on the feed which is then translated into observed growth (Gupta *et al.,* 2007).

Within the condition of the experiment, microbial infested feedstuffs diets was an unacceptable source of plant and animal diets on *Clarias gariepinus* fingerlings due to the toxic effects on their survival. The fishes adapted to the diets within 2-4 days of commencement of the feeding trial, though the acceptability of the diets varied. The haematological indices indicated a sharp rise in white blood cell for diets containing contaminants. These parameters are valuable in monitoring feed toxicity especially with feed ingredients that affect the formation of blood in culture fisheries (Oyawoye & Ogunkunle, 1998). This justifies the need to understand the physiological concept of fish health and survival in relation to blood and the quality of dietary protein fed (Onyia *et al*., 2013).

Haematological parameters are very important factors used for the evaluation of fish physiological status. Their changes depend on fish species, age, and the cycle of the sexual maturity of spawners and diseases, Golovina (1996). Haematological components of blood are also valuable in monitoring feed toxicity especially with feed constituents that affect the formation of blood in culture fisheries Oyawoye and Ogunkunle (1998).

The Packed Cell Volume (PCV) was within the range (24.70 – 25.49 %), with diet C (Contaminated feedstuffs) being the highest. PCV also known as haematocrit is a useful tool in fisheries and aquaculture management for checking anaemic condition in aquatic species. The mean value of PVC for fish fed formulated diet fall within the recommended range of 20 – 38% for fish as reported by Erondu *et al*. (2003). The PCV range for the feeding trials were lower than reports of Adewolu and Aro, (2009), Sotolu and Faturoti (2009), Adeyemo *et al*., (2003), Abalaka (2013), Agbabiaka *et al*., (2013b), Mamman *et al.* (2013), Dienye and Olamuji (2014), Adewole and Olaleye (2014), Afia and Ofor (2016) and higher than results of Ayoola (2011).

White blood cells are the defence cells of the fish body. The increase in WBC (leucopomia) for fish fed diet C (Contaminated feedstuffs) could be attributed to the increase in leucocytes synthesis in the haematopoietic tissue of the kidney and perhaps the spleen as a defense mechanism against the destruction of bacteria and fungi. Lymphocytes are the most numerous cells comprising the leucocytes, which function in the production of antibodies and chemical substances serving as defense against infection Ayoola (2011). The primary consequence of observed changes in leucocyte count in stressed fish is suppression of the immune system and increased susceptibility to disease Wedemeyer and Wood (1974). This is true since the amount has implication in immune response and the ability of the animal to fight infection, Douglas and Jane (2010).

High count for Red blood cells was observed in fish fed with all formulated diets in all the graded diets. This could be attributed to the level of protein in all formulated diets to meet the fish nutrient requirements as haemoglobin is a property of protein of which RBC is a carrier. This might have increased the production rate of RBC. The result of RBC count for the treatments disagrees with results of Adeyemo *et al.* (2003), Osuigwe *et al*., (2005) and agrees with results of Ayoola (2011), Agbabiaka *et al*., (2013b), Dada and Ikwerowo (2009), Dienye and Olamuji

(2014), Adewole and Olaleye (2014), and Afia and Ofor (2016). However, the values recorded for RBC for all the treatments were not within the range of normal haematology of a healthy fish, Fagbenro *et al*., (1993) and Rastogi (2007). Erythrocyte count greater than 1.00×10^6 mm^{-3} is considered high and indicative of high oxygen carrying capacity of the blood, which is characteristic of fishes capable of aerial respiration and with high activity Jimoh *et al*., (2014).

The haemoglobin concentration (Hb) showed no variation among the treatments with highest Hb recorded for C. gariepinus fed all diets compared to initial. Physiologically, haemoglobin is crucial to the survival of fish being directly related to oxygen binding capacity of blood. However, Teixeria (2000) cited by Afia and Ofor (2016) stated that referenced values determined for haematological tests should be carefully interpreted once there is a wide range of physiological variations since these variations might be influenced by environmental conditions, sex, age, feeding system and feeding. Low haemoglobin level might decrease the ability of fish to enhance its activity in order to meet occasional demands, Joshi *et al*., (2002). The range of Hb (8.60 – 8.73 g/dl) for the treatments diets were high and were not similar with results of Musa et *al.*, (2013), Adeyemo *et al*., (2003), and Gabriel *et al*., (2004).

Mean corpuscular haemoglobin concentration (3.38 – 3.52 g/dl) obtained in the current study was different from the initial and the values were different from results of Omitoyin (2006), Adeyemo *et al* (2003), Agbabiaka *et al*., (2013b) and Afia and Ofor (2016). This might be due to contaminants in the feedstuffs, which consequently influenced blood parameters values.

The Mean corpuscular haemoglobin range (22.74 – 23.94 pg) from the present study compared unfavourably with results of Afia and Ofor (2016) and favourably with results of Terry *et al*., (2000), Gabriel *et al*., (2004), Ochang *et al*., (2007), Ayoola (2011), Mamman *et al*., (2013), Adewole and Olaleye (2014) and Dienye and Olamuji (2014). This could be attributed to the environment condition and status of feedstuffs as well as other environmental factors the cultured fish may have been exposed to during the experimental period.

Mean corpuscular volume (MCV) range of (65.98 – 70.14 fl) obtained from the treatments diets were different from results of Ayoola (2011), Ayeloja *et al*. (2012), Mamman *et al*. (2013), Afia and Ofor (2016). The present result compared favourably with reports of Adeyemo *et al*. (2003), Ochang *et al.* (2007), Sotolu and Faturoti (2009), Agbabiaka *et al*. (2013b), Dienye and Olamuji (2014), Adewole and Olaleye (2014).

Proteins play an important role in the maintenance of blood nutrients (Shwetha *et al*., 2012). Total protein range (5.63-6.55g/dl) obtained from treatment diets with diet B (contaminated feedstuffs) which has the high value from the study could link to the protein content of the formulated diet which disagree with the finding of Yadav *et al.* (2003) who reported a decrease in serum total protein content in *Channa punctatus* induced with stem-bark extract of *Croton tiglium*. Decrease in total protein in fish exposed to toxic levels of toxicants could be attributed to either a state of hydration and change in water equilibrium in the fish or a disturbance in proteinsynthesis within the liver or both (Gluth and Hanke, 1984).

Values of potassium ion (K^+) concentration were lower than 13.36±4.55 mmol/l concentrations reported by Owolabi (2011) as well as

13.24±2.45 mmol/l (Lawali *et al*., 2015). The range from the study (1.70-1.85 u/l).

Values of sodium ion (Na^+) concentration were within the range of 1.10 - 74.54 mmol/l recorded for juvenile *C. gariepinus* fed coconut (*Cocosnucifera*) water (Ayotunde *et al*., 2015). The range from the study (30.53-32.92 u/l).

Creatinine value from the study ranged from 1.63-1.66 mg/l. Creatinine values were comparatively above 0.35 – 0.97 µmol/L recorded for *C. gariepinus* juveniles exposed to paraquat dichloride (Ogamba *et al.*, 2011), the low values suggested that creatinine was effectively used up by fish muscle in response to the presence of anti-metabolites in the diets.

Urea concentration observed in the present study (1.77 – 2.77 u/l) which is in line with the range of 2.33 - 3.23 µmol/l who reported for *C. gariepinus* juveniles exposed to paraquat dichloride (Ogamba *et al.*, 2011). The liver is the main source of urea; thus, the lower urea levels have been suggested to be an indication of the impaired functioning of the liver (Hlophe and Moyo, 2014).

There were slightly high values of alanine aminotransferase (ALT) in the study with range of (18.98-21.69 u/l) which suggested that the blood serum enzyme in the experimental fish efficiently utilized amino acids for metabolic purposes, confirming the observation of Adesina (2008). Transaminases are important enzymes for monitoring the health status of fish (Racicot *et al.*, 1975) and leak out into the bloodstream from dying or damaged liver cells. Increased levels of transaminases in the blood serum of fish are usually associated with dying or damaged liver cells while a decrease could suggest leakage of enzymes into the serum (Yilmaz *et al*., 2006; Ozovehe, 2013).

The range values of aspartate aminotransferase (AST) in the study were (9.91-10.15 u/l). Aminotransferase levels in fish increase in response to stress (Tiwari and Singh, 2004). The values of aspartate aminotransferase (AST) in the study disagree with that of Dienye and Olumuji (2014) who reported elevated AST, ALT and ALP activities in fish fed 30% *M. oleifera* leaf meal diet and above which suggested hepatic cellular damage leading to their leakage into the bloodstream (Mousa *et al*., 2008).

The water quality parameters in the present study were not affected by the diet forms and were within the range recommended for African catfish culture Boyd. (1996) Olurin *et al*. (2006). These results highlight that, feeding *C. gariepinus* with commercial and locally formulated diets does not cause significant deterioration of water quality parameters provided the diets are formulated correctly and the fish fed optimally. Although the water temperatures from feeding trials were not different significantly, these were however, within the optimal range (<40^0C) described for aquaculture FEPA (2003). The body temperature of fish changes according to that of its environment, affecting its metabolism, physiology and ultimately affecting the production Bhatnager and Devi (2013). Temperature results agree with earlier works of Ajani *et al*. (2011) and Ekanem *et al*. (2012) and dissagree with results of Mustapha *et al*. (2014) and Limbu (2015). Dissolved oxygen is needed for aerobic generation of energy for body maintenance, growth, survival, behaviour and physiology of aquatic organisms Afia and ofor (2016). The dissolved oxygen range of 6.11 – 6.13 mg/l obtained from this experiment agree with results of Ajani *et al*. (2001), Ekanem *et al*. (2012), Mustapha *et al*. (2014), Limbu (2015), Afia and Ofor (2016) and were within tolerable range for fish culture. The ideal pH for biological productivity is 7.0 to 8.5; fish becomes stressed in water with pH ranging from 4.0 to 6.5 and 9.0

to 11.0 Ekubo and Abowei (2011). The mean pH level obtained in this study for both feeding trials is assumed not to have affected the fish growth as it was within desirable limits for catfish culture. The result agrees with results of Ajani *et al*. (2011), Ekanem *et al*. (2012) and Limbu (2015) and slightly lower than result of Mustapha *et al*. (2014). The study revealed that, there were no significance differences in the value of ammonia (0.01mg/l) as it does not affect fish culture in the experiment.

CONCLUSION

It was observed that, microbial infested diets had no detrimental effects on growth of *Clarias gariepinus* fingerlings but has negative impact on its survival as high mortality rate was recorded on the experiment. The research also revealed that, the contaminated diets affected the haematological and biochemical content of the experimental fishes especially the white blood cell and biochemical test.

Recommendation

1. Microbial infested feedstuffs have detrimental effects on the life and survival of *Clarias gariepinus* fingerlings and should be avoided for fish production process.

2. There should be proper selection of feedstuffs ingredients to avoid the use of microbial infested feedstuffs for formulation of diets.

3. Research should be conducted on feedstuffs sold on the markets for microbial infestation.

4. This type of research should be conducted on other available culturable species like, tilapia, *H. bidorsalis* and carp.

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Evaluation of Nigerian Bitumen Yield and Composition by Hydrous and Anhydrous Pyrolysis: A Case Study of Ondo State Bitumen

Abdulkadiri Raji & Muhammad Umar Garba

Corresponding Author's Email: abdulkadirraji.ar@gmail.com, Phone No.: 08065610919

Department of Chemical Engineering, Federal University of Technology (FUT), Minna, Nigeria

ABSTRACT

Petroleum is the world's most important source of energy. There is an ever-increasing demand for energy and this has resulted to depletion and decline in production due to the fact that petroleum is extracted much faster than its rate of formation, hence the need to source for unconventional sources of crude oil. This research aims to establish the superiority of crude oil produced from bitumen by hydrous pyrolysis technique over crude oil produced from bitumen by anhydrous pyrolysis technique. The bitumen sample collected from Agbabu in Odigbo local government area of Ondo State was subjected to pyrolysis by two processes – hydrous pyrolysis and anhydrous pyrolysis. Initial protocol tests were carried out on the bitumen sample including TGA, FT-IR, SEM-EDX and GC-MS to determine the thermal behavior, functional groups, elemental composition and nature of the Saturates, Aromatics, Resins and Asphaltenes (SARA) composition of the bitumen. Anhydrous Pyrolysis was carried out with temperature ranging from 200^{o}C to 400^{o}C and time from 30 minutes to 60 minutes. Hydrous Pyrolysis was also carried out with temperature ranging from 300 ^{o}C to 500 ^{o}C and time from 30 minutes to 60 minutes. The synthetic crude obtained from both processes were subjected to further test to determine the viscosity, density, specific gravity, calculation of the API gravity and GC-MS analysis. The bitumen was found to have high viscosity of 28cSt and low API gravity of 8.6 which corresponds to standard for heavy oil. The bitumen SARA components determined are 35 % saturates, 37 % aromatics, 26 % resins and 2 % asphaltenes. At the end of the study, it was established that the yield of synthetic crude oil from hydrous pyrolysis has a greater API gravity of 29.20, lower sulfur content 0.08 %, lower viscosity 3.04cSt and a higher concentration of saturates 65 % than the synthetic crude obtained from anhydrous pyrolysis which respectively has 23.99, 0.16 %, 3.28cSt and 50 %. From this research, exploitation of Nigerian bitumen becomes more economically viable such as seen in other countries around the world like Canada.

Key words: Nigeria, bitumen, anhydrous pyrolysis, hydrous pyrolysis, yield, composition.

INTRODUCTION

Petroleum has become the world's most important source of energy as it is used in supplying energy to power industries, generate electricity to heat homes and provide fuel for vehicles and airplanes to carry goods and people all over the world, also refined products of petroleum find application in the manufacture of chemical products, such as plastics, fertilizers, detergents and paints (Shedrach *et al.*, 2018). However, there is an increasing scarcity of

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conventional oil reserves due to depletion and decline in production as argued in the studies of Dr. Marion King Huppert (Ogundele *et al.*, 2011) based on the fact that petroleum is extracted much faster than its rate of formation, hence the need to source for unconventional sources of crude oil. (Lewan *et al.*, 2018). These unconventional sources of petroleum are oil-shale, heavy oils, tar sands and bitumen. Bitumen in Nigeria is found across Lagos, Ogun, Ondo and Edo states. Ondo State has one of the largest deposits of bitumen in the whole world and these have not been exploited for petroleum production (Arogundade & Ogunsuyi, 2021).

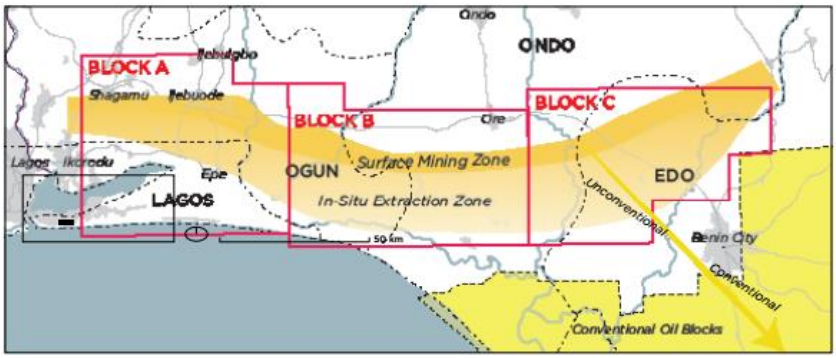

Fig. 1: Bitumen in Nigeria across four states namely Lagos, Ogun, Ondo and Edo state (Shedrach *et al.*, 2018).

Bitumen can be subjected to pyrolysis (hydrous or anhydrous) to produce petroleum. Whereas Anhydrous pyrolysis is thermal decomposition in an oxygen free and water-free environment, in Hydrous_pyrolysis the heated material is in contact with water and the general name for all hydrothermal pyrolysis processes with water either in the liquid phase, vapour phase or steam is Aquathermolysis. (Lewan *et al.*, 2018).

This research aims to establish the superiority of crude oil produced from bitumen by hydrous pyrolysis techniques over crude oil produced from bitumen by anhydrous pyrolysis techniques.

EXPERIMENTAL METHODS

Materials and Methods

The materials used in this work include: Viscometer, thermometer, stopwatch, water-bath, open-cup, digital density meter and beaker.

Methodology

The bitumen sample was collected from Agbabu which is situated in Odigbo local government area of Ondo State. Laboratory tests were carried out on the bitumen to determine its density, specific gravity, API gravity, viscosity and flash point. The Saybolt Furol Viscometer was used to determine the viscosity of the bitumen. The flash point of the bitumen was determined with the 'open cup' test apparatus. Density of the Bitumen was determined using Anton-Paar digital density meter and the specific gravity was determined with the help of a 10mL beaker. The following Spectro-Analytical Techniques were used for characterization of the bitumen sample: Thermo-Gravimetric Analysis (TGA), Fourier Transform Infrared Spectroscopy (FT-IR), Scan Electron Microscopy (SEM-EDX) and Gas

Chromatography Mass Spectrometry (GC-MS) respectively to determine the thermal behavior, functional groups, elemental composition and nature of the Saturates, Aromatics, Resins and Asphaltenes (SARA) composition of the bitumen. Hydrous and anhydrous pyrolysis experiment was carried out using the SA2-series 1200-degree lab-scale Nitrogen atmosphere horizontal tubular furnace pyrolysis manufactured by Chinese company Samlab and supplied by Vacutec, a South African equipment supplying company. The sulphur content was determined by the use of Energy Dispersive X-ray Fluorescence (EDXRF). The crude oil sample is placed in the beam emitted from an X-ray source. The resultant excited characteristic radiation is measured and the accumulated count is compared with counts from previously prepared calibration standards. This corresponds to ASTM D4294 standards for testing.

RESULTS

Table 1: **Results of viscosity, density, specific gravity, API gravity and flash point of bitumen sample**

Parameter	Units	Bitumen
Viscosity @40 ^{o}C	St	2800
Density	g/cm^3	0.97
Specific Gravity	-	1.01
API Gravity	^{o}API	8.60
Flash point	^{o}C	255

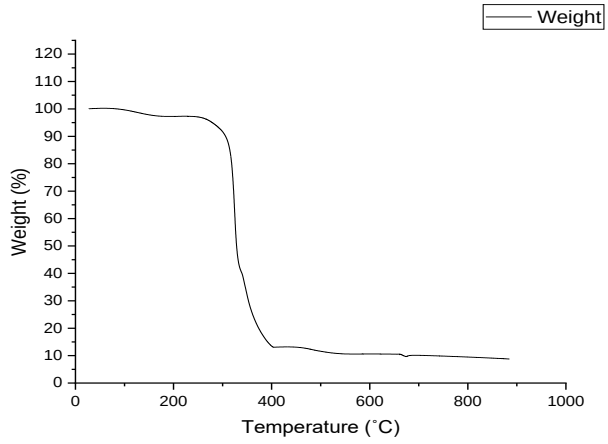

Fig. 2: **TGA of bitumen sample**

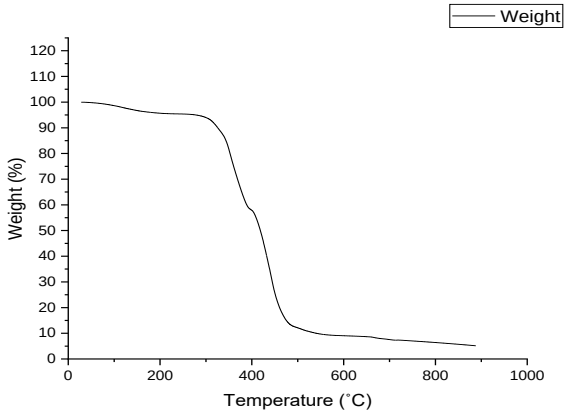

Fig. 3: **TGA of bitumen plus water**

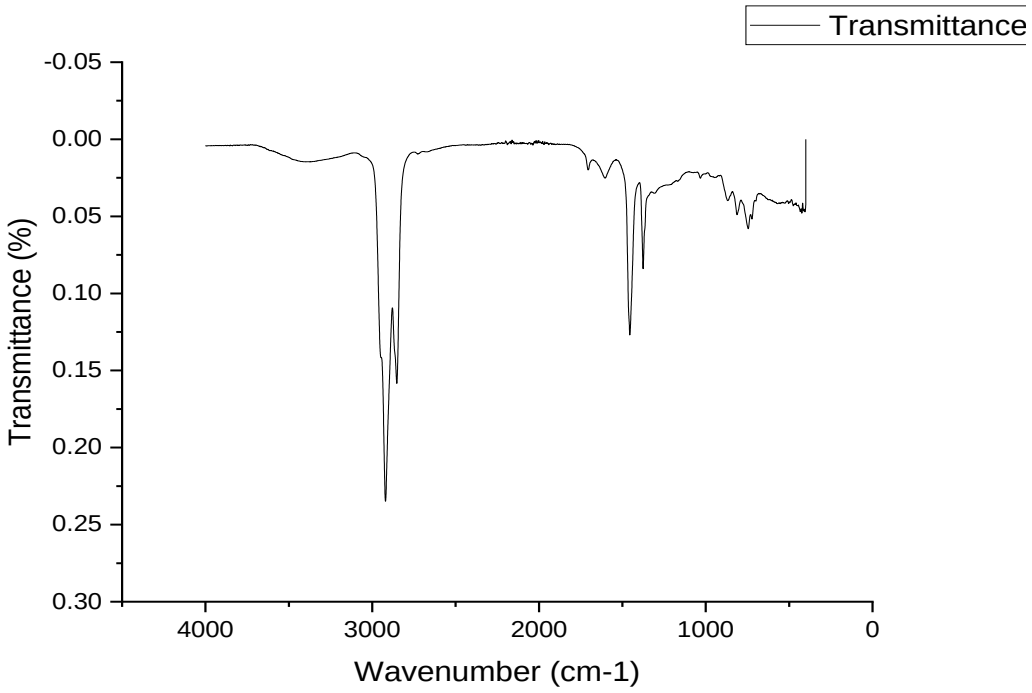

Fig. 4: **FT-IR of the bitumen sample**

Table 2: **FT-IR band assignment for bitumen sample**

Peak (cm^{-1})	**Functional group**	**Source compound**
2925	C-H Symetric stretch in CH_3	Alkane – Saturates
2860	C-H Stretch in CH_2	Alkane – Saturates
1730	C=O Stretch in carboxylic group	Carboxylic acid – Resinate
1650	C=C Stretch from Aromatic compound	Aromatics
1480	C-CH_3 Methylenic asymmetric	Alkane – Saturate
1395	C-[R] Asymmetric	Aromatic amines
1050	SO_2 Sulphoxides	Organic sulphates
890	C=H bond from alkene	Alkene – saturates
830	Two adjacent H on ring	Aromatics – para
770	Four adjacent hydrogens on ring	Aromatics – meta
750	Aromatic bending H-C modes	Aromatics – ortho

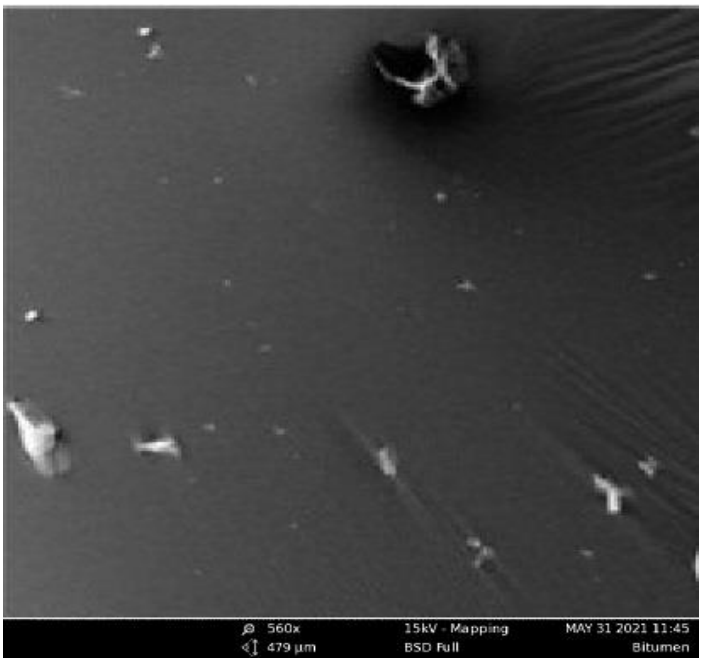

Fig. 5: **SEM of bitumen sample**

Table 3: **Elemental composition of the bitumen sample from EDX**

S/NO.	Element Symbol	Element Name	Weight Concentration
1	C	Carbon	82.34%
2	H	Hydrogen	8.31%
3	N	Nitrogen	3.43%
4	O	Oxygen	2.76%
5	S	Sulphur	2.16%
6	V	Vanadium	120ppm
7	Ni	Nickel	80ppm
8	Fe	Iron	50ppm
9	Al	Aluminium	5ppm
10	Ca	Calcium	30ppm
11	Cu	Copper	5ppm
12	Na	Sodium	40ppm
13	Mg	Magnesium	20ppm
14	Mn	Manganese	22ppm
15	Si	Silicon	15ppm

Table 4: **GC-MS of bitumen sample**

S/No	Retention Time	Area Pct (%)	Compound Identified	Chemical Formula	SARA Group/ Molecular weight
1	3.29	0.510	2,6-dimethyloctane	$C_{10}H_{22}$	Saturate 142.28
2	4.061	0.163	Dodecane	$C_{12}H_{26}$	Saturate 170.33
3	7.574	0.372	2,4-bis(1,1-dimethylethyl)phenol	$C_{14}H_{22}O$	Aromatic 206.32
4	10.329	0.468	2,6,10,14-tetramethylpentadecane	$C_{19}H_{30}$	Saturate 268.51
5	10.926	0.202	Benzo[a]pyrene	$C_{20}H_{12}$	Aromatic 252.31
6	15.274	0.458	2-propylcyclohexane	C_9H_{18}	Saturate 126.24
7	15.928	0.404	Tridecane	$C_{13}H_{28}$	Saturate 184.36
8	20.362	2.138	1-methyl-3-ethyladamantane	$C_{13}H_{22}$	Saturate 178.31
9	20.441	2.082	1,5,4,6-tetramethyladamantane	$C_{14}H_{24}$	Saturate 192.34
10	25.61	0.190	nonane, 2-methyl	$C_{10}H_{22}$	Saturate 142.28
11	25.76	0.001	Pyrene	$C_{16}H_{10}$	Aromatic 202.25
12	27.83	0.673	3-methyloctane	C_9H_{20}	Saturate 128.25
13	27.962	0.492	2-methyl-2-butene	C_5H_{10}	Saturate 70.13
14	28.979	0.170	O-cresol	C_7H_{12}	Saturate 96.17
15	29.035	0.168	Heptadecane	$C_{17}H_{36}$	Saturate 240.50
16	29.038	0.566	1,2-benzenedicarboxylic acid	$C_8H_6O_4$	Aromatic 166.14
17	29.1592	0.632	2,6,10,14-tetramethylhexadecane	$C_{20}H_{42}$	Saturate 282.54
18	30.25	0.512	Benzo[b]flouranthene	$C_{20}H_{12}$	Aromatic 252.31
19	30.43	0.802	Cyclohexadecane	$C_{16}H_{32}$	Saturate 224.42
20	30.6948	0.503	1,2-dimethyl-5-nitroadamantane	$C_{12}H_{19}NO_2$	Resinate 209.28

Table 5: **GC-MS for synthetic crude oil E-I from anhydrous pyrolysis experiment**

S/NO	Retention Time	Area Pct (%)	Compound Identified	Chemical Formula	SARA Group/ Molecular weight
1	3.79	0.510	1,2-diethylcyclooctane	$C_{12}H_{24}$	Saturate 168.32
2	4.125	0.163	2,2–dimethyloctane	$C_{10}H_{22}$	Saturate 142.28
3	4.386	0.372	1,2-benzenedicarboxylic acid	$C_8H_6O_4$	Aromatic 166.14
4	5.257	0.468	n-decane	$C_{10}H_{22}$	Saturate 142.28
5	5.869	0.202	1-ethylcyclopentene	C_7H_{12}	Saturate 96.17
6	10.234	0.458	1,3,5-trimethyladamantane	$C_{13}H_{22}$	Saturate 178.31
7	10.728	0.404	Benzo[a]anthracene	$C_{18}H_{12}$	Aromatic 228.29
8	12.365	2.138	2,4-dimethylhexane	C_8H_{18}	Saturate 114.23
9	15.248	2.082	2,4,4-trimethyl-2-pentene	C_8H_{16}	Saturate 112.21
10	15.61	0.190	Methylcyclohexane	C_7H_{14}	Saturate 98.19
11	17.176	0.001	1,3,5-trimethylbenzene	C_9H_{12}	Aromatic 120.19
12	20.832	0.673	2,3–dimethyl–1,3-butadiene	C_6H_{10}	Saturate 82.14
13	20.916	0.492	Naphthalene	$C_{10}H_8$	Aromatic 128.17
14	27.828	0.170	Hexadecanoic acid methyl esther	$C_{17}H_{34}O_2$	Resinate 270.45
15	25.535	0.168	1,3-dimethyladamantane	$C_{12}H_{20}$	Saturate 164.29
16	25.238	0.566	Tridecane	$C_{13}H_{28}$	Saturate 184.36
17	27.592	0.632	4-ethyl-1,2-dimethylbenzene	$C_{10}H_{14}$	Aromatic 134.22
18	30.625	0.512	Benzo[a]pyrene	$C_{20}H_{12}$	Aromatic 252.31
19	30.443	0.802	1,7-dimethyl-4-cyclodecane	$C_{15}H_{30}$	Saturate 210.40
20	30.698	0.503	3-methyl-1-adamantaneatic acid pyridine	$C_{13}H_{20}O_2$	Resinate 208.30

Table 6: **GC-MS result for synthetic crude oil E-II from hydrous pyrolysis experiment**

S/NO	Retention Time	Area Pct (%)	Compound Identified	Chemical Formular	SARA Group/ Molecular weight
1	3.29	0.510	Methylcyclohexane	$CH_3C_6H_{11}$	Saturate 98.19
2	4.061	0.163	m-Ethylmethylbenzene	$C_6H_3(CH_3)_3$	Aromatic 120.19
3	4.274	0.372	n-Decane	$C_{10}H_{22}$	Saturate 142.28
4	4.529	0.468	1,2,3 Trimethylbenzene	$C_6H_3(CH_3)_3$	Aromatic 120.19
5	4.859	0.202	Benzene	C_6H_6	Aromatic 78.11
6	4.974	0.458	1,4- Diethylbenzene	$C_6H_4(C_2H_5)_2$	Aromatic 134.22
7	5.328	0.404	2,4-dimethylhexane	$C_5H_9(CH_3)_3$	Saturate 114.23
8	5.362	2.138	p-Xylene	$C_6H_4(CH_3)_2$	Aromatic 106.16
9	5.441	2.082	o-Xylene	$C_6H_4(CH_3)_2$	Aromatic 106.16
10	5.61	0.190	2,4,4-Trimethyl-2-pentene	$C_4H_4(CH_3)_4$	Saturate 112.21
11	5.76	0.001	(S)-3,4-Dimethylpentanol	$C_7H_{16}O$	Resinate 116.2
12	5.83	0.673	Ethylcyclopentane	$C_5H_9C_2H_5$	Saturate 98.19
13	5.962	0.492	Benzene, 1,2,3,4-tetramethyl, o-Cymene	$C_{10}H_{14}$	Aromatic 134.22
14	5.979	0.170	Benzonitrile	$C_6H_5(CN)$	Resinate 103.12
15	6.035	0.168	D-Limonene	$C_{10}H_{16}$	Resinate 136.23
16	6.038	0.566	2-Propanone, methyl-2-propenylhydrazone	$C_7H_{14}N_2$	Resinate 126.20
17	6.1592	0.632	2-methyl-nonane	$C_{10}H_{22}$	Saturate 142.28
18	6.4	0.512	Sodium cyclopentadienide	C_5H_5Na	Saturate 88.08
19	6.43	0.802	1-Ethylcyclopentene	C_7H_{12}	Saturate 96.17
20	6.6948	0.503	1-methylethyl- cycloundecane	$C_{14}H_{28}$	Saturate 196.37
21	6.7574	0.361	2-Heptenal-(Z)	$C_7H_{12}O$	Resinate 112.17
22	7.55	1.080	2-Methyl-1-heptene	C_8H_{16}	Saturate 112.21
23	7.62	1.244	Isoprene	C_5H_8	Saturate 68.12
24	8.18	1.513	2-Methyl-2-butene	C_5H_{10}	Saturate 70.13
25	10.41	1.212	2,3,4,5,6-Pentamethylpyridine	$C_{10}H_{15}N$	Aromatic 149.23
26	10.45	0.985	1H-Indene, 2,3dihydro-1,1,5trimethyl-	$C_{12}H_{16}$	Aromatic 160.26
27	12.207	0.411	Naphthalene, 2,7dimethyl	$C_{12}H_{12}$	Aromatic 156.22
28	12.53	0.988	3-Methyl-octane	C_9H_{20}	Saturate 128.25
29	12.67	0.304	2,2-Dimethyloctane	$C_{10}H_{22}$	Saturate 142.28
30	12.9	1.015	2,3-Dimethyl-1,3-butadiene	C_6H_{10}	Saturate 82.14

Table 7: **Results for the synthetic crude oil obtained by anhydrous pyrolysis (E-I) and hydrous pyrolysis (E-II)**

Properties	Unit	Synthetic Crude E-I	Synthetic Crude E-II
Density	25 °C	0.89	0.87
Specific gravity	25 °C	0.91	0.88
API gravity	°API	23.99	29.2
Viscosity @ 40°C	St	328	304
Sulphur Content	%	0.16	0.08
Pour Point	°C	21	18
Flash Point	25 °C	98	78

DISCUSSION

The results obtained for determination of viscosity, density, specific gravity, API gravity and flash point for the bitumen as reported in **Table 1**, showed that the viscosity of 2800St was high, which signifies the bitumen is of category of heavy oils. The API gravity of 8.6 confirms that the bitumen is of category of heavy oils and these values are in close range with viscosity 3200St, density 1.03g/cm^3, specific gravity 1.02, and API gravity 7.2 reported in previous studies (Shedrach *et al.*, 2018) and (Arogundade & Ogunsuyi, 2021). The TGA for the bitumen and bitumen plus water, **Figure 2** and **Figure 3**, showed that there was significant material decomposition from around 280 °C up to 400 °C and 370 °C up to 500 °C respectively. The most dominant functional group as shown in **Figure 4** and reported in **Table 2** are the -CH_3, -CH_2 methyl group of alkanes. The SEM for the bitumen, **Figure 5**, showed a uniform morphology with the metallic impurities coming up as bright or shiny spots. The elemental composition of the bitumen shows Carbon with the highest percentage of 82.34 % and trace metals constitute only 1%, with Vanadium being the most notable present and these values correspond with the range for Carbon 80 %-85 % reported in earlier studies (Ogunsuyi *et al.*, 2011). From **Table 4**, GC-MS for bitumen, it is deduced that the SARA composition for the bitumen was 35 % Saturates, 37 % Aromatics, 26 % Resin and the n-heptane non-dissolvable Asphaltenes was 2 %, these were in agreement with reported findings by Shedrach *et al.*, 2018.

The GC-MS result, **Table 6**, for synthetic crude oil E-II from Hydrous Pyrolysis showed a higher percentage of saturates 65 % and aromatics 25 % and a lower percentage of resins 9.5 % than the GC-MS result, **Table 5**, for the synthetic crude oil E-I from Anhydrous Pyrolysis with saturates 50 %, aromatics 30 % and resins 19.5 %. The determined non-dissolvable asphaltenes for both crude oils were 0.5 %. Furthermore, as reported in **Table 7**, the synthetic crude oil E-II has a higher API gravity of 29.2, a lower density 0.87 g/cm^3, lower viscosity 304St and lower sulfur content of 0.08 wt% than the synthetic

crude oil E-I. Hence, the yield from hydrous pyrolysis was more physically, chemically and isotopically similar to crude oils obtained from natural maturation conditions and hydrous pyrolysis have also shown to increase gas generation and reduce maturation time. It is recommended that further research should be carried out on bitumen from other parts of Nigeria and these should be exploited for petroleum production as exemplified in other parts of the world such as Athabasca, Canada.

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Determination of Trace Elements in Africa Locust Bean (*Parkia biglobosa*)

***[1]Idris M. A., [1]Shuaibu M. D. & [2]Ismail A. M.**

[1]Pre-ND Department, College of Agriculture Mokwa, Niger State, Nigeria.
[2]Ministry of Education, Niger State, Nigeria.

*Corresponding Author's Email: idris2the@gmail.com, Phone No.: 08036874408

ABSTRACT

Most fruits are nutritional to human and animals. This research work determines some trace metals in Africa locust beans (*Parkia biglobosa*) and to investigate it value of heavy metal contamination. The fruit was collected from Mokwa local market and was separated into outer yellow pulp and inner seed and were analyzed for trace metal concentration of Cu, Zn, Pb, Fe, and Mn using atomic absorption spectrometry (AAS), unicamp – 969 model connected to computer. The result obtained shows that locust bean pulp (seed) have the highest concentration of all the trace metals determined with Fe having the highest value 966.8 ppm, followed by Zn with 86.21 ppm, then Cu, 67.65 ppm and lastly Mn with 55.20 ppm. Although Cu has the least concentration of 32.7 in the outer yellow pulp, Mn have the highest 78.5 ppm followed by Fe with 76.8 ppm then Zn with 43.5 ppm. The variation in the concentration of these trace metals in the samples may be due to the level of the metals in the soil and / or the ability of the plants to absorb or retain the metals.

Keywords: Locust Bean Seed, Trace Elements, Analysis, Consumption and Spectrometry.

INTRODUCTION

Heavy metals are trace metals which are detrimental to human health and having a density of at least five times that of water. Once liberated into the environment through the air, drinking water, food, or countless varieties of man-made chemicals and products, heavy metals are taken into the body via inhalation, ingestion and skin absorption (Abitogum and Olasehinde, 2012). If heavy metals enter and accumulate in body tissues faster than the body's detoxification pathways can dispose of, then a gradual build-up of these toxins occurs. High concentration exposure is not a necessity to produce a state of toxicity in the body, as heavy metal accumulation occurs in body tissues gradually and, over time, can reach toxic concentration levels, much beyond the permissible limits. (Suruchi and Khanna, 2011).

The African locust bean pulp is sweet to taste when ripe, this indicates the presence of natural sugars and thus a potential energy source. It has a yellow colour indicates the presence of phyto-nutrients, possibly carotenoids, which are impotant precursor of retinol (vitamin A). A sour taste when unripe, which indicates the presence of ascorbic acid (vitamin C) (Gernah *et al*. 2007). The parkia tree plays a vital ecological role in recycling of nutrients from deep soils, by holding the soil particles to prevent soil erosion with the aid of roots. The fruit may also be used as an

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ingredient for the preparation of various stew, soup and sausages for the consumption of cereals pressed in to cakes and preserved for later use or used in the preparation of some indigenous drinks (Muller). The fruit pulp is consumed by rural Africa which is an indication of it is non-toxic (Akoma *et al*., 2001). Africa locust bean *(Parkia biglobosa)* seed has been found to be very useful especially in the fermented form and it is widely eaten among all the tribes across the country. However, the yellow dry powdery fruit pulp has not attracted much attention. Literature has revealed that the fruit pulp is used in rural communities in Africa during emergencies, when the grain stores are empty (Edem and Miranda, 2011). The pulp is also used as an ingredient in the preparation of various stews, and soups (Odebunmi *et al.*, 2010).

Minerals are essential inorganic elements required for a variety of functions in the body which include giving structure to the skeleton, muscle contractions, blood formation, synthesis of protein and energy. They are found in red blood cells, cell membranes and are components of hormones and enzymes (Tova, 2004). Micronutrients are necessary for normal growth and development of plants as well as people, they are the ingredients or activators of various enzymes and play a catalytic role in many physiological processes (Salami and Non, 2002). The elements such as iron, manganese, zinc or copper take part in photosynthesis, decarboxylation, and nitrogen fixation processes as well as protein and carbohydrates metabolism. Antioxidants found in onion protect the human organism against free radicals which are responsible for ageing and cardiovascular diseases (Rabinowitch and Currah, 2002).

Locust bean *daddawa* is also a good source of iron and sodium. Iron functions as a component of haemoglobin in the transport of oxygen in the system. As an essential trace element commonly found in the soil as a mineral. In most cases, iron in the body exists in complex forms bound to proteins either as porphyrin, haem-compounds, or as ferritin and transferring although free inorganic iron may occur in small quantities (Swaminathan, 2006). It plays an important role in the formation of haemoglobin, cytochrome, enzymes and also for the transportation of oxygen to the tissues for oxidation of carbohydrates, proteins and fats in the body (Adeyeye and Otokiti,1999). Apart from the cultural, food and nutritional values of *parkia biglobosa,* it also serve as a great source of income to the locals that process it in to finished products for consumption (Gloria *et al* 2011).

METHODOLOGY

Sample Preparation

Sample preperation of freshly harvested pakia biglobosa was purchased at Mokwa Township Main Market of Niger State. The pulp was properly removed from the seed using a wooden mortar and pistle and was dried at room temperature in the labouratory for two weeks.The dried pulps were grounded and sieved using two millimeter size mesh. The powdered samples of the yellow pulp (fruit) and seed pulp (beans) ware stored in plastic containner for laboratory analysis.

Laboratory Analysis

Two grams each of samples collected were digested using 25cm^3 mixture of 4:1 HNO_3 and $HClO_4$ acid. The mixtures were allow to stand over night to allow the initial reaction to subside. It was digested at a temperature of 100^OC for 2 hours, the digests were then filtered into 100cm^3 volumentric flask and dilluted to the lower meniscus with distiled water (Salami and Non, 2002). The trace metals; Fe, Zn, Cu, Mn, and Pb

were determined spectrophometrically using Atomic Absorption Spectroscopy (AAS) unicamp – 969 model.

RESULT AND DISCUSSION

Table 1: showed variation in the level of trace metals analysed in the sample's Yellow Pulp (fruit) and Seed Pulp (Beans) of freshly harvested Africa locust beans (pakia biglobosa) from Mokwa Township Main Market of Niger State. The variation may be due to difference in the ability of the plant parts to precipitate and absorb the micronutrient from the soil or probably it may be as a result of the level of contamination of the soil by the metals. This research revealed that the elemental concentration distribution sequence in the yellow pulp (fruit) of pakia biglobosa are in the order Mn > Fe > Zn > Cu , whereas in the seed pulp (beans) Fe > Zn > Cu > Mn. Minerals and trace metals are required in humans in trace amounts to maintain good health and excess of it might be toxic (Aletor and Omodara, 1994).

The sequence of the values obtained, showed that iron (Fe) have the highest concentration of 966.8 ppm in the seed pulp (beans) and remain the second most concentrated trace metal in the yellow pulp (fruit) with a mean concentration of 76.50 ppm. Manganese (Mn) have the highest mean concentration of 78.50 ppm in the yellow pulp (fruit), whereas it mean concentration of 55.20 ppm is the lowest concentration in the seed pulp (beans). From the values of the concentrations obtained in the yellow pulp, zinc (Zn) and copper (Cu) have almost half their mean concentration in the seed pulp (beans). Lead (Pb) was below detection limit in the yellow pulp and seed pulp (beans) of the samples.

Table 1: Mean concentration in (ppm) of the trace metals: Cu, Zn,Pb, Fe and Mn determined in the samples.

Trace Elements	Yellow Pulp	Seed Pulp (Beans)
	Mean ± SD	Mean ± SD
Cu	32.70 ± 0.03	67.65 ± 0.11
Zn	43.51 ± 0.10	86.21 ± 0.06
Pb	ND	ND
Fe	76.80 ± 0.12	966.8 ± 0.23
Mn	78.50 ± 0.06	55.20 ± 0.03

ND means not detected

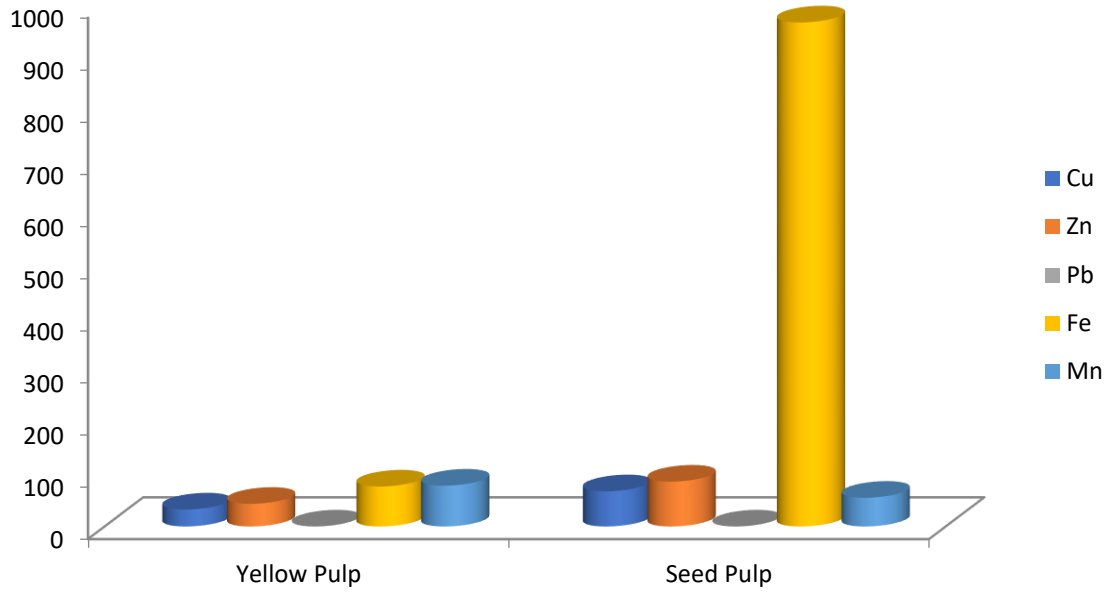

Figure 1: Bar Chart Representation of Values Presented in Table 1

CONCLUSION

The results of this study revealed the presence of trace metals in both yellow pulp (fruit) and seed pulp (beans) of the Africa locust beans (pakia biglobosa) sampled. As a good source of iron, zinc, copper and manganese among others, it could serve as alternative source to replace animal protein as revealed in other research works. It is therefore concluded from this study that significant amount of the trace metals analysed are present with exception of lead which was below detection limit.

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Development of A Portable Solar Powered Arduino Microcontroller-Based Weather Station

*[1,2]Ughanze I. J., [1]Ibrahim A. G., [1]Eichie J. O. & [3]Ugwu C. A

[1]Department of Physics, Federal University of Technology Minna, Niger State, Nigeria
[2]R and D Department, Scientific Equipment Development Institute Minna, Niger State, Nigeria
[3]Electrical Maintenance Unit, Scientific Equipment Development Institute Enugu, Enugu State, Nigeria

*Corresponding author's Email: ughanzeifeanyi@yahoo.com, Phone no.: +2347065936880

ABSTRACT

Real-time weather parameter acquisition is a challenge in our society today. This project created a solar-powered Arduino microcontroller weather station that measures atmospheric temperature, relative humidity, pressure, wind speed, and wind direction using appropriate electronic sensors such as DHT22 sensors for temperature and humidity, BMP180 sensors for pressure, three-cup anemometer with reed switch sensor for wind speed and a wind vane with a continuous variable resistor for wind direction. The output of the sensors is processed by an Arduino at mega 2560 microcontroller, which then sends the data to the LCD for display and to the Wi-Fi module, which uploads the data to an online server so that out-of-site end users can receive the measured weather data.

Keywords: Weather station, Microcontroller, Electronic Sensors, Wi-Fi module, solar panel.

INTRODUCTION

Weather has always had a big impact on people's lives, shaping their cultures, habits, attitudes, and behaviour, as well as their environs in general. Man has always attempted to determine the causes of various weather situations that he encounters, as well as to monitor and forecast what the weather will be at any particular time. Taking weather and attempting to forecast it correctly can have a significant impact on the human race's existence and prosperity (Ukhurebor *et al*., 2017).

Weather refers to the temperature of the atmosphere, including how hot or cold it is, how wet or dry it is, how quiet or stormy it is, and how clear or foggy it is. These recurrent fluctuations in atmospheric condition are due to weather elements. Here are some examples of weather parameters: Atmospheric temperature, atmospheric pressure, relative humidity, solar radiation, cloudiness, rain fall, wind speed and wind direction. (Karthik *et al*., 2015).

Climate change is now widely acknowledged as a danger to food security. Losses in productive regions as a result of climate change have the potential to be catastrophic. Climate elements that are not under human control should be addressed quickly and effectively in agricultural areas. Precision agriculture and smart agriculture are becoming increasingly popular for this purpose. Farmers plan their production under unpredictable conditions since numerous

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aspects, such as weather, yield levels, crop prices, agricultural inputs, food demand, and production costs, are difficult to anticipate. Estimates can be made based on measurements of weather parameter conditions derived from these factors (Muhittin, 2020). The relevance of weather tracking in communications, air transportation, industries, meteorology and atmospheric physics research cannot be overstated.As a result, there is a constant need for an effective technique of monitoring atmospheric parameters (Anthony *et al.,* 2017).

A microcontroller weather station uses sensors to read and record atmospheric information without the need for external assistance. The collected atmospheric parameters can be processed as wired data, which can then be downloaded into a computer or transmitted wirelessly through a server. (Ughanze *et al.,* 2019).

A sensor is a device that measures a physical quantity and converts it into an analog or digital signal that an observer or instrument can read (Anuj *et al.*, 2010)

The Internet of Things (IoT) is a term that refers to the network of devices that connect It allows physical devices, buildings, vehicles, and other components like as sensors and actuators to communicate with one another. All items, such as electrical circuits, sensors, actuators, and electronics equipment, can connect to the internet and exchange data using the Internet of Things. It allows the physical environment to be connected to computer-based systems. The Internet of Things (IoT) framework enables objects to communicate with one another (Maulik and Irshad, 2018).

REVIEW OF MAJOR COMPONENTS UTILSED IN THE DEVELOPMENT OF THE WEATHER STATION

1. A solar cell is an important component of a solar panel. A photovoltaic module is made up of numerous solar cells connected in series and parallel. To improve continuous atmospheric data collecting, a monocrystalline solar panel delivers DC voltage to the system (Ami *et al*., 2015).

2. Battery: A battery is made up of two or more chemical cells connected in series. A battery is made up of various elements that are used to convert chemical energy into electrical energy (Jacob *et al.,* 2018). This device's design makes use of a deep cycle battery. This is because if it discharges to its lowest energy level, it can be recharged more quickly and in a shorter amount of time. As a result, even when there is no sun light, the battery will have sufficient storage capacity to power the entire load of the device (Saleh *et al*., 2015)

3. Charge controller: A charge controller functions as a switch between the solar panel and the battery. While the battery is fully charged, the charge controller trips the terminals of the battery to prevent overcharging and the battery from draining when not in use. This improves the battery's performance, extends its life, and decreases the chance of a fire (Saleh *et al.,* 2015).

4. Microcontroller: The Mega 2560 is an ATmega2560-based microcontroller board. It contains 54 digital input/output pins, 16 analog inputs, 4 UARTs (hardware serial ports), a 16 MHz crystal oscillator, a USB connection, a power jack, an ICSP header, and a reset button. It comes with everything you'll need to get started with the microcontroller; simply plug it

into a computer with a USB cable or power it with an AC-to-DC adapter or battery. Most shields created for the Uno and previous boards Diecimila are compatible with the Mega 2560 board. Arduino Mega, for example. In this project, I utilized an Arduino Uno. Arduino is based on the ATmega328 microcontroller. A 16 MHz ceramic resonator, a USB connector, a power jack, an ICSP header, and a reset button are all included in the package. Our Arduino uses an Atmega16U2 chip designed as a USB-to-serial converter instead of the FTDI USB-to-serial driver chip(Aarti *et al.,*2017).

5. DHT22 sensor is a low-cost digital temperature and humidity sensor that assesses the surrounding air using a capacitive humidity sensor and a thermistor before sending a digital signal to the data pin. DHT22 sensor has the following features as shown in Table 1.

Table 1: Features of DHT22

Specification	Values
Model	DHT22
Power supply	3.36V DC
Output signal	Digital signal via single-bus
Sensing element	Polymer capacitor
Operating range	Humidity 0-1000%RH;temperature -40-80 Celsius
Accuracy	Humidity+2%RH(Max+5%RH);temperature <+-0.5 Celsius
Resolution or sensitivity	Humidity 0.1RHtemperature 0.1 Celsius
Repeatability	Humidity +-1%RHtemperature 0+.2Celsius
Humidity hysteresis	+-0.3%RH
Long-term stability	+-5%RH/year
Sensing period	Average; 2s

The microcontroller and the DHT22 communicate over a single bus. When the microcontroller transmits the start signal, DHT22 switches from low power consumption to operating mode. Once the microcontroller sends the start signal, DHT22 sends a response signal including relative humidity and temperature data. (Ughanze *et al*., 2019)

BMP180 sensor

It's a barometric pressure sensor with an I2C interface that can also be used to measure altitude because pressure fluctuates with altitude. SDA, SCL, GND, and Vin are the four pins. (2016, Amber.)

The BMP180 sensor measures atmospheric pressure and outputs an analog signal to the

microcontroller. The microprocessor translates the BMP180's analogue signal to a digital signal, which is then shown on the LCD. The I2C communication protocol is used by the BMP180. The specifications of the BMP180 atmospheric pressure sensor are enumerated below:

- Operating voltage of BMP180: 1:3V – 3.6
- Input voltage of BMP180MODULE: 3:3V to 5:5V
- Peak current : 100uA
- Consumes 0.1uA standby
- Maximum voltage at SDA, SCL : VCC + 0.3V
- Operating temperature: - 400C to 800C

6. A wind vane is a mechanically assembled device which measures wind direction with the aid of a resistive sensor embedded in it. Eight switched exist in the wind vane and each is connected to different resistor sensors. Weather rack calculates the resistance value that each resistor produces when calculating the voltage on the resistors. Wind vane often times reads and records values for 8 directions but many read up to 16 directions if when two contacts are closed at the same time. (Beth and Kong, 2017).
7. An Anemometer a mechanically assembled device which has three cup to calculate wind speed by calculating the rotation of the anemometer cups at a set interval. If usually have a speed sensor switch which is interfaced with a magnet, so that for each rotation the sensor switch will close and open. The magnet is usually placed on the shaft of the rotating cup symmetry and this enables the rotation of the three cups if there is effect of wind on it (Dipak and Ajij, 2016)

METHODOLOGY

System Design, Materials and Methods

This study made use of a number of components, which when put together form an embedded system. These components were carefully chosen. Figure 1 depicts the overall device as a block diagram.

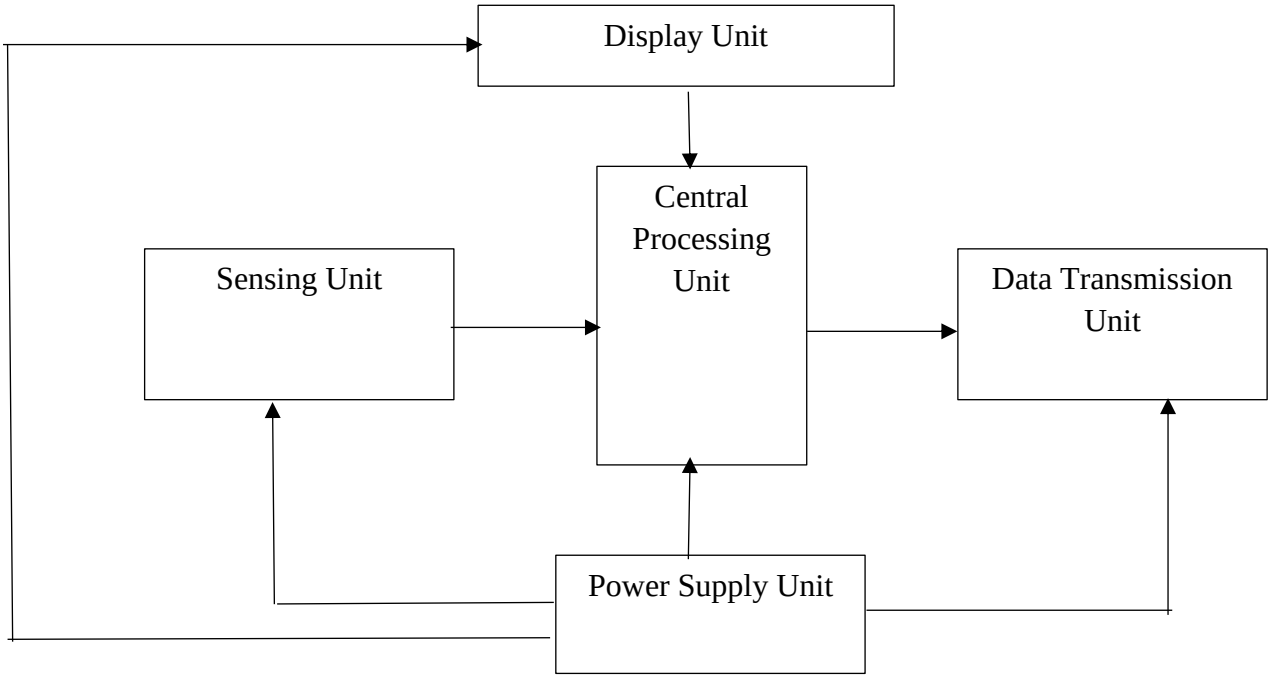

Figure 1: Block diagram of the weather station.

The weather station is made up of five major units, as shown in figure 1, which are: (1) Power Supply Unit (2) Sensing Unit (3) Central Processing Unit (4) Display Unit (5) A Data Transmission Unit.

1. Power supply section

The power supply portion provides the device with electrical energy. Renewable energy is suitable for a device that can be monitored 24 hours a day, seven days a week. As a result, solar energy will be used to power the device, which will be created by the tremendous energy of the tropical African sun. A monocrystalline solar panel provides DC voltage to the system to improve continuous atmospheric data collection.

2. The sensing unit is in charge of keeping track of atmospheric parameters. The following sensors were used in this study. DHT 22 sensors, BMP 180 sensors, a three-cup anemometer and a wind vane built locally

The DHT22 Sensors read and record atmospheric temperature and relative humidity as digital signals, which they transfer to the microcontroller as an output signal via proper pin connections between the microcontroller and the DHT 22 sensors. While the BMP 180 sensor measures air pressure as an analogue signal, this signal is delivered to the microcontroller as an input signal via the microcontroller's analogue input pin. The three-cup anemometer uses a reed switch sensor to measure wind speed and provide it to the microcontroller through analogue input pin interface. A wind vane detects the wind direction with the use of a continuous variable resistor, and the wind direction monitored is recovered as an input signal by the microcontroller through its analogue pin interfaced with the wind.

The central processing unit is the heat of the weather station, Atmega 2560 microcontroller was utilized, it process all the analogue and digital signals which is retrieved from the atmospheric sensors into useful Format for display on the LCD and also transmits to communication link which uploads the weather information to an online sever

4. Display Unit

The microcontroller is connected to a 20 x 4 LCD that displays the microcontroller's processed atmospheric parameters. The displayed atmospheric data is mostly for on-site weather station users, and it is achieved by connecting an appropriate pin between the LCD and the microcontroller's input pin.

5. Data Transmission Unit.

The ESP8266 Wi-Fi module acts as a communication link for the microcontroller to submit processed weather parameters to an online server. Real-time atmospheric temperature, relative humidity, atmospheric pressure, wind speed, and wind direction are shown in graphical and digital format on the internet server enabling out-of-sit users to assess from anywhere on the planet.

RESULTS

To verify the correct functionality of the system after the design of the whole circuit, proteus software was utilised for the virtual implementation of the device. Figure 2 shows a screen shot of the simulation.

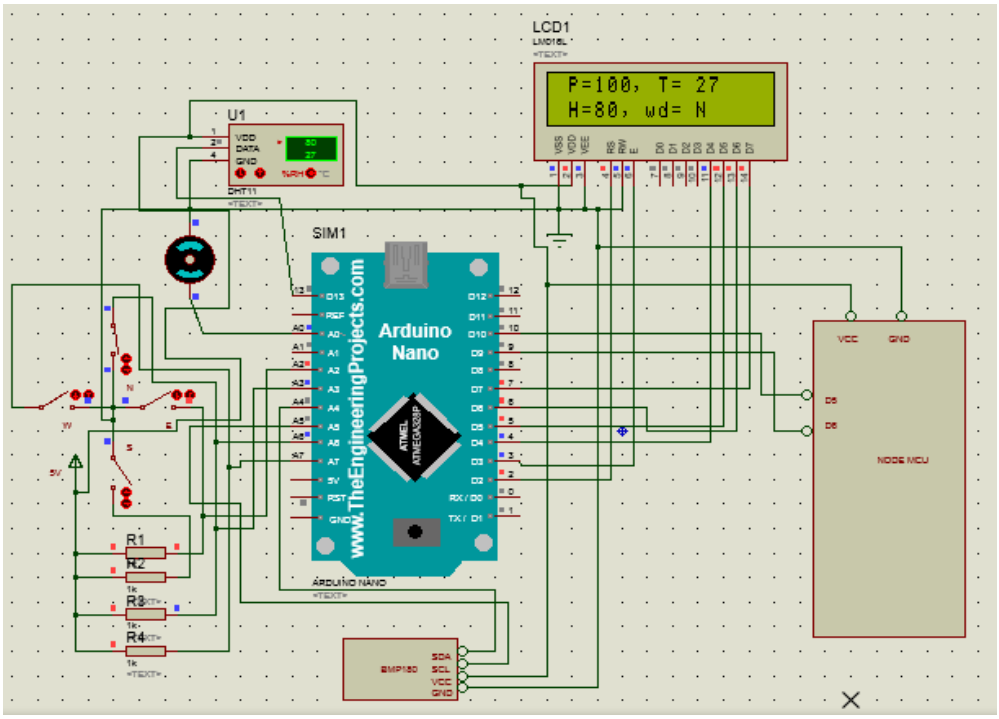

Figure 2: A screen shot of the simulation

Figure 2 indicates that simulation worked fine and displayed measured weather parameters such as atmospheric temperature, relative humidity, atmospheric pressure and wind direction on the LCD.

Figure 3 shows how electronic hardware components were carefully mounted and soldered on the board

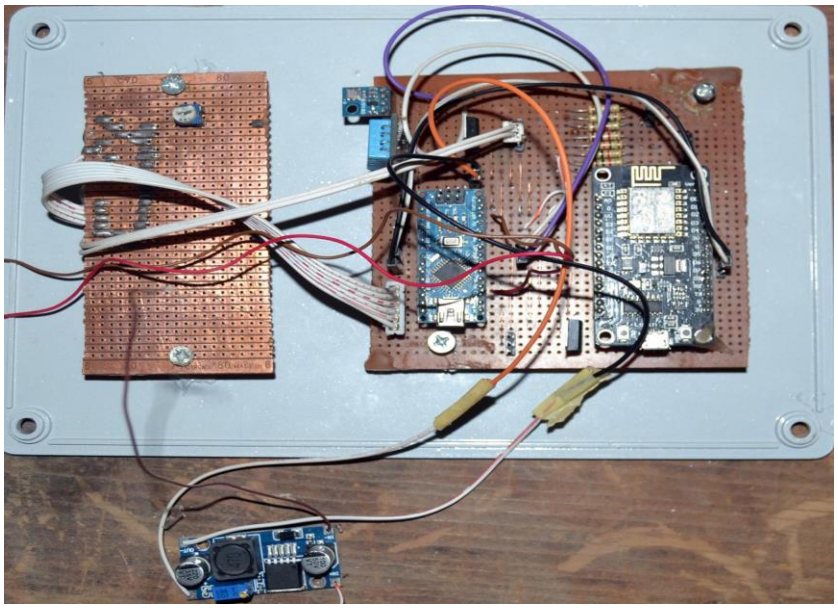

Figure 3: Electronic hardware components soldered on the board

Figure 4 shows the constructed weather station

Figure 4: Constructed weather station

CONCLUSION

The designed weather station is a low-cost, solar-powered weather monitoring device with flexibility, portability, scalability, and user-friendly operations. It can offer real-time data on atmospheric temperature, relative humidity, air pressure, wind speed, and wind direction, among other meteorological variables.

With the help of an online data logger, we can keep track of all the atmospheric parameters measured. The device also incorporates an LCD for monitoring atmospheric data in real time on-site.

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Antimicrobial and Phytochemical Activities of Henna Plant

***Olusola L. F., Agholor K., Mustapha A. & Ahmed A.**

Department of Biological Sciences, Niger State Polytechnic, Zungeru, Niger State, Nigeria

Email: lucyemma366@gmail.com, sola2015.oe@gmail.com Phone no. +2348131593008

ABSTRACT

Anti-microbial and phytochemical activities of *Lawsonia Interims* (henna leaves) was successfully carried out in this study. The sample used for the study was obtained from Bussun, Gussoro in Shiroro Local Government area, Kuta town of Niger State. The leaves were dried at room temperature and blended to powder. *The* methanolic extracts of the leaves powder were obtained and reconstituted using Dimethyl Sulfoxide (DMSO) to obtain different concentration. The antimicrobial activity of the different concentration of the extract was determined using agar well diffusion techniques. The phytochemical test conducted on the henna leaves extracts (*Lawsonia inermis*) indicated that chemical properties such as alkaloids, cardiac glycosides, flavonoid, tannins terpenoids were present. The result of the study also demonstrated that Henna is very effective against bacteria such as *Staphylococcus aureus, Pseudomonas aeruginosa and Streptococcus pyogenes Salmonella typhi* and *Escherichia coli*. About 1.0mg/mL concentration of henna leaves extract was highly effective against *Staphylococcus aureus* closely followed by *Pseudomonas aeruginosa* and *Streptococcus pyogenes* but less effective against *Salmonella typhi* and *Escherichia coli* with zone of inhibitions of 11mm, 14mm, 17mm, 19mm and 21mm at 0.2mg/mL, 0.4mg/mL, 0.6mg/mL, 0.8mg/mL, and 1.0mg/mL concentration of henna leaves respectively. The study concluded that henna plant has effective antimicrobial activities. The study recommended that people should be encouraged to consume henna leaves extract as it has ability to eliminate bacteria such as *Staphylococcus aureus*. Cosmetic industries should make use of henna in producing body creams and lotion. This would go a long way in reducing skin infection from bacteria.

Keywords: Antimicrobial; Phytochemical; Activities; Henna leaves; Extracts

INTRODUCTION

World is endowed with a rich wealth of medicinal plants. Man cannot survive on this earth for long life without the plant kingdom because the plant products and their active constituents played an important role. There is a widespread belief that green medicines are healthier and more harmless or safer than synthetic ones. Medicinal plants have been used to cure a number of diseases. Though the recovery is slow, the therapeutic use of medicinal plant is becoming popular because of its inability to cause side effects and antibiotic resistant microorganisms. Seeking healing by using plants is an ancient practice (Chaudhary *el at.*, 2016; Jallad and Jallad., 2016).

The role medicinal plants play in biological activities, such as antibacterial, antifungal,

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insecticides and herbicides cannot be overemphasize. Henna is one of the medicinal plant widely used across the globe, particularly Africa and Asia. It is much ranched shrub or small tree, cultivated for its valuable leaves, although its stem, bark and root also have been used in traditional medicine for 9,000 years (Al-riwawi, 2018). Henna *is* a perennial plant commonly called Lawsonia *inermis*, it is a native to North Africa and South East Asia, and often cultivated as an ornamental plant throughout India, Persia, and along the African coast of the Mediterranean Sea.

Henna grows better in tropical savannah and tropical arid zones, in latitude between 15° and 25° North and South, produces highest dye content in between temperature 35 to 45°C. The optimal soil temperature ranges for germination are 25 to 30°C (Chaudhary *et al*., 2012; Chaudhary *et al*., 2016). Henna leaves are very popular natural dye to color hand, finger, nails and hair. The dye molecule, Lawson is the chief constituents of the plant; its highest concentration is detected in the petioles (0.5-1.5%) (Abdulmoneim, 2016; Chopra, 2015).

There are two types of natural henna, red henna and black henna. Red henna is a commercial name of *Lawsonia inermis* and the leaves powder gives red color for skin, hair and nails, while the natural black henna is a commercial name of *Indigofera tinctoria* that gives black color for hair, skin and nail. *Lawsonia inermis* is a flowering plant which belongs to *lythraceae* family (Babu, and Subhasree, 2009; Singh and Pandey, 2017).

Many studies on Henna (*Lawsonia inermis*) leaves extractions showed that it had antibacterial activity against Gram positive bacteria (*Bacillus spp.*, *Staphylococcus aureus*, *Staphylococcus epidermidis* MRSA, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Bacteriodes fragilis*, *Clostridium perfringens*, *Streptococcus mutans*, *Micrococcus*, *Streptococcus salivarius*, *Bacillus subtilis*, *Staphylococcus epidermidis* and *Streptococcus gordonii*); and against Gram negative bacteria (*Escherichia coli*, *Salmonella spp*, *Klebsiella spp*., *Shigella sonnei*, *Pseudomonas, aeruginosa*, *Citrobacter frewndii*, *Vibrio cholerae*, *Neisseria meningitides*, *Haemophilus influenzae*, *Aeromonas hydrophila*, *Micrococcus spp* and *Corynebacterium diphtheriae*) among the studies include the study of (Al-riwawi, 2018; Al-Arnaoutt and Al- Amoutt, 2015; Yegit, 2017).

In folk medicines, henna has been used as astringent, antihemorrhagic, intestinal antineoplastic, cardio-inhibitory, hypotensive, sedative and also as therapeutic against amoebiasis, headache, jaundice and leprosy **(**Saadabi, 2016; Tommasi, 2016). It is obvious that medicinal plants, Henna (*Lawsonia inermis*) inclusive, has a great potential to serve as antimicrobial agent. Despite the fact that Africa, particularly Nigeria is blessed with Henna (*Lawsonia inermis*), the plants has been underutilized, thus neglected for foreign alternatives.

Nowadays, people barely applied Henna (*Lawsonia inermis*) on their skins again as a result of civilization and other factors like lack of awareness of the skin benefit it has (Khan and Nasreen, 2016; Muhammad and Muhammad, 2017). Aside this, medicine extract from natural plants like Henna (*Lawsonia inermis*) leaves are not well considered by the public for the treatment of diseases due to the fact that people preferred going to Hospital for chemically made drugs or medicine which thus has effect on human body in the long run (Nayak *et al.,* (2007)

However, various researchers (Yigit, 2018; Subharea, 2019; Ramoun *et al*., 2016; Al-Rimawi 2018) had attempted in various ways to study the antimicrobial activities of Henna (*Lawsonia inermis*) in Nigeria and outside Nigeria. Additionally, Hennas are used in the traditional systems of medicine to cure skin infections and burn wound, it possesses anti-inflammatory activity due to the presence of ethanol, high amount of flavanol, phenolic acid and Quinones. Therefore, this study seeks to evaluate the antimicrobial activities of Henna (*Lawsonia inermis)* against pathogenic bacteria.

METHODOLOGY

Sample Collection

The sample (specimen) was collected during the season of their abundance. Sample of *Lawsonia Interims* leaves were collected from Bussun, Gussoro in Shiroro Local Government area, Kuta town of Niger State. The sample collected were washed with clean water (distilled water) and dried at room temperature for 3 weeks (21 days). Motar was used to ground the sample (Henna) into powder. Sample bottles were used to store the powder for further action.

Phytochemical Screening

Chemical tests were carried out on the ethyl alcohol crude extract of lawsonia plant using standard procedure to identify the constituents.

Alkaloids: To identify the presence of alkaloids, 2ml of extract and 2ml of wagneras reagent was added. Brownish precipitate indicated the presence of alkaloids.

Cardiac glycosides: To know the presence of cardiac glycosides, 2ml of extract was dissolved with 2ml of chloroform followed by adding concentrated Sulphuric acid carefully and layer was formed. Deep reddish brown colour at the interface of steroid ring indicated the presence of cardiac glycosides.

Flavonoids: To determine the presence of flavonoids, 2ml extract was treated with 2ml of 10% lead acetate. Yellowish green colour indicated the presence of flavonoids.

Tanins: To identify the presence of tanins, 2ml of extract with 0.1% ferric chloride. Brownish green indicated the presence of tanins.

Terpenoids: To know the presence of terpenoids, 2ml of extract was dissolved with 2ml of chloroform and concentrates sulphuric acid carefully and layer was form. A reddish brown colour indicated the presence of terpenoids (Adamu *et al*., 2007)

Preparation of Sample

The extraction of Henna leaves was carried out using 200g powdered leaves in 150ml of solvent (methanol) contained in a sterile beaker covered and wrapped with masking tape. The extraction was allowed to proceed for 24hrs at room temperature 28^0C. After 24hrs, the extract was filtered using a clean whatman filter paper. The filtration was carried out for 1hr, 20minuites.

Preparation of Concentration of the Leaves Extract

Preparation of concentration of the leaves extract was conducted using sterile dilution technique, 0.2g, 0.4g, 0.6g, 0.8g and 1.0g of methanol, extract were weighed and dissolved separately in 5ml of Dimethyl Sulfoxide (DMSO) inside test tubes. The tubes containing the various concentration were labeled immediately and streptomycin was used as a control.

Preparation of the Inoculums

Bacteria isolates; *staphylococcus aureus, Escheriechia coli, Pseudomonas aeruginosa, Salmonela typhi,* and *streptococcus pyogenes* were obtained from the Federal University of Technology Minna Microbiology laboratory, Mueller Hinton Agar was prepared as directed by the manufacturer, 38g into 1000ml of distilled water. The cultured used were 24hrs old, having incubated at 37^0c. The standard bacteria colonies were inoculated on Mueller Hinton Agar media and spread evenly on the plates and left to dry completely, subsequently, a cork borer (4mm) was sterilized in ethanol and used to cut a well into each of the plates by rotating the plate gently. The plates were labelled and the wells were filled up with 1ml of the plant extracts using a sterile pasture pipette, antibiotic disk (streptomycin) was used as positive and negative control. It was allowed to stand for (60 minutes) for effective diffusion. They were then incubated at 37^0c for 24 hours in an incubator and then the diameters of the zone of the inhibition were measured to the nearest mm.

RESULTS

Table 1: Phytochemical Analysis of Henna Leaves (*Lawsonia inermis*) Result

Phytochemical test	Result
Alkaloids	+
Cardiac glycosides	+
Flavonoid	+
Tannins	+
Terpenoids	+

Key: Present (+) Absent (–)

Table 2: Antimicrobial Activities of Henna Leaves (*Lawsonia inermis*)

	Zone of inhibition				
Organism	0.2mg/mL	0.4mg/mL	0.6mg/mL	0.8mg/mL	1.0mg/mL
Salmonella typhi	8mm	11mm	12mm	14mm	16mm
Staphylococcusaureus	11mm	14mm	17mm	19mm	21mm
Escherichia coli	9mm	11mm	11mm	13mm	16mm
Pseudomonas aeroginosa	10mm	11mm	12mm	16mm	20mm
Streptococcus pyogenes	9mm	10mm	14mm	15mm	18mm

DISCUSSION

Table1shown above presents the result of phytochemical analysis conducted on henna leaves (*Lawsonia inermis*). The result revealed that chemical properties such as alkaloids, cardiac glycosides, flavonoid tannins and terpenoids were presents in the henna leaves (*Lawsonia).* While Table 2 presented the level of concentration of henna leaves and their zone of inhibition against selected bacteria; *Escherichia coli*, *Pseudomonas aeruinosa*, *Salmonella typhi*, and *Staphylococcus aureus.* At 0.2mg/mL, 0.4mg/mL, 0.6mg/mL, 0.8mg/mL, and 1.0mg/mL concentration of henna leaves, the zone of inhibition against *Salmonella typhi*, were 8mm, 11mm, 12mm, 14mm and 16mm respectively. Thus, the concentration of 1.0mg/mL gave the highest zone inhibition of 16mm while 0.2mg/mL gave lowest zone of inhibition against *Salmonella typhi*.

At 0.2mg/mL, 0.4mg/mL, 0.6mg/mL, 0.8mg/mL, and 1.0mg/mL concentration of henna leaves, the zone of inhibition against

Staphylococcusaureus, were 11mm, 14mm, 17mm, 19mm and 21mm respectively. Thus, the concentration of 1.0mg/mL gave the highest zone of inhibition of 21mm while 0.2mg/mL gave lowest zone of inhibition of 11mm against *Staphylococcus aureus*

At 0.2mg/mL, 0.4mg/mL, 0.6mg/mL, 0.8mg/mL, and 1.0mg/mL concentration of henna leaves, the zone of inhibition against *Escherichia coli*, stood at 9mm, 11mm, 11mm, 13mm and 16mm respectively. Thus, the concentration of 1.0mg/mL gave the highest zone inhibition of 16mm while 0.2mg/mL gave lowest zone of inhibition of 9mm against *Escherichia coli*

Concentration of henna leaves at 0.2mg/mL, 0.4mg/mL, 0.6mg/mL, 0.8mg/mL, and 1.0mg/mL, against *Pseudomonas aeroginosa* shows the zone of inhibition of 10mm, 11mm, 12mm, 16mm and 20mm respectively. Thus, the concentration of 1.0mg/mL gave the highest zone inhibition of 20mm while 0.2mg/mL gave lowest zone of inhibition of 10mm against *Pseudomonas aeroginosa.*

In addition, concentration of henna leaves at 0.2mg/mL, 0.4mg/mL, 0.6mg/mL, 0.8mg/mL, and 1.0mg/mL, against *Streptococcus pyogenes* shows the zone of inhibition of 9mm, 10mm, 14mm, 15mm and 18mm respectively. Thus, the concentration of 1.0mg/mL gave the highest zone inhibition of 18mm while 0.2mg/mL gave lowest zone of inhibition of 9mm against *Streptococcus pyogenes.* By implication, concentration of henna leaves at 1.0mg/mL is highly effective against *Staphylococcusaureus* but less effective against *Salmonella typhi* and *Escherichia coli*. This can be as a result of the fact that *Staphylococcusaureus* is usually found on human skin while henna leaves (*Lawsonia inermis*) are known to be effective on human skin than any other part of human body.

Based on the analysis conducted on anti-microbial activities of henna leaves (*Lawsonia inermis*), it could be clearly seen that concentration of henna leaves (*Lawsonia inermis*) at 1.0mg/mL is highly effective against *Staphylococcusaureus* closely followed by *Pseudomonas aeroginosa* and *Streptococcus pyogenes* but less effective against *Salmonella typhi* and *Escherichia coli* with zone of inhibition of 21mm, 20mm, 18mm, 16mm and 16mm respectively. The effectiveness of henna leaves (*Lawsonia inermis*) against *Staphylococcus* at 1.0mg/mL can be attributed to the fact that *Staphylococcusaureus* is usually found on human skin which agrees with the finding of Al-Rimawi, (2018) that henna leaves (*Lawsonia inermis*) are effective on human skin than any other part of human body. Therefore, the result of this study revealed that henna plant is effective against bacteria such as *Staphylococcusaureus, Pseudomonas aeroginosa* and *Streptococcus pyogenes.*

This study equally agreed with the result of Al-Rimawi (2018) who confirmed that henna (*lawsonia inermis*) both red and black henna showed variable antimicrobial activity against tested bacteria *Staphylococcus epidermis, Pseudomonas. aeruginosa* and *Candida albicans.* In addition this study was also supported by the study of Rahmoun (2013) who found that Henna leaves (*Lawsonia inermis*) has effective antimicrobial activity against twelve bacteria and three Candida species using the Agar disc diffusion method. The bacteria observed to be sensitive to henna leaves include *Staphylococcusaureus, Pseudomonas aeroginosa, E. coli,* and *Streptococcus pyogenes,*

though their methodology is differs with the presence study.

CONCLUSION

Bases on the finding of the study it can be concluded that henna possess great antimicrobial activity against bacteria such as *Escherichia coli*, *Pseudomonas aeruinosa*, *Salmonella typhi*, and *Staphylococcus aureus.* Though it is more effective in *Staphylococcus aureus* than any other bacteria observed with zone of inhibition ranging from 11mm, 14mm, 17mm, 19mm and 21mm at 0.2mg/mL, 0.4mg/mL, 0.6mg/mL, 0.8mg/mL, at 1.0mg/mL concentration of henna leaves respectively.

Recommendations

Based on the finding of the study, it could be recommended that

i. People should start applying Henna (*Lawsonia inermis*) on the skin because it has serious effect to kill skin bacteria like *Staphylococcus aureus*
ii. Cosmetic industries should make use of Henna (*Lawsonia inermis*) in producing body creams and lotion. This would go a long way in reducing skin infection from bacteria.
iii. Frequent consumption of Henna (*Lawsonia inermis*) as vegetable should be encourage by people, this would assist in curing, protecting or preventing human body system against bacteria such as *Pseudomonas aeruinosa*, *Salmonella typhi* etc.

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Weed Persistence Index, Weed Control Efficiency and Harvest Index in Onion (*Allium cepa* l.) Field as Affected by Plant Population and Weed Control Treatments in Sudan Savanna, Nigeria

[*1]Garba Y., [1]Waziri A., [2]Isah, A. S., [1]Majin N. S. & [3]Uthman A.

[1]Department of Crop Production, Ibrahim Badamasi Babangida University, Lapai, Niger State
[2]Department of Agronomy, Ahmadu Bello University Zaria, Nigeria.
[3]Department of Biochemistry, Ibrahim Badamasi Babangida University, Lapai, Niger State.

*Correspondence address: gyahaya4@gmail.com. 09031112831

ABSTRACT

Field experiment was conducted in farmer's field at Birnin Kebbi during the 2017/2018 and 2018/2019 dry seasons to examine the effect of weed persistence index, weed control efficiency and harvest index as influenced by plant population and weed control treatments in onion (*Allium cepa* L.) field under Sudan Savanna agro-ecological zone of Nigeria. The experiment was factorial arranged, conducted in a Randomized Complete Block Design with three plant population (500,000, 333,333 and 250,000 plants per hectare) and weed control treatments (Pre-emergence application of pendimethalin (1.0, 1.5 and 2.0 kg a.i ha^{-1}), butachlor (2.0, 2.8 and 3.6 kg a.i ha^{-1}), Post-emergence application of fluazifop-p-butyl at 2.0 kg a.i ha^{-1}, oxyfluorfen at 1.0 kg a.i ha^{-1}, weeding at 3 and 6 WAT, weeding at 3, 6 and 9 WAT, weed free and weedy check replicated three times. Results showed that plant population of 500,000p/ha recorded lowest weed persistence index and highest weed control efficiency. Weed persistence index was lowered with all the application rates of pendimethalin and butachlor, while pendimethalin at 1.0 kg a.i. ha^{-1} followed by one hoe weeding and weed free recorded highest weed control efficiency. Harvest index was increased under the influence of pendimethalin and butachlor at 1.0 and 2.0 kg a.i. ha^{-1} followed by one hoe weeding respectively. It is therefore concluded that all the aforementioned treatments are recommended for weed persistence index, weed control efficiency and harvest index in Birnin Kebbi, Sudan Savanna Agro-ecological Zone of Nigeria.

Key words: weed persistence index, weed control, weed control efficiency, harvest index, plant population

INTRODUCTION

Weeds are recognized worldwide as an important type of undesirable economic pest. It is a plant growing out of place, which is a plant growing where it is not wanted. Ekwealor *et al*. (2019) described weeds as plants that are unwanted in a given situation and could be harmful, dangerous or economically detrimental. According to Das (2008), weeds have been existing on earth ever since man started domesticating and cultivation of plants

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about 10,000 B.C which was expressed as the beginning of farming where farmers began to replace hunters. Weeds are controversial plants that are neither all bad nor all good and therefore, there are not strangers to man because, they have been on earth since time immemorial (Das, 2008). Dekker (2011) attested that most of the common and widespread weed species we now have come as a consequence of crop domestication, planting and cultivation. Rashid *et al*. (2005) reported that many weed species have moved out of their natural geographic ranges and spread around the world in tandem with human migrations and commerce. The seed bank of weeds in an agricultural field is made up of different species but in a given year, the infestation is typically dominated by a few species. The species that dominate the infestation are those best adapted to current management practices and have several characteristics that assure their survival (Mahmood *et al*., 2012).

Farmers control weeds to increase plant growth, to improve crop yield and quality, and to reduce habitat for insect and disease vectors. They may also control weeds to prevent a buildup of weed seeds in the soil. Weeds are one of the major plant protection problems in onion fields. They compete with onions for light, nutrients, water and space while also harboring several harmful insects and pathogens (Uygur *et al*., 2010)**.** Controlling weed development during the onion crop cycle is essential to obtain high yields and marketable products. Kraehmer and Baur (2013) reported that the effect of weeds on crops reduces crop yields and its quality, delaying or interfering with harvesting and causes many billions of dollar worth of crop losses annually.

Weeds control methods vary greatly with the status of agriculture and the nature of the cropping system adopted. Sathya *et al*. (2018) indicated that in modern agriculture, application of herbicides are commonly used as an alternative method to traditional methods of hand weeding at initial period of crop growth for better control of weeds. The objective of this research was to compare weed persistence index and weed control efficiency in onion as affected by plant population and weed control treatments.

METHODOLOGY

Experimental site

Field experiment was conducted in Farmer's field during the 2017/2018 and 2018/2019 dry seasons at Birnin Kebbi, Kebbi State to evaluate weed persistence index and harvest index in onion as affected by plant population and weed control treatments in Sudan Savanna, Nigeria. Birnin Kebbi lie on latitude 12°25'N and longitude 4°15'E. The area enjoys a tropical type of climate generally characterized by annual temperature range of 25-40 °C. The mean annual rainfall is about 500- 700 mm (Gindi *et al*., 2013).

Treatment and experimental design

The treatments consisted of three plant population (500,000, 333,333, 250,000 plants/ha^{-1}) and twelve Weed control treatments (Pendimethalin at 1.0 kg a.i ha^{-1}, pendimethalin at 1.5 kg a.i ha^{-1}, pendimethalin at 2.0 kg a.i ha^{-1}, butachlor at 2.0 kg a.i ha^{-1}, butachlor at 2.8 kg a.i ha^{-1}, butachlor at 3.6 kg a.i ha^{-1}, fluazifop-p-butyl at 2.0 kg a.i ha^{-1}, oxyfluorfen at 1.0 kg a.i ha^{-1}, hoe weeding (Hw) at 3 and 6 Weeks after transplanting (WAT), Hw at 3, 6 and 9 WAT, Weed free and weedy check) arranged in a Randomized Complete Block Design and replicated three times. The experimental field was irrigated, manually

ploughed and plot size of 2m × 3m was constructed. Poultry manure at the rate of 4 t/ha was uniformly incorporated on each plot during construction and application of herbicides was done 2 days after transplanting (DAT) based on the treatments design. The plots were irrigated 4 -5 days throughout the growing period.

Data collection

Data collected during the experiment include weed parameters (dry weed weight weed index), growth parameters (plant height, leaf area index, crop vigor score) and yield parameters (total bulb weight, above ground biomass, marketable bulb yield and cured bulb yield).

Data analysis

The data collected was subjected to analysis of variance (ANOVA) using statistical analysis soft ware (SAS, 2013) at 5% level of probability and means were separated using Duncan Multiple Range Test (DMRT).

Weed persistence index (WPI):

Weed persistence index is the relationship between weed dry weight in control plot and that of the treated plots indicating relative dry matter accumulation of weeds per count in comparison to control and was obtained using the formula below as described by Rana and Kumar (2014):

$$\mathrm{WPI} = \frac{\mathrm{W_T}}{\mathrm{W_C}} \times \frac{\mathrm{W_{PC}}}{\mathrm{W_{PT}}}$$

Where:-

W_C = Weed dry weight in control plot (unweeded) plot

W_T = Weed dry weight in treated plot

W_{PC} = Weed population in control (unweeded) plot

W_{PT} = Weed dry weight in treated plot

Weed control efficiency (WCE)

Weed control efficiency (WCE) denotes the magnitude of weed reduction due to weed control treatment. It was obtained by using the formula suggested by Mani *et al.* (1973) and expressed in percentage.

$$\mathrm{WCE}\ (\%) = \frac{\text{Weed density in control plots} - \text{weed density in treated plots}}{\text{Weed density in control plots}} \times 100$$

Harvest index (HI)

This was expressed as the ratio of total bulb dry weight to the total biomass dry weight and expressed in percentage.

$$\mathrm{HI} = \frac{\text{Total cured bulb weight per plot (kg)}}{\text{Total biomass dry weight (kg)}} \times 100$$

RESULTS

Effect of plant population and weed control treatments on weed persistence index and weed control efficiency

Table 1 presents weed persistence index (WPI) and weed control efficiency (WCE) during the 2017/2018 and 2018/2019 dry sessions. Weed persistence index was significantly affected by plant population in 2017/2018 only. Result showed that plant population of 500,000 p/ha recorded the lowest weed persistence index, while plots planted with 250,000 p/ha recorded

the highest WPI. Weed control efficiency was not significantly affected by plant population in both seasons. Weed control treatments significantly affect weed persistence index and weed control efficiency in both seasons. The result indicated that in 2017/2018, plots sprayed with pendimethalin at 1.0 kg a.i ha^{-1} followed by one hoe weeding recorded the lowest weed persistence index. Similar lowest weed persistence index was recorded in plots sprayed with pendimethalin at 1.5 kg a.i ha^{-1} followed by fluazifop-p-butyl at 2.0 kg a.i ha^{-1}, butachlor at 2.8 kg a.i ha^{-1} followed by oxyfluorfen at 1.0 Kg a.i. ha^{-1} and weed free plots. The highest weed persistence index was observed in the weedy check in 2017/2018. All weed control treatments recorded similar lowest weed persistence index in 2018/2019, though butachlor at the rate of 2.0 kg a.i ha^{-1} followed by one hoe weeding and butachlor at 3.6 kg a.i. ha^{-1} were at par, while the highest weed persistence index in 2018/2019 was recorded in weedy check.

In consideration of weed control efficiency, weed free plots recorded the highest weed control efficiency in 2017/2018, though the result was at par with those results obtained in plots with application of pendimethalin at 1.0, 1.5 and 2.0 Kg a.i. ha^{-1}, butachlor at 2.0 and 2.8 Kg a.i. ha^{-1}, fluazifop-p-butyl at 2.0 Kg a.i. ha^{-1} and oxyfluorfen at 1.0 Kg a.i. ha^{-1}. The lowest weed control efficiency is recorded in the weedy check plots. In 2018/2019, application of pendimethalin at the rate of 1.5 kg a.i. ha^{-1} followed by fluazifop-p-butyl at 2.0 kg a.i. ha^{-1} significantly produced the highest weed control efficiency. Similar highest weed control efficiency was recorded when only pendimethalin at 2.0 kg a.i. ha-1 and weed free plots. Weedy check plots recorded the lowest weed control efficiency at harvest in 2018/2019.

Effect of plant population and weed control treatments on Harvest index

The effect of plant population and weed control treatments on harvest index at Birnin Kebbi during the 2017/2018 and 2018/2019 dry seasons and pooled is presented in Table 2. Significant difference was observed in plant population on harvest index in both years and at pooled. Plant population of 500,000 p/ha recorded the highest harvest index in both years and at pooled while plant population of 250,000 p/ha recorded the lowest harvest index in both years and at pooled. Harvest index was significantly affected by weed control treatments. Result showed that, in 2017/2018 dry season, pendimethalin at 1.0 and 1.5 kg a.i. ha^{-1} followed by one hoe weeding and fluazifop-p-butyl at 2.0 kg a.i. ha^{-1} respectively recorded similar highest harvest index. The result was at par with all other treatments except oxyfluorfen at 1.0 kg a.i. ha^{-1} and weeding at 3, 6 and 9 WAT and represents the lowest harvest index. In 2018/209 plots sprayed with fluazifop-p-butyl at 2.0 kg a.i. ha^{-1} recorded the highest harvest index. All other treatments were at par except plots that received weeding at 3 and 6 WAT and weed free, while the lowest harvest index was recorded in plots sprayed with butachlor at the rate of 2.8 kg a.i. ha^{-1} followed by fluazifop-p-butyl at 2.0 kg a.i. ha^{-1}. Result at pooled indicated that Fluazifop-p-butyl at 2.0 kg a.i. ha^{-1} recorded the highest harvest index, while the lowest was observed in plots weeded twice

Table 1. Effect of plant population and weed control treatments on weed persistence index and weed control efficiency in onion at Birnin Kebbi

		WPI		WCE (%)	
Treatment	Rate (Kg a.i. ha^{-1})	2017-2018	2018-2019	2017-2018	2018-2019
Plant population (plants/ha^{-1})					
500,000		0.1601^{b}	0.1201	73.14	79.39
333,333		0.1694^{ab}	0.1332	71.39	81.03
250,000		0.1885^{a}	0.1329	70.99	77.59
SE±		0.0091	0.0088	1.52	2.02
Weed Control (WC)					
Pendimethalin	1.0 fb 1HW	0.0567^{e}	0.0233^{d}	83.30^{ab}	90.49^{ab}
Pendimethalin fb Fluazifop-p-butyl	1.5 fb 2.0	0.0386^{e}	0.0227^{d}	81.89^{ab}	94.62^{a}
Pendimethalin	2.0	0.0834^{de}	0.0230^{d}	76.97^{abc}	92.19^{a}
Butachlor	2.0 fb 1HW	0.1172^{cd}	0.0994^{c}	79.97^{ab}	84.24^{abc}
Butachlor fb Oxyfluorfen	2.8 fb 1.0	0.0597^{e}	0.0285^{d}	83.31^{ab}	90.53^{ab}
Butachlor	3.6	0.1656^{c}	0.0582^{cd}	71.05^{cd}	87.30^{ab}
Fluazifop-p-butyl	2.0	0.0736^{de}	0.0279^{d}	81.33^{ab}	80.32^{bc}
Oxyfluorfen	1.0 fb 1HW	0.1223^{cd}	0.0361^{d}	77.57^{abc}	84.49^{abc}
Hoe weeding	3 and 6 WAT	0.2408^{b}	0.1919^{b}	65.48^{d}	73.52^{c}
Hoe weeding	3, 6 and 9 WAT	0.0767^{de}	0.0294^{d}	75.75^{bc}	79.75^{bc}
Weed free	-	0.0371^{e}	0.0045^{d}	85.48^{a}	94.61^{a}
Weedy check	-	1.000^{a}	1.0000^{a}	0.00^{e}	0.00^{e}
SE±	-	0.0183	0.0183	3.04	4.04
Interaction					
P.P*WC		NS	NS	NS	NS

Means with the same letter (s) in a treatment column are not significantly different at 5% level of probability using Duncan Multiple Range Test (DMRT). NS= not significant, *= significant at 5% level.

Table 2. Effect of plant population and weed control treatments on harvest index at Birnin Kebbi during the 2017/2018 and 2018/2019 dry seasons

		Harvest index (%)		
	Rates (Kg a.i. ha^{-1})	2017/2018	2018/2019	Pooled
Plant population (plants/ha^{-1})				
500,000		63.63^{a}	68.62^{a}	66.12^{a}
333,333		48.87^{b}	53.51^{b}	51.19^{b}
250,000		38.32^{c}	44.20^{b}	41.26^{c}
SE±		2.43	4.39	2.29
Weed control (WC)				
Pendimethalin	1.0 fb 1HW	48.42^{ab}	63.44^{abc}	55.93^{abc}
Pendimethalin fb	1.5 fb 2.0	53.79^{ab}	64.10^{abc}	58.95^{ab}
Fluazifop-p-butyl				
Pendimethalin	2.0	59.56^{a}	59.26^{abc}	59.41^{ab}
Butachlor	2.0 fb 1HW	60.31^{a}	56.74^{abc}	58.53^{ab}
Butachlor fb Oxyfluorfen	2.8 fb 1.0	50.47^{ab}	38.54^{c}	44.50^{bc}
Butachlor	3.6	50.01^{ab}	46.83^{abc}	48.42^{bc}
Fluazifop-p-butyl	2.0	53.58^{ab}	75.62^{a}	64.60^{a}
Oxyfluorfen	1.0 fb 1HW	43.25^{b}	48.90^{abc}	46.08^{bc}
Hoe weeding	3 and 6 WAT	40.15^{b}	46.13^{bc}	43.14^{c}
Hoe weeding	3, 6 and 9 WAT	51.56^{ab}	51.01^{abc}	51.28^{abc}
Weed free	-	45.39^{ab}	45.74^{bc}	45.52^{bc}
Weedy check	-	46.87^{ab}	69.01^{ab}	57.94^{abc}
SE±	-	4.86	8.78	4.58
Interaction				
PP*WC	-	NS	NS	NS

Means with the same letter (s) in a treatment column are not significantly different at 5% level of probability using Duncan Multiple Range Test (DMRT). NS= not significant, *= significant at 5% level

DISCUSSION

Lowest weed persistence index was observed when plant population of 500,000p/ha was applied. This result could be as a result of weed suppressing ability of onion when planted in a closed spacing. Khan *et al*. (2021) reported that increasing planting density reduced the weed infestation that could be explained as the higher population pressure and intercrop competitive ability of Bt cotton reduced the weed persistence. The use of herbicide as sole or in combination or manual hoe weeding decreases weed population in onion. This described the report of Kumar *et al*. (2019) who stated that significant lowest weed persistence index was observed when pre emergence application of pendimethalin + imazethapyr was applied for the control of weeds in pegion pea. Mishra *et al*., (2016) also revealed that weed persistence index was maximum in hand weeding treatment and or followed by combination of herbicides (Cyhalofopbutyl and Pyrazosulfuron) in rice. Keeping weed free at all times increases harvest index, likewise application of Pendimethalin at

the rate of 1.5 kg a.i. ha^{-1} followed by fluazifop-p-butyl at 2.0 kg a.i. ha^{-1} resulted in highest harvest index. This result is in consonant with the report of Kalhapure *et al*. (2013) who reported that combination of integrated weed management of chemical and cultural weed control is found to be effective in onion production. The lowest weed control efficiency was recorded against the control plots. Similar lowest weed control efficiency was reported by Kumar (2014) on weed management studies in onion. Harvest index of onion was influenced more when planted under plant population of 500,000 p/ha. This indicates that increase in harvest index could be as a result of increase in plant population of onion. This result confirmed the result of Kamithi *et al*. (2009) who stated that an increase in plant population density increased the harvest index (HI) of Chickpea (Cicer arietinum L.). In terms of weed control treatments, the application of butachlor at 2.0 Kg a.i. ha^{-1} followed by one hoe weeding and fluazifop-p-butyl at 2.0 kg a.i. ha^{-1} was superior over other treatments. Sepahvand *et al*. (2014) reported that grain yield, harvest index, seed rows per cob, seeds per row and cob weight of corn were increased by weed control treatments

CONCLUSION

Increased plant population of onion up to 500,000 p/ha resulted in decrease in weed persistence index and highest harvest index. Application of all rates of pendimethalin and butachlor including weed free treatment recorded lowest weed persistence index. However, highest harvest index was achieved with the use of pendimethalin at 2.0 kg a.i. ha^{-1}, though, butachlor at the rate of 2.0 kg a.i. has^{-1} followed by weeding once at 6 WAT and application of fluazifop-p-butyl 2.0 kg a.i. ha^{-1} recorded similar highest harvest index. Weed control efficiency was achieved with the practice of weed free. It is therefore recommended that plant population of 500,000 p/ha was appropriate for lowest weed persistence index and highest harvest index. Application of pendimethalin at 2.0 kg a.i ha^{-1} or butachlor at 2.0 kg a.i ha^{-1} followed by one hoe weeding including fluazifop-p-butyl at 2.0 kg a.i ha^{-1} produced better harvest index. All rates of pendimethalin or butachlor reduces weed persistence index, while weed control efficiency was in good standing with the practice of weed free treatment.

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Utilization of Shea Caterpillar, *Cirina butyrospermi* in the Practical Diet of Hybrid African Catfish, *Clarobranchus* Fingerlings

Alabi, A. T., Sadiku S. O. E. & Oyero J. O.

Correspondence Email address: aat4_u@yahoo.com

Department of Water Resources, Aquaculture and Fisheries Technology, Federal University of Technology, P.M.B, 65 Minna, Niger State.

ABSTRACT

A 56-days feeding trial was conducted to evaluate Utilization of Shea Caterpillar, *Cirina Butyrospermi* in the Practical Diet of Hybrid African Catfish, *Clarobranchus* fingerlings through their growth performance and nutrient utilization. Five isonitrogenous diets were formulated, containing 40 % crude protein and 9.5 % lipid. SCM and FM were included at different inclusion levels; DI (0 % SCM, 100 % FM); DII (25 % SCM, 75 % FM); DIII (50 %: 50 % SCM: FM); DIV (75 % SCM, 25 % FM) and DV (100 % SCM, 0 % FM). These diets with one commercial catfish reference diet (CRD) skretting, were fed to the experimental fish. The experiment was completely randomized in triplicate, and twenty fish were allocated per each (50 cm by 30 cm by 20 cm) white plastic buckets, with the mean initial body weight 2.52±0.43 per fish. The fish were fed at 3 % of their body weight at two equal meals per day throughout the experimental period. At the end of the study, there was significant difference ($P<0.05$) in the growth and body composition parameters evaluated. Fish fed diet DI had the highest mean final weight 20.34±0.59, mean weight gain 16.84±0.61, and protein efficiency ratio 2.59±0.16; and fish fed diet DII had highest specific growth rate 2.70±0.18 and lowest feed conversion 0.58±0.07 with no significant difference ($P>0.05$) between diet DI and DII. Fish fed diet DV had the lowest mean final weight 16.48±0.19**,** mean weight gain 12.96±0.19, specific growth rate 1.29±0.30, protein efficiency ratio 1.46±0.07 but highest in feed conversion ratio 6.81±4.51. However, there was significant difference ($P<0.05$) in the survival rate of the fish, fish fed diet DII had the highest survival rate 95.00±5.00 while fish fed diet DV had the lowest survival rate 85.00±8.86. The ANPU of the experimental fish was significantly different ($P<0.05$), DVI (CRD) had the highest ANPU (22.05±0.36), followed by DIV (18.91±0.2), while the DI and DII had the least ANPU 13.13±0.04 and 15.62±0.55 respectively. 25%, to 75% inclusions level of Shea caterpillar *Cirina butyrospermi* is recommended for the diet of hybrid catfish *Clarobranchus* in terms of growth performance.

Key words: Fishmeal (FM), *Clarobranchus,* Shear Caterpillar (SCM), feed utilization,

INTRODUCTION

The request for high quality fish and fishery products is increasing significantly every year across the globe, due to their nutritional benefits, that they supply plentiful of beneficial

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healthy substances (FAO, 1986; and Jag *et al.,* 2018). Aquaculture is among the major contributors to the world's food security (Austin, 2013). Its production however, is of great significant in the agricultural industry globally, it is growing and will continue to grow as the demand for fish and fish products increases as the supply from natural sources decreases (Delbert and Gatlin, 2010). As in more traditional forms of animal production, nutrition plays a critical role in intensive aquaculture because it influences not only production costs but also fish growth, health and waste production (Delbert and Gatlin 2010). Akankali and Nwafili (2015) reported fish feed and fertilizer play the key role for fish production in the conventional fish culture system, representing about 60-70% of production costs. According to (Rumsey, 1993 and Orire *et al.,* 2015), feed production cost has been considered to be the highest cost in aquaculture practices today, which often ranges from 30 – 70% of total production cost. At present, a large percentage of high quality fish feeds are said to be imported, with about 45,000 metric tons imported in 2010 (FDF, 2012). Among the major constraints facing aquaculture is quality feed (Arnauld, 2016), which includes feed sources especially, protein source (fishmeal and soy meal) which imposes a greater environmental challenges (Isaac *et al.,* 2018). Fishmeal has been linked to overfishing of ocean resources, and furthermore, it is a resource dependent on catch from the ocean, its production is variable both quantitatively and qualitatively (Sánchez-Muros *et al.,* 2014) and the price of fishmeal is often high (Falaye *et al*., 2014). Therefore, the cost effectiveness in feed production especially fishmeal as well as its unavailability has brought to notice the need to research for various alternative protein sources in feed production (Alceste, 2000 and Orire *et al.,* 2015). The use of insects as feed ingredient in fish production in reducing feeding costs (Sealey *et al.,* 2011) and the possibility of substantial replacement of fishmeal by insect meal without compromising the growth, feed conversion and product quality of fish (Stadtlander *et al*., 2017). It is therefore pertinent to exploit not only the known unconventional feed ingredients but also to determine and introduce new and lesser known plants and animal feed resources (Bamphitlhi and John, 2012).

The Shea Caterpillar, *Cirina butyrospermi*

Cirina butyrospermi, the pallid emperor moth or shea defoliator, is a moth of the family *Saturniidae*. The species was first described by John O. Westwood in 1849 as *C. forda* and as C. *butyrospermi* by Vuillet (1911). It is found in West Africa, including Nigeria, Ghana, Zimbabwe, the Democratic Republic of the Congo and South Africa. The adults are pale creamy brown with a small darker spot on each hind wing but lacking true eyespots. There is one generation per year.

Collection and processing of Shea caterpillar *Cirina butyrospermi,*

*C. butyrospermi*larva is collected from the shea butter tree, *Vitellaria paradoxa,* its only host in Nigeria and throughout the West African sub region (Agbidye and Nongo**,** 2009). The eggs are found on the host plant from May to June and the larvae from June to August each year. The larvae are particularly harvested from shea butter trees in July and August each year (Odeyemi and Fasoranti, 2000; Ande and Fasoranti, 1997). The larvae are either collected from the leaves on the trees or pitfall traps made round the bases of trees with the larvae and descending larvae trapped and collected (Mbata and Chidumayo, 2003, Fasoranti and Ajiboye, 1993). These Caterpillars are either pushed

inside out with a thin stick or punctured and the contents squeezed out. Frequently, and especially if large quantities are harvested, they are boiled and dried out in the sun and stored for later use or sold in the local markets. The insect is widely used as an ingredient in vegetable soup (Fasoranti and Ajiboye, 1993). *C. butyrospermi* known as Kontoro in Nupe, Monimoni or Ikanni in Yoruba and 'Igyô' in Tiv, is reported to be widely consumed and marketed in Nigeria (Agbidye *et al.*, 2009).

Statement of the Research Problems

i. The fish feed industry is still relying upon fishmeal and fish oil from the industrial fishing operations despite the increasing demand for food fish and the decline in capture fisheries (Elezuo, 2011).
ii. Fishmeal is used as the main dietary protein source in aquaculture, because of its known nutritional quality and palatability properties (Hardy and Tacon 2002). And the resulting volatility in price linked to fishmeal poses risks for economic viability of the aquaculture sector (Kobayashi *et al.,* 2015).
iii. Total dependent on fishmeal as the only protein source will affect the whole operation of aquaculture system and consequently reduce the production.
iv. The limitation of feedstuffs of plants' origin as a result of the presence of alkaloids, glycosides, oxalic acids, phytates, protease inhibitors, haematoglutinin, saponegin, monosine, cyanoglycosides and so on, which negate growth and other physiological activities at higher level inclusion.

The Objectives of the Study

i. To evaluate the nutritive value of shea caterpillar (*C. butyrospermi*,).
ii. To determine the suitability of shea caterpillar (*C. butyrospermi*,), as a replacement of fishmeal in the practical diets of African catfish (*Clarobranchus)*.

METHODOLOGY

Location of the Study

The experiment was carried out at a fish farm, Ikhlas Agro Nigeria, Limitd, Oke-Oyi, Ilorin-East, Kwara State. The location is between Latitude 8º34'44.86"N and Longitude 4º42'53.35"E. The experimental was done in accordance with the Department of Water Resources, Aquaculture and Fisheries Technology, Federal University of Technology, Minna protocol.

Experimental design

The experiment consisted of five treatments with a commercial reference diet, each in three replicates in a complete randomized design. Each bucket was stocked with twenty hybrid catfish *Clarobranchus* fingerlings.

Experimental set-up

The experimental set-up consists of twenty one white plastic buckets (50cm x 30cm x 20 cm) installed in a wooden frame. The frame was nailed and brazed firmly to prevent it from collapse.

Experimental fish

450 hybrid of African catfish, *Clarobranchus* fingerlings were collected from Ikhlas Agro Nigeria, Limited, Oke-Oyi, Ilorin Kwara State, The fish were acclimatized in a (50x30x20 cm) rectangular white plastic buckets for a week and

fed commercial fish feed (Skretting) throughout the week of acclimation.

Experimental diets

Five different diets with varying inclusion level were formulated and pelletized with shea caterpillar, fishmeal, yellow maize, rice bran with vitamins premix. The inclusion levels of Shea Caterpillar Meal (SCM) as a replacement for Fishmeal (FM) was as designated as thus; DI (0% SCM, 100 FM); DII (25% SCM, 75% FM); DIII (50%:50% SCM:FM); DIV (75% BCM, 25%FM) and DV (100% SCM, 0% FM), with a commercial catfish reference diet (CRD) designated DVI.

Experimental procedure

The fish were fed with the experimental diet at 3% body weight per day and later adjusted as the fish grows. Water exchange was done on a daily bases with the siphoning of faecal matter and uneaten feed. And water quality parameters was also monitored on weekly bases for temperature using clinical thermometer, dissolved oxygen according to the method of winker's (21, 22), hydrogen iron concentration (pH) with EIL 7045/46 pH meter and the conductivity was monitored by the use of conductivity meter.

Table 1: Proximate composition of some ingredients used for the experimental diet (dry basis)

Composition (%)	FM	SCM	MM	RBM
Crude protein	72	65.68	10.06	11.03
Ash	6	3.01	0.49	10.30
Lipid	10	12.34	10.92	11.50
Moisture	9	16.81	10.03	7.20
Crude fibre	0.00	3.70	0.44	13.60

FM: Fishmeal (Danish fishmeal, Hanstholm Prime, 2019)
SCM: Shear caterpillar meal
MM: Yellow maize meal
RBM: Rice bran meal

Table 2a: **Inclusion level, in % of ingredient used in Shea Caterpillar Diet at 40% c.p**

INGREDIENTS	DI	DII	DIII	DIV	DV
SCM%	0.00	11.80	23.60	35.39	47.19
FM%	47.19	35.39	23.60	11.80	0.00
RBM%	23.91	23.91	23.91	23.91	23.91
MM%	23.91	23.91	23.91	23.91	23.91
VIT PREM%	5.00	5.00	5.00	5.00	5.00

N.B: 3% is reserved for Vitamins Premix and MM and RBM are in 1:1.

Table 2b: **Proximate composition of experimental diet fed *Clarobranchus* for 56 days**

Composition (%)	Diets					
	DI	**DII**	**DIII**	**DIV**	**DV**	**DVI (CRD)**
Crude Protein	39.07	38.78	37.65	37.56	37.19	45.00
Crude fibre	4.70	4.98	5.01	5.41	5.87	4.00
Ash	9.34	10.91	11.41	10.76	12.13	8.00
Moisture	6.19	6.39	6.87	7.37	7.52	8.00
Lipid	10.73	10.84	9.47	9.87	9.54	8.00
NFE	29.28	28.02	27.99	28.03	27.31	

Where **DVI** represented Commercial Diet Reference (CRD) which is Blue Crown feed.
Source: (Blue Crown 2019)

Evaluation of growth parameters

Growth performance and nutrient utilization were analyzed in terms of Final Weight Gain (FWG), Feed Efficiency percentage (FE%), Specific Growth Rate (SGR), Feed Intake (FI), Protein Efficiency Ratio (PER), Apparent Net Protein Utilization (APNU) and percentage survival rate (%SR). These parameters were calculated with the following formulae described and used by various researchers, Halver (1989), Bondi (1989) and Maynard *et al.,* (1979) as reported by Bake *et al.,* (2014).

Body weight gain $= \frac{\text{final weight (g)} - \text{initial weight (g)}}{\text{intial weight (g)}}$

Percentage weight gain $= \frac{\text{initial weight(g)}}{\text{final weight (g)}} \times 100$

Feed efficiency (%) $= \frac{\text{weight gained (g)}}{\text{feed fed (g)}} \times 100$

Specific growth rate (%)$= \frac{\text{In final weight (g)} - \text{In initial weight (g)}}{\text{feeding period (Day)}} \times 100$ (Brown, 1957)

Feed intake (mg/fish/day)$= \frac{\text{feed feed (g)/Number of fish}}{\text{feeding period (Day)}}$

Protein efficiency ratio$= \frac{\text{wet body gain (g)}}{\text{protein intake (g)}}$ (Osbome *et al.,* 1919)

Apparent Net Protein Utilization (APNU)$= \frac{\text{P2} - \text{P1}}{\text{Total Protein intake}} \times 100$ (Bender and miller, 1953)

Where, **P1** is the Initial Carcass Protein before the fish was fed experimental diet

P2 is the final Carcass Protein after the fish was fed experimental diet

$$\text{Survival rate (\%)} = \frac{\text{number of fish stocked} - \text{mortality}}{\text{number of fish stocked}} \times 100$$

Statistical analysis

Data obtained were subjected to one way analysis of variance (ANOVA) using Turkey's test (Steel, 1981; 1984) at 5% probability level. Multiple parameter means comparison of treatments was according to Duncan multiple range tests (Duncan, 1955). All statistics analyses including regression were executed using the software Minitab Release 14 and graphical analyses will be plotted with Microsoft Excel Window 2007.

RESULTS

Table 3: Growth performance, nutrient utilization and body parameters of hybrid of *Clarobranchus* fingerlings fed different inclusion levels Shea caterpillar meal (SCM)

DIETS	MIW	MFW	MWG	SGR	FCR	PER	SR	ANPU
DI	2.50±0.0^{a}	20.34±0.5^{a}	17.84±0.6^{a}	2.63±0.1^{a}	0.62±0.0^{a}	2.59±0.1^{a}	89.00±5.00^{b}	13.13±0.04^{f}
DII	2.57±0.8^{a}	20.03±0.7^{b}	17.46±0.6^{a}	2.70±0.1^{a}	0.58±0.0^{a}	2.55±0.1^{a}	95.00±5.00^{a}	15.62±0.55^{e}
DIII	2.47±0.0^{a}	18.14±0.7^{d}	15.67±0.1^{b}	2.11±0.1^{b}	0.89±0.1^{b}	1.97±0.2^{b}	90.00±5.00^{b}	18.40±1.7^{c}
DIV	2.52±0.6^{a}	17.01±0.3^{e}	14.49±0.3^{c}	1.64±0.2^{e}	1.93±0.9^{d}	1.63±0.1^{c}	90.00±5.00^{b}	18.91±0.2^{c}
DV	2.52±0.0^{a}	16.48±0.19^{f}	13.96±0.1^{d}	1.29±0.3^{f}	6.81±4.5^{e}	1.46±0.0^{c}	85.00±8.86^{c}	17.17±0.5^{d}
DVI	2.55±0.0^{a}	18.28±0.0^{c}	15.73±0.1^{b}	2.04±0.1^{c}	0.93±0.1^{b}	2.12±0.0^{b}	86.00±5.77^{c}	22.05±0.3^{b}

Values in the same column with different superscript letters are significantly different (p<0.05) from one another.

MIW:-Mean Initial Weight
SR:- Survival Rate
MFW:-Mean Final Weight
MWG:-Mean Weight Gain
SGR:-Specific Growth Rate
FCR:-Feed Conversion Ratio
PER:-Protein Efficiency Ratio
ANPU:- Apparent Net Protein Utility

DI:- 0% inclusion of shea caterpillar meal
DII:- 25% inclusion shea caterpillar meal
DIII:- 50% inclusion shea caterpillar meal
DIV:- 75% inclusion shea caterpillar meal
DV:- 75% inclusion shea caterpillar meal
DVI:- Skretting (CRD)
DVII:- Blue Crown (CRD)

Table 4: Carcass composition of hybrid of hybrid of *Clarobranchus* fingerlings fed different inclusion levels Shea caterpillar meal (SCM)

Parameters (%)	Initial	Final					
		DI	DII	DIII	DIV	DV	DVI
Fat	12.68±1.1^{e}	11.68±1.13^{g}	15.07±1.58^{b}	14.76±1.91^{c}	12.75±2.16^{d}	15.31±1.45^{a}	12.06±2.61^{f}
Ash	17.52±0.10^{d}	19.01± 1.23^{a}	16.52±2.94^{e}	15.60±2.46^{g}	18.10±1.24^{b}	15.66±1.01^{f}	17.82±4.57^{c}
MC	11.27±1.40^{b}	12.58±4.71^{a}	6.82±0,86^{g}	7.79±1.82^{e}	8.03±0.65^{d}	7.43±0.41^{f}	8.68±1.20^{c}
CP	34.54±0.31^{g}	35.75±0.90^{f}	37.62±5.32^{e}	42.29±3.64^{b}	41.71±1.82^{c}	41.12±6.13^{d}	44.62±3.15^{a}
CF	1.14±0.20^{a}	0.58±0.03^{d}	0.50±0.02^{f}	0.48±0.02^{g}	0.53±0.08^{e}	0.72±0.03^{b}	0.59±0.21^{c}

Values in the same row with different superscript letter are significantly different (p<0.05) from one another

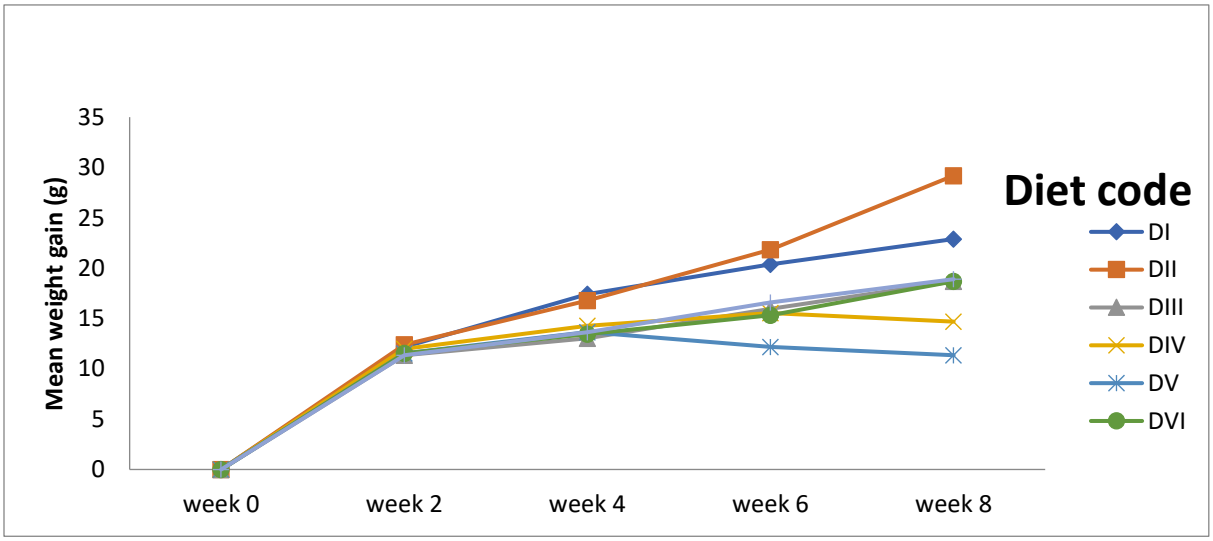

Figure 1: Growth Response of hybrid of *Clarobranchus* fingerlings fed different inclusion levels of Shea caterpillar meal (SCM) for eight weeks

Proximate composition of some ingredients used for the experimental diet (dry basis)

The proximate analysis results for some feed ingredients used in the formulation of the experimental diets is shown in Table 3.1 and indicated that Shea caterpillar meal is lower in crude protein (65.68 %), compared to Danish fishmeal with higher crude protein (72.00%). The lipid content of SCM used in the diets formulation was 12.34 %, which was higher than that of Danish fishmeal, 10.00 %. The percentage composition of the ingredients used in the diets formulation is shown in Table 3.1a, indicated that the DI had 0 % and 100 % inclusion level of SCM and FM respectively; DII had 25 % and 75 % inclusion level of SCM and FM respectively; DIII had 50 % and 50 % inclusion level of SCM and FM respectively; DIV had 75 % and 25 % inclusion level of SCM and FM respectively; and DV had 100 % and 0% inclusion level of SCM and FM respectively. The commercial reference feed,

DVI had crude protein 45.00 % and 8.00 % lipid content.

Proximate composition of experimental diet fed experimental fish for 56 days

The Proximate composition of experimental diet fed experimental fish presented in Table 3.2b showed that crude protein was highest in DI (39.07 %) and lowest in DV (37.19 %). Crude fibre was highest in DV (5.87 %) and lowest in DI (4.7 %). Ash level was lowest in DI but highest in DV, 9.34 % and 12.13 % respectively. Diet DI of the experimental diet has the lowest moisture content (6.19 %), while diet DV has the highest (7.5 %). Similarly, diet DII has the highest lipid content of 10.84 % while diet DIII has the lowest of 9.47 %.

The proximate composition of shea caterpillar meal in this present study revealed that the crude protein and the lipid contents were 65.68 % and 10.92 % respectively. The protein content of shea caterpillar in this study was higher than the values reported earlier by Morgane *et al.,* (2016) and Yapo *et al.,* (2017) but was lower compare to the value reported by Agbidye *et al.,* (2009). The differences might be attributed to differences in environmental conditions such as soil types, harvesting time and the processing conditions.

Growth performance, nutrient utilization and body parameters of hybrid of *Clarobranchus* fingerlings fed different inclusion levels Shea caterpillar meal (SCM)

The mean growth performance, nutrient utilization and body parameters of the experimental fish, is as shown in table 4. There was no significance difference (P>0.05) in the mean initial weight of the fish used for the experiment. However, there was significant difference (P<0.05) in the mean final weight, the final mean weights varied between 20.34 g - 16.48 g. Fish fed 100 % SCM, diet (DV) had the least mean final weight (16.48 g), while fish fed 100 % FM, diet (DI) had the highest mean final weight (20.34 g), followed by the fish fed diet DII (20.03g). Similarly, there was significant difference (P<0.05) in the mean body weight gain, fish fed diet (DI) had the highest mean body weight (16.84 g) and the least was DV (12.96 g).

In the specific growth rate, there was significant difference (P<0.05), fish fed diet DII (2.70 %) and diet DV (1.29 %) had the highest and the lowest respectively. Feed conversion ratio is significantly difference (P<0.05), fish fed diet DV had the highest (6.81 %) while the least was DII (0.58 %). There was also significant difference (P<0.05) in protein efficiency ratio among the treatments, diet DI had the highest (2.89%), and diet DV has the least protein efficiency ratio (1.46%). The survival rate of the experimental fish were significantly different (P<0.05), the fish fed DII had the highest survival rate, (95.00%), while diet DVI, which was commercial reference diet (CRD) had the least (83.33%).

There was no significant difference (P>0.05) between the MWG, SGR, FCR and PER of diets I and II, this showed that the fish was able to digest and convert the diets into body tissue with some degree of efficiency. However, the MWG, SGR, FCR and PER of diets III, IV and V was significant difference (P<0.05). There was significant difference (P<0.05), in the apparent net protein utilization (ANPU) of the experimental fish, as diet VI (CRD) had the highest 25.77±1.00, followed by diet VII (CRD) 22.05±0.36. While the experimental diets, DI and DII had the least ANPU 3.13±0.04 and 7.62±0.55 respectively.

Carcass composition of hybrid of *Clarobranchus* fingerlings fed different inclusion levels Shea caterpillar meal (SCM)

Table 4.2 reflects the carcass composition of the experimental fish. There was significant difference (P<0.05) in the fat content of hybrid of *Clarobranchus* carcass fed experimental diets. DV had the highest fat content (15.31±1.45) and the least was DI (11.68± 1.13). Considering the ash content of the experimental fish, there was a significant difference (P<0.05), diet DI had the highest figure, (19.01± 1.23), followed by DIV (18.10±1.24) and diet DIII was the least, (15.60±2.46). The moisture content also, was significant difference (P<0.05), diet DI had the highest moisture content while diet DII was the least, 12.58±4.71 and 6.82±0.86 respectively. The protein content of the experimental fish showed significant difference (P<0.05), the CRD diet, DVI had the highest, 44.62±3.15, while diets DIII, DIV and DV followed, 42.29±3.64, 41.71±1.82, and 41.12±6.13 respectively. Diet DI was the least, (35.75±0.90).

DISCUSSION

The quality and quantity of protein in fish diet is of paramount importance because it plays crucial role in influencing the growth rate of fish, provided all other physiological requirements are satisfied (Sogbesan *et al.,* 2006 and Bake *et al.,* 2015). However, the growth performance and nutrient utilization as shown in Table 4 indicated that *Clarobranchus* fingerlings fed DI and DII gave a better growth performance and nutrient utilization than the other treatments even the commercial catfish reference diet (CRD). This may likely be due to the high protein content of the Danish fishmeal used in the diet DI and DII. Although generally, all the fish fed the experimental diets, accepted the feeds, acceptability of the diets however differed among the treatments. This agrees with earlier reports (Riche *et al.,* 2001; Riche and Garling Jr., 2004; Ahmad, 2008), suggested that when alternative protein sources are used in fish diet, palatability and attractiveness of the diets are usually affected. As suggested by Watanabe *et al*., (1987) and quoted by Bake *et al.,* (2015), proper utilization of dietary protein is dependent on the good quality or amino acid balance of the protein sources.

The mean initial weight of all the experimental fish were not significantly different indicated homogeneous group distribution of the fingerlings fish stocked. It is observed from the study that 25 % inclusion of SCM attained best growth while 100 % inclusion level of SCM exhibited least growth response. In the present study, there was no feed rejection during the experimental period, the suitability of inclusion of alternative ingredient in a fish diet in terms of growth performance and nutrient utilization has been reported to vary highly among fish species and experimental conditions (El-Sayed, 1999; Bake *et al.,* 2013; Bake *et al.,* 2015). From the present study, the percentage survival rate was good among all the treatments, and this could be attributed to good handling, good water quality management, proper processing and the suitability of SCM meal inclusion in the diet of *Clarobranchus*, with the least being (83.33%) and (95.00%) was the highest.

Within the condition of this experiment, shea caterpillar meal (*Cirina butyrospermi)* was an acceptable source of animal protein in the diet of *Clarobranchus.* The fishes adapted to the diets within 2 – 4 days of commencement of the trial though, acceptability of the diet varied. The health and survival of fish has often been reported in term of relationship between the

weight and length increases (Onyi *et al.,* 2013). The low growth response by experimental fish fed diet DV, 50 % inclusion level of SCM could be as a result of low feed intake. The best weight gain and growth response were obtained by fish fed 0 %, 25 % inclusion level of SCM, the weight gain of fingerlings is usually a reliable indicator of nutritional adequacy of the diet (Cho and Bureau, 2000) and there were significant differences between fish fed. The result on the survival rate, indicated that, all the treatment performed well though, there were significant differences among the treatment.

This study has demonstrated that the inclusion of shea caterpillar up to 75 % has significant effect on the growth of *Clarobranchus* fingerlings, hence can replace fishmeal thereby improve its growth performance and feed efficiency. This is in an agreement with suggestion by various authors that the synergetic effect of combination of different protein sources perform better than single protein source in fish diets (ugwumba *et al.,* 2001 and Sogbesan *et al.,* 2005).

CONCLUSION

In conclusion, it can be deduced from the study that shea carterpillar meal would replace fishmeal in the diet of hybrid catfish *Clarobranchus* up to 75 % without any adverse effects on its growth and body compositions.

RECOMMENDATION

i. 25 % inclusions level of Shea caterpillar *Cirina butyrospermi* is recommended for the diet of hybrid catfish *Clarobranchus* in terms of growth performance.

ii. More research should be carried out on the cultivation and conservation of shea caterpillar to make it readily available at all time and avoidable in price.

iii. Further work on utilization of *Cirina butyrospermi* meal as a lipid source for better growth and economy sustenance of aquaculture is recommended.

iv. This type of research should be conducted on other available cultivable species of fish in aquaculture.

Furthermore, the increased use of insects as food and feed is expected to require more volume than can be harvested from nature. Therefore, farming the insects as mini-livestock is advisable. The high environmental impacts connected with meat production and the increase in demand up till 2050 require dietary changes. Insect-based substitutes as protein source are potentially more sustainable but require more advanced cultivation and processing techniques (Smetana *et al.,*2015). Such advancement is expected as the whole sector of insects as food and feed is just emerging. In comparison to current production practices, this potential abundant food source can contribute to a more sustainable food and feed production, as certain insects can be reared on organic side streams, including manure. However, food and feed safety issues need to be considered. Insect production has great potential with respect to sustainably providing food for the growing population. However, further technological development of this sector and monitoring of the effects of these developments on the environmental impact of insect production are needed.

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Phytochemical Constituents of *Lannae bateri* Leaves Extract and Evaluation of its Effect on the Biochemical Parameters of Guinea Pig

Ejoba R.

Department of Biochemistry, Faculty of Natural Sciences, Kogi State University Anyigba, Nigeria, PMB 1008

*Corresponding author: gideon4.ayeni@gmail.com; Tel: +234-806765704; +234-7068317885

ABSTRACT

The plant, *lannea bateri* had been used for the treatment of diseases locally among inhabitants of Africa's Sub-continents, particularly in the treatment of epilepsy, paralysis with no empirical validation. However, a comprehensive knowledge of the phytochemical constituents of this plant mediating the acclaimed bioactivities in both *in vitro* and *in vivo* experiment have not been fully elucidated. The aim of the present study is to elucidate the phytochemical constituents of the plant's crude extract and carry out biochemical evaluation upon administration, on serum liver parameters in a model employing guinea pig. Phytochemical analysis of *L. bateri* crude extract showed the presence of alkaloids, flavonoids, steroids, tannins and saponins at a varying proportions. Assessment of the plant's crude extract on animal model, attenuate the activities of the liver marker enzymes, alkaline phosphatase (ALP), aspartate and alanine amino transaminases (AST & ALT), respectively. The results showed a significant ($p < 0.05$) reduction in the activities of enzymatic parameters compared with the reference control animal group, under the condition of the investigation. In the same vein, administration of the crude extract of *L. bateri* were found to maintain the intracellular concentration of potassium, sodium and chloride ions. To this end, the reporting data however, has given adequate credence for the cautious application of indigenous medicinal plants in the treatment of various liver related ailments.

Keywords: Transaminases, *Lannae bateri*, *in vitro*, Phytochemistry, Administration.

INTRODUCTION

Medicinal plants had been known and used since the ancient era. A lot of herbal plants including the species *Lannea bateri* were used in the treatment of diverse ailments (Adewumi et al, 2001; Odugbemi and Akinsolire, 2016). Eventually this metamorphosed to the modern medicine, where drugs, injections and surgery were now used in the treatment of human and animal diseases (Evans, 1996). *Lannea bateri* belongs to the family of Anacardiacea and it is a deciduous tree, that grow within 5 – 18m tall. The leaves of this plant had been used in the treatment of epilepsy, convulsion paralysis, ulcers, haemophoids etc (Garba et al, 2015). The aim of the research work is to determine its phytochemical constituent as well as establishing the biochemical basis of its healing properties.

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METHODOLOGY

Preparation of plant extract

The leaves of the plant *Lannea bateri* was extracted using cold ethanol (80%). The leaves (50 g of blended sample) were macerated in 200 mL of ethanol (80%) for a period of 48 hours. The extract was filtered and evaporated to dryness. The residue left was 28.5 g. This was dissolves in water.

Phytochemical Screening of the Ethanolic Extract of *Lannea bateri* leaves

Phytochemical screening of the plant extract for the presence of bioactives agents such as Alkaloids, Tannins, Saponins, Terpenoids, Flavonoid and Steroids were carried out to examine their presence in accordance to the methodologies of Harborne (1998), Sofowora (1991) and El-Olemyl *et al.,* (1994)

Phytochemical Screening

Akaloids

0.2 g of the extract was warmed with 2% H_2SO_4 for two minutes and filterd. A few drops of dragon doff reagent was added. A red precipitate indicate the presence of alkaloids.

Terpenoids

0.2 mL of the extract was mixed with 2.0mL of chloroform and conc. H_2SO_4 was gently added. A reddish brown colouration indicate the presence of terpenoids

Tannins

A little quantity of the extract was mixed with water and heated on water bath and filtered. A few drops of ferric chloride was added. A dark green solution indicates tannins.

Saponins

0.2 g of the plant extract was shaken with 5.0 mL of distilled water and heated to boil. Frothing, bubbles and persistent foaming indicates the presence of saponins.

Steroids

0.2 mL of acetic acid was added to 0.5 g of the extract and then 2 mL of H_2SO_4. Colour change from violet to blue or green indicating the presence of steroids.

Flavonoids

0.2 g of the extract was dissolved in NaOH and HCl was added. A yellow precipitate indicate the presence of flavonoids.

Extract Administration

Twenty Guinea pigs of both sexes were divided into a group of four (**A, B, C, D**) containing pigs. Group **A** serves as control group, while group **B, C** and **D** were treated with 200 mg/kg body weight, 400 mg/kg body weight and 600 mg/kg body weight, respectively for a period of 28 days. The Guinea pigs were sacrificed and the blood were taken. The serum electrolytes (K^+, Na^+ and Cl^-) was determined by using a protocol of Krieg, Gunsser and Becker (1986).

and the serum enzymes; Aspartate aminotransferase (AST), Assay for Alanine Aminotransferase **(**ALT) and Assay for Alkaline Phosphatase (ALP) were assayed using a reported methods of Henry (1974) and Roy (1970).

Assayed for Serum Electrolytes

Potassium (K^+)

Serum potassium ion (K^+) is determined using sodium tetraphic nylboron. This produced a colloidal suspension. The turbidity is proportional to the K^+ concentration.

Sodium (Na^+)

Serum sodium ion (Na^+) is precipitated as triple salt when treated with a ferricyanide to produce a chromophore whose absorbance is inversely proportional to the concentration of Na^+ present.

Chloride (Cl^-)

Serum chloride ion (Cl^-) react with mercuric ions. The Cl^- displaced the thiocyanate ions from the mercuric ions. The thiocynate ions react with ferric ions producing a colour complex which absorbance is taken at 480 nm. The intensity of the colour is directly proportional to the Cl^-.

(V) Assays for Serum Enzymes

(a) Aspartate aminotransferase (AST)

The method of Henry (1974) was adopted. The enzyme catalyses the reaction of Aspartate and oxoglutarate to produce oxaloacetate and glutamate. The oxaloacetate formed is reacted with NADH to yield NAD^+ by an oxidation reaction with enzyme lactate dehydrogenate with activity taken at 340 nm.

(b) Assay for Alkaline Phosphatase (ALP)

The method of Roy (1970) was adopted. The enzyme is acted upon by Amp-buffered sodium thymopthalein monophosphate. This stops the enzyme activity and a blue chromogen is produced which is measured photo metrically.

(c) Assay for Alanine Aminotransferase (ALT)

The method of Henry (1974), was adopted. The enzyme react with oxoglutarate to produce pyruvate and glutamate. The pyruvate is converted to lactate by lactate dehydrogenate converting NADH to NaD^+. The rate of oxidation of NADH taken at 340 nm to give the enzyme activity.

Statistical Analysis

The data were represented in mean ± standard deviation. Results were statistically analysed by one way analysis of variance (ANOVA) using Instat Software, Bonferroni multiple comparison test, Graph Pad Instat Software (San Diego, USA). A p-value less than 0.05 ($P < 0.05$) was considered significant.

RESULTS AND DISCUSSION

Table 1: Result of Phytochemical Screening of *Lannea bateri*

Test	Inference
Terpenoids	–
Alkaloids	+++
Tannins	+
Saponins	+
Flavonoids	+++
Steroids	++

Key: Absent = (–), Moderate = (+), Abundant = (++), High concentration = (+++), Excessive concentration= (++++)

Table 2: Result of serum electrolytes

Group	Potassium (K^+)	Sodium (Na^+)	Chloride (Cl^-)
A (Control)	5.85±2.40[a]	216.50±41.80[a]	26.70±31.50[a]
B (200 mg/kg bw)	7.20±1.80[a]	214.30±42.60[b]	62.40±29.70[a]
C (400 mg/kg bw)	5.90±2.65[a]	198.60±50.65[a]	80.30±20.60[b]
D (600 mg/kg bw)	9.25±3.25[b]	180.30±25.30[b]	92.45±36.30[b]

Value expressed in mean ± Standard deviation (SD) of four replicates (n=4). [a, b] Different alphabets over the bars for a given concentration are significantly different from each other ($p < 0.05$).

Table 3: Results of serum enzymes

Group	AST (mg/dl)	ALP (mg/dl)	ALT (mg/dl)
A (Control)	275.60±80.26[a]	120.65±6.40[a]	22.85±5.30[a]
B (200mg/kg bw)	350.40±120.67[b]	128.75±23.60[a]	35.40±7.85[b]
C (400mg/kg bw)	286.65±30.75[a]	136.40±40.30[b]	23.20±10.80[b]
D (600mg/kg bw)	348.40±41.85[b]	155.55±14.60[b]	28.56±12.50[b]

Value expressed in mean ± Standard deviation (SD) of four replicates (n=4). [a, b] Different alphabets over the bars for a given concentration are significantly different from each other ($p < 0.05$).

DISCUSSION

The result of the phytochemical screening indicates high concentration of flavonoids and alkaloids in the plant extract *(L. bateri*). These bioactive agents made the plant important and may be implicated in the treatment of disease such as ulcer, antioxidant, paralysis, epilepsy, convulsion, etc (Cai and Sun, 2003, Anderson and Teuber, 2001, Madziga *et al*, 2010, Fin, 2010). The serum electrolytes, potassium (K^+), Sodium (Na^+) and chloride (Cl^-), showed a significant different at ($p<0.05$) of the control and the treated groups of the Guinea pigs.

Regarding the observed serum electrolytes (Na^+, K^+ and Cl^-) evaluated, elevated levels of the serum electrolytes particularly, Na^+ and K^+ being, a major components of extracellular and intracellular fluids, depicts renal compromised function when considering the roles of tubular and glomerular in a normal kidney (Ibrahim, 2001). Therefore high serum level of Cl^- in renal regulation are thought to be index of electrical neutrality an inverse relationship between Cl^- and HCO_3^- (Ibrahim 2001). The extract showed significant ($p < 0.05$) reduction in serum concentration of alanine aminotransferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP) as compared to the control groups of Guinea pigs. The extract from

the plant *L. bateri* is important for these enzymes.

CONCLUSION

Lannea bateri possessed relevant phytochemicals of diverse classess. The Crude extract demonstrated promising ameliorative effect through the attenuating activities of the liver marker enzymes and serum electrolytes.

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Conflict of Interest

Authors declare no conflicting interest arising from this study.

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In vitro antioxidant properties of free and bound phenolic extract of *Celosia argentea, Corchorus olitorius, Amaranthus hydridus* and *Jatropha tajorensis*

[*1]Innalegwu D. A., [1,2]Evans E. C., [1]Gara T. Y. & [1]Muhammad F. M.

[1]Department of Biochemistry, Federal University of Technology, P.M.B. 65, Minna, Niger State, Nigeria.
[2]Centre for Genetic Engineering and Biotechnology, Federal University of Technology, P.M.B. 65, Minna, Niger State, Nigeria

[*]Corresponding author: a.daniel@futminna.edu.ng; +2347032867209

ABSTRACT

Polyphenols have attracted a lot of interest recently due to their antioxidant property. The present study is aimed at investigating the antioxidant properties of free and bound phenols of methanol leaf extracts of *Celosia argentea, Corchorus olitorius, Amaranthus hydridus* and *Jatropha tajorensis*. Total flavonoids and phenolic contents of the extracts was determined using spectrophotometric method while the antioxidant activity of crude extract, free and bound phenols (FP and BP) was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. The flavonoid contents ranged between 35.90±4.89 mg/100g in *C. olitorius* to 292.67±8.39 mg/100g in *A. hydride* while the phenolic contents ranged from 264.43±4.47 mg/100g in *J. tajorensis* to 431.98±4.90 mg/100g in *C. argentea*. The DPPH scavenging activity of the crude, free and bound phenolic extracts of the extratcs gave an IC_{50} of 383.52±1.05, 114.66±3.24 and 279.06±4.51 µg/mL for crude, FP and BP for *J. tajorensis* respectively, 331.29±1.33, 109.74±3.86 and 195.89±5.12 µg/mL for crude, FP and BP of *C. argentea* respectively, 379.46±3.11, 180.34±3.12 and 227.50±4.34 µg/mL for crude, FP and BP of *C. olitorius* respectively and 136.34±2.05, 135.47±1.88, 193.95±3.56 µg/mL for *A. hydridus* respectively which are significantly higher ($p<0.05$) with the standard (Ascorbic acid) with an IC_{50} of 12.66±3.33 µg/mL. From the result obtained, it is rational to attribute the wide usage of these vegetables in folkloric medicine to its high phenolic content. Hence, information from this study could be exploited in the global fight against degenerative diseases, whose etiology has been linked to oxidative stress.

Keywords: Polyphenols, antioxidants, Free phenols, Bound phenols, Degenerative diseases

INTRODUCTION

Polyphenols are a wide and complex group of secondary plant metabolites essential for the physiology of plants, having functions in growth, structure, pigmentation, pollination, allelopathy, and resistance for pathogens and predators (Manach *et al.,* 2004). Polyphenols have attracted the interest of researchers because of their antioxidant capacities. They have long been recognized to possess anti-allergic, anti-inflammatory, antiviral and anti-proliferative, anticarcinogenic and antioxidant properties (Atansuyi *et al.,* 2012). Reports have shown that

Innalegwu D. A., Evans E. C., Gara T. Y. & Muhammad F. M. (2021). *In vitro* antioxidant properties of free and bound phenolic extract of *Celosia argentea, Corchorus olitorius, Amaranthus hydridus* and *Jatropha tajorensis*. 2nd Faculty of Natural Sciences Annual Conference. IBB University Lapai held between 30th August to 2nd September 2021. Pp 96-106

there is an inverse relationship between the intake of flavonoids and the risk of coronary heart disease, lung cancer (Atansuyi *et al.,* 2012) and stomach cancer (Garcia-Closas *et al.,* 1999).

According to Nathan AND Ding (2010), free radicals and other small reactive molecules are important regulators of many physiological and pathological processes. Increased levels of these reactive molecules can cause oxidative damage to biological macromolecules and disrupt the cellular reduction–oxidation (redox) balance (Dowling and Simmons, 2009). Yoshihara *et al.,* 2010 have reported that oxidative stress caused by the accumulation of free radicals in the body has been responsible for many illnesses including cardiovascular diseases, cancer, neurodegenerative disorders, and aging.

In view of the recognition of the potent antioxidant properties of polyphenols, researchers have been tailoring their efforts towards identifying plants polyphenols with potent antioxidant properties that could be exploited for the management of degenerative diseases.

Green leafy vegetables (GLVs) are micronutrient dense nature's gift to mankind that provide more vitamins per mouthful than any other food (Anchalsingh, and Andnitisha, 2014). They are said to be rich sources of calcium, iron, β-carotene, vitamin C, dietary fibre and many trace minerals (Anchalsingh, and Andnitisha, 2014). It has been reported that some GLV contain immense varieties of bioactive non-nutritive health enhancing factors such as antioxidants, phytochemicals and essential fatty acids (Anchalsingh, and Andnitisha, 2014). This according to ethnomedicinal usage is increasing their recognition of the roles which their phytonutrients content play in the prevention of non-communicable diseases (Anchalsingh, and Andnitisha, 2014; Doughari *et al.,* 2009). Various studies reported the presence of abundant phenolic compounds such as quercetin, kaempferol and acacetin in GLVs (Anchalsingh, and Andnitisha, 2014).

Amaranth hybridus is a gluten-free pseudocereal thrives in all temperate-tropical areas of the world particularly in Mexico and South America (Rastogi *et al*., 2013). Moreover, in certain regions of the world, such as eastern Africa, amaranth leaves are consumed as a vegetable because it is a fast-growing plant available most of the year (Karamac *et al*., 2019). There has been an increase renewed interest in this ancient and highly nutritious food crop due to the excellent nutritional value of seed and leaves (Venskutonis *et al.,* 2013). Amaranth proteins have a well-balanced amino acid composition (Karamac *et al*., 2019), high bioavailability (Venskutonis *et al.,* 2013), and good functional properties (Lopez *et al*., 2019). Dietary fiber, vitamins and precursors of vitamins (ascorbic acid, riboflavin, tocols, carotenoids), as well as minerals (Ca, Fe, Mg, K, Cu, Zn, and Mn) are other important nutrients present in seeds and leaves of amaranth (Karamac *et al.,* 2019). Their contents are high compared to these in some cereals and green leafy vegetables (Karamac *et al*., 2019).

Celosia argentea L. is an herbaceous plant which belongs to the family *Amaranthacaceae* and one of the leading leaf vegetables in south-western Nigeria, where it is known as sokoyòkòtò' in Yoruba language meaning make husbands fat and happy (Kanu *et al.,* 2017). It is also known as red soko 'because it has red pigment on the leaves which differentiates it from the green soko'(Malomo *et al.,* 2011). *C. argentea* impact the anthocyanin- red colour into soup, making it less popular than green (Kanu *et al.,* 2017). The

entire plant is used in treatment of ulcers, piles, bleeding nose, inflammation, gynecologic disorders, mouth sores, eye diseases, glandular swellings, eczema, constipation and as an aphrodisiac (Rub *et al.,* 2015). The seeds are used in the treatment of blood diseases, diarrhea and the roots are well known for their anti-diabetic activity (Kanu *et al.,* 2017). The in vitro and in vivo antioxidant activity of the plant is reported to be due to abundance of phenolics making *Celosia argentea* as a potential source of cheap, natural antioxidants (Rub *et al.,* 2015).

Jatropha tanjorensis is a perennial herb, a member of the *Euphorbiaceae* family, commonly called 'hospital too far', catholic vegetable, Jatropha. It is used locally in soups as well as medicinally in treating anaemia, skin disease, malaria fever (Oduola *et al*., 2005; Omoregie and Osagie, 2011). Meanwhile, *J. tanjorensis* has received a lot of attention due to its potential health benefits, availability and affordability (Omoregie and Osagie, 2007; Omobuwajo *et al*., 2011). *J. tanjorensis* have also been shown to exhibit antibacterial activity (Iwalewa et al., 2005). In fact, earlier reports have shown that *J. tanjorensis* is rich in antioxidant nutrients like phosphorus, selenium, zinc and vitamins C and E (Omobuwajo *et al.,* 2011).

Corchorus olitorius (malvaceae), is a plant native to both tropical and subtropical regions throughout the world with mallow leaves commonly consumed as a leafy vegetable (Adedosu *et al.,* 2015). The leaves are used in ethnomedical practices to treat ache and pain, dysentery, malaria, enteritis, fever, gonorrhea, pectoral pains and tumors (Abdul Sadat *et al.,* 2017). Hence this research is aim at investigating the antioxidant activity of free and bound phenols in methanol extract of *Celosia argentea, Corchorus olitorius, Amaranthus hydridus* and *Jatropha tajorensis*.

Materials and Methods

Materials

Fresh leaves of *Amaranthus hybridus, Celosia argentea, Jatropha tajorensis* and *Corchorus olitorius* were obtained from Kure market in Minna, Niger State, Nigeria. The leaves were washed, and dried at room temperature for 7 days at the Biochemistry Laboratory of Federal University of Technology, Minna, Niger State**.** All chemicals and reagents used in this study are of analytical grade and were obtained from NAHSON Chemicals, Minna, Niger State.

Plant Processing and Extraction

The dried leaves were pulverized into powder using kitchen type blender to obtained a fine powder. Fifty grams (50 g) each of the powdered sample was extracted exhaustively with 20 mL of methanol for 2 hours at 45^0C using reflux extractor. The extract was filtered using muslin cloth and further filtration with filter paper to obtain a fine filtrate (Kabiru *et al.,* 2012). The filtrate was dried at a reduced temperature of 40^0C using water bath to obtain a semi-solid paste. The yield of the extract was calculated using the formula below:

$$\%\, Yield = \frac{Weight\ of\ extract}{Weight\ of\ dry\ sample}\ X\ 100$$

……. Equation (1)

Extraction of Free and Bound Phenols (Polyphenols)

The extraction of the soluble free phenols (FP) was carried as reported previously by Chu *et al.*, 2002 and reported by Atansuyi *et al.,* 2012 with minor modifications. Four grams (4 g) of the methanol extract was solubilized in methanol-water (80:20, v/v), sonicated and homogenized

at room temperature for 1 h 30 min. The solution was filtered through Whatman filter paper, using a Buchner funnel under vacuum. The filtrate was then evaporated using a rotary evaporator under vacuum at 40°C to obtain the FP extract. On the other hand, bound phenols (BP) was extracted according to the method of Krygier *et al.,* 1982 as reported by Atansuyi *et al.*, 2012 with slight modifications. Briefly, residues recovered from the extraction of FP was dried and hydrolyzed with 4 M NaOH at room temperature under shaking. The mixture was acidified to pH 2 with concentrated HCl, extracted four times with ethyl acetate, pooled together and evaporated at 40°C to dryness under vacuum to yield BP extract. The yield of extraction was calculated using equation (1) above.

Determination of total phenol content

The total phenol content of the extracts was determined according to Singleton *et al.,* (1999) using gallic acid as standard. Zero-point five milliliter (0.5 mL) of 1 mg/mL of crude methanol extract, FP and BP were oxidized with 2.5 ml of 10% Folin-Ciocalteau's reagent (v/v) and neutralized by 2.0 ml of 7.5% sodium carbonate. The reaction mixture was incubated for 40 min at 45°C and the absorbance was measured at 765 nm. The amount of phenol present in the extracts was expressed as gallic acid equivalents (GAE).

Determination of total flavonoid content

The total flavonoid content of the extracts were determined as reported by Meda *et al.* (2005) with slight modification. Zero-point five milliliter (0.5 mL) of 1 mg/mL of crude methanol extract, FP and BP were mixed each with 0.5 mL methanol, 50 µL of 10% $AlCl_3$, 50 µL of 1 M potassium acetate and 1.4 mL water and allowed to incubate at room temperature for 30 min. Thereafter, the absorbance of the reaction mixture was subsequently measured at 415 nm and the total flavonoid was calculated using quercetin as standard, and expressed as quercetin equivalent (QE).

Antioxidant assay using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging ability

The free radical scavenging ability of the extracts against DPPH radical was evaluated as described by Gyamfi *et al.* (1999). Briefly, an appropriate dilution (50 – 250 µg/mL) of the crude, FP and BP (1 ml) was mixed with 1 mL of 0.4 mM methanolic solution containing DPPH radicals. The mixture was left in the dark for 30 min and the absorbance was measured at 516 nm. The DPPH free radical scavenging ability was subsequently calculated with respect to the reference (which contains all the reagents without the test sample).

RESULTS AND DISCUSSION

Results

Table 1: Percentage yield of crude methanol extract of the vegetables

Extracts	% yield
C. argentea	20.40
C. olitorius	18.00
A. hybridus	15.00
J. tajorensis	18.40

Table 2: Total flavonoids and phenolics contents (mg/100g) of plant extracts.

Samples	Phenols	Flavonoids
C. argentea	431.98±4.90[d]	159.75±1.41[b]
C. olitorius	269.68±11.34[a]	35.90±4.89[a]
A. hybridus	407.05±3.41[b]	292.67±8.39[c]
J. tajorensis	264.43±4.47[a]	165.40±7.06[b]

Values are expressed in mean ± standard error of mean of triplicate determination
Values are present in mean ± standard error of mean. Values with same superscript on the same column have no significant difference at p<0.05

Table 3: IC_{50} of methanol, free and bound phenolic extracts of the four selected vegetables

Extracts	MeOH Extracts	Free Phenols	Bound Phenols
C. argentea	331.29±1.33[c]	109.74±3.86[a]	195.89±5.15[b]
C. olitoius	379.46±3.11[c]	180.34±3.12[a]	227.50±4.34[c]
A. hybridus	136.34±2.05[a]	135.47±1.88[a]	193.95±3.56[b]
J. tajorensis	383.52±1.05[c]	114.66±3.24[a]	279.06±4.51[b]
Ascorbic acid	12.66±3.33*		

Key: MeOH = Methanol
Values are present in mean ± standard error of mean. Values with same superscript on the same row have no significant difference at p<0.05

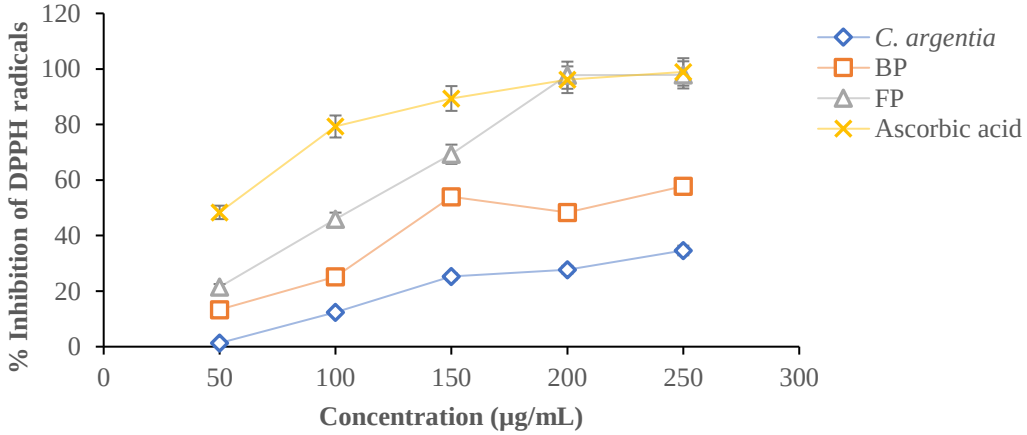

Figure 1: DPPH radical scavenging activity of free phenol (FB) and bound phenol (BP) of methanol extract of *C. argentea.*

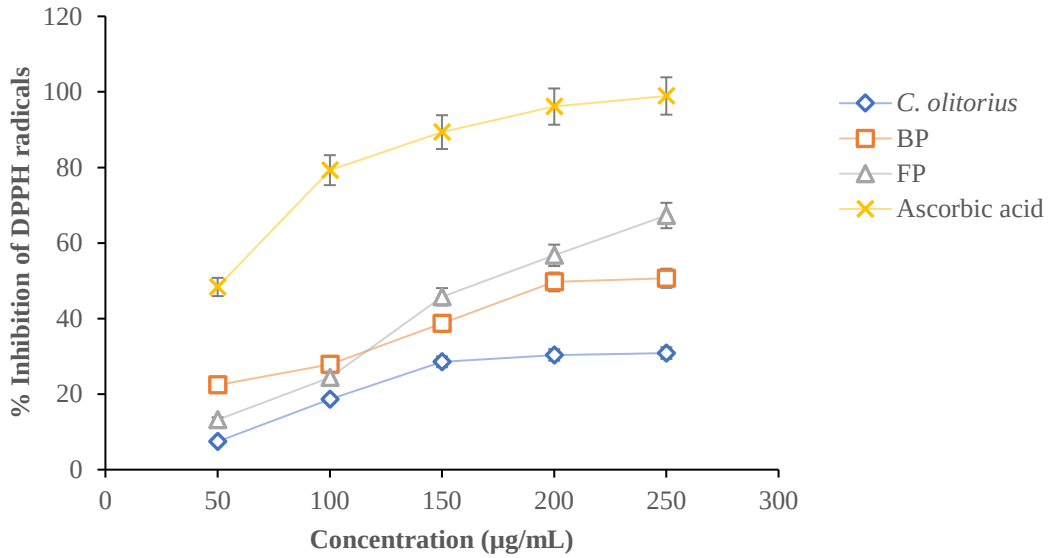

Figure 2: DPPH scavenging activity of free phenol (FB) and bound phenol (BP) of methanol extract of *C. olitorius*

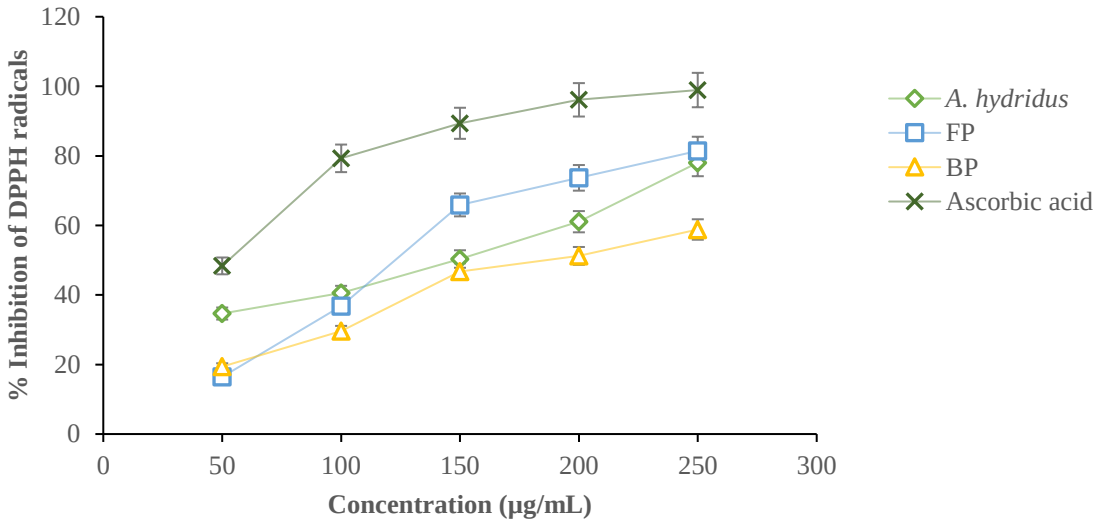

Figure 3: DPPH scavenging activity of free phenol (FB) and bound phenol (BP) of methanol extract of *A. hybridus*

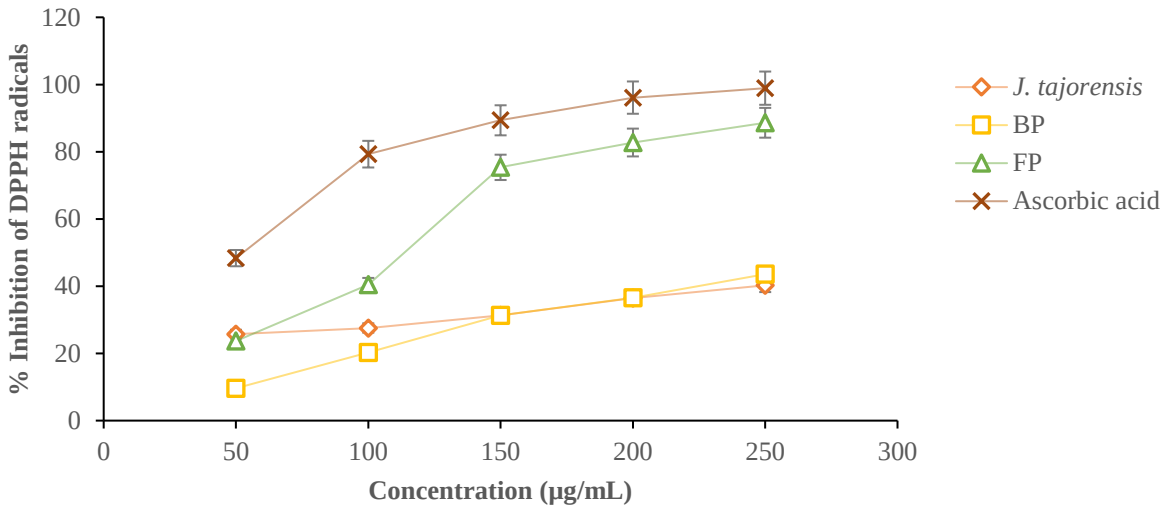

Figure 4: DPPH scavenging activity of free phenol (FB) and bound phenol (BP) of methanol extract of *J. tajorensis*

DISCUSSION

Globally, interest in natural product that could ameliorate the menace of oxidative stress has been on the rise due to cost and unavailability of conventional synthetic drugs (Manach *et al.,* 2004). Hence, efforts are now being tailored at discovering plants with potent antioxidant properties which could be harnessed and exploited for therapeutic purposes. Antioxidant properties of plants are intricately related to their phytochemicals (Atansuyi *et al.,* 2012; Doughari *et al.,* 2009). Recently, these bioactive substances, especially the polyphenols, have been found to be responsible for the antioxidant properties of plants (Omobuwajo *et al.,* 2011). Hence, an increased interest in the isolation of the polyphenolic components of plants that could be used in the management of degenerative diseases.

The % yield of methanol extract of *C. argentea, C. olitorius, A. hybridus* and *J. tajorensis.* Highest yield (20.40 %) was recorded in *C. argentea* followed closely by *C. olitorius* and *J. tajorensis* with 18.00 and 18.40 % respectively while *A. hybridus* recorded the least yield (15.00 %) (Table 1). Yield of extract play a major role in the choice of plant and is affected by factors such as time/season of collection, place of collection, and the species of plant. Hence the reason for the variation in the yield recorded above.

Table 2 shows the phenolics and flavonoids contents of the extract. *C. argentea* contain the highest amount of phenols (4.31.98±4.90 mg/100) followed by 407.05±3.41, 269.68±11.34 and 264.43±4.47 mg/100g for *A. hybridus, C. olitorius* and *J. tajorensis* respectively. High flavonoids content (292.67±8.39 mg/100g) was also recorded in *A. hybridus* followed by *J. tajorensis, C. argentea* and *C. olitorius* with flavonoids content of 165.40±7.06, 159.75±1.41 and 35.90±4.89 mg/100g respectively. The amount of phenols and flavonoids content of these plants justify their wide usage particularly among the Yoruba's of the South-Western part of Nigeria (Alegbejo, 2013)**.** This may also explain the rationale behind their widespread usage in folkloric medicines for the treatment of ailments. However, it is not enough to know

what is responsible for the pharmacopotency of these leaves, without unraveling the mechanism involved in their therapeutic effects. Hence, we tested the *in vitro* antioxidant properties of free phenols (FP) and bound phenols (BP) of extracts of *Amaranthus hybridus, Celosia argentea, Jatropha tajorensis* and *Corchorus olitorius* with a view of gaining an insight into the mechanism(s) involved in their antioxidant action.

The DPPH radical scavenging activity has been extensively used for screening antioxidants ranging from fruits, cereals and vegetable juices or extracts (Ayoola *et al.,* 2006). Therefore, the ability of the FP and BP extracts to scavenge DPPH radicals were investigated and presented in Table 3 and Figure 1–4. DPPH is an unstable diamagnetic molecule that attains stability through protonation. This stability is visually noticeable by an abrupt discoloration from purple to golden yellow. Antioxidant potentials of plant extracts is judged based on their median inhibition concentration (IC_{50}). According to Blois, 1958 classification of IC_{50} as reported by (Fidrianny *et al.,* 2014), extracts with an IC_{50} <50 µg/mL is said to be a very strong antioxidant, 50-100 µg/mL as strong antioxidant, 101-150 µg/mL as medium antioxidant, while extracts with IC_{50} >150 µg/mL as a weak antioxidant (Fidrianny *et al.,* 2014). Based on this classification, free phenolic extract of *C. argentea, A. hybridus* and *J. tajorensis* are classified as medium antioxidant with an IC_{50} of 109.74±3.86, 135.47±1.88 and 114.66±3.24 µg/mL respectively while free phenolic extract *C. olitoius* is a weak antioxidant with IC_{50} of 180.34±3.12 µg/mL. Methanol and bound phenolic extracts of the plant shows a weak inhibition of the DPPH radicals with IC_{50} value > 150 µg/mL (Table 3). Although the reason behind this observation is not completely understood, it is logical to speculate that the amount of phenols and flavonoids present in these extracts maybe responsible for their antioxidant activity which have been reported to be a natural antioxidant in plants (Doughari *et al.,* 2009; Fidrianny *et al.,* 2014). Furthermore, the observation further justifies the widely speculated report that one of the mechanisms of antioxidant activity of polyphenols is through radical scavenging (Ayoola *et al.,* 2006). Phytochemicals can act as antioxidants by protecting cell membrane and cellular oxidative processes from damages that may give rise to diseases (Atansuyi *et al.,* 2012). Hence, antioxidants are usually assessed by their ability to offer protective shields to lipids intentionally assaulted with peroxidants.

CONCLUSION

Phytochemical screening of methanol extracts of *C. argentea, C. olitorius, A. hybridus* and *J. tajorensis* shows that phenols was higher in all the extract than flavonoids. The antioxidant activity also shows that free phenols have lower IC_{50} than the bound phenols hence high antioxidant capacity. The extracts of these plants can be a good source of antioxidants for the management of oxidative stress and other related diseases.

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Characterization of Zinc Oxide (ZnO) Nanoparticles Synthesized by Thermal Treatment Method

***Gene A. S., Gomina M., Salihu A. S., Musa H. I. & Muhammed H.**

Department of Physics, Ibrahim Badamasi Babangida University Lapai, Nigeria.

*Corresponding author: genesalahudeen@gmail.com, Phone number: +2348120557889

ABSTRACT

The present study reports the particle size distribution, physical and optical properties of zinc oxide nanoparticles (ZnO NPs) synthesized by thermal treatment method. The samples were calcined at different temperatures in the range of 450 to 650 ^{o}C. Local starch was used as capping agent to control the agglomeration of the nanoparticles. The particle size distribution of the synthesized nanoparticles was analyzed by Zeta size analyzer which shows that the particle sizes increase from 100 nm at 450 ^{o}C to 1000 nm at 650 ^{o}C. The optical properties of the synthesized nanoparticles were studied using UV-vis absorbance spectroscopy, where Blue shift in wavelength of the absorption peaks was observed, which implies an increase in particle sizes as calcination temperature increases. Raman spectroscopy using Nd: YAG at 599 nm confirmed the presence of ZnO nanocrystals for all the samples calcined at temperature 450, 550 and 650 ^{o}C, and also confirmed the increase of crystal sizes as the calcination temperature increases.

***Keywords*: Synthesis, characterization, ZnO-Nanoparticles, thermal method**

INTRODUCTION

Nanomaterials are attractive subjects of continuous scientific interest and have been deeply investigated in materials sciences, because of their physio-chemical properties and their wide range of applications as a humidity sensor (Bayhan and Kavaso, 2006), semiconductors (Honeybourne and Rasheed, 1996), magnetic materials (Bid and Pradhan, 2003), catalysts (Gabr *et al.,*1992), super hard materials (Bayhan *et al.,* 1997), high temperature ceramics (Hulbert *et al*., 2007), among others. In particular, zinc oxide (ZnO) is commonly used as catalytic materials (Gabr *et al.,* 1992), humidity sensors (Bayhan *et al.,* 1997), and magnetic material (Parhi and Manivannan, 2008). Various methods previously reported on the synthesis of ZnO, include mechanical activation (Stanojevic *et al.,* 2007), micro emulsion method (Niu *et al.,* 2004), chemical method (Sparza *et al.,* 2011), microwave method (Parhi and Manivannan, 2008), sol-gel method (Zhang and Chen, 2003), thermal method (Stefanescu *et al*., 2011), solution method (Chandra and Patil, 1992), ultrasonic spray pyrolysis (Marinkovi *et al*., 2001), ball milling method (Marinkovi *et al*., 2005), and combustion method (Bangale and Bamane, 2011). Most of the methods are difficult to employ on a large-scale production due to the complicated procedures, longer reaction times, high reaction temperatures, toxic reagents and by-products involved in these syntheses

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methods (Naseri *et al.,* 2012). The synthesis of ZnO nanoparticles by a thermal treatment method is reported in this study; aqueous solution of zinc nitrates, native starch, and deionized water was prepared. The solution was dried at 70 ^{o}C for 24 h., followed by grinding and calcination at temperatures in the range of 450 to 650 ^{o}C. This method has the advantages of simplicity, cheaper, no unwanted by products, and being environmentally friendly (Naseri *et al*., 2010). In this paper, we have investigated the effect of calcination temperature on the particle size distribution, optical and physical properties of ZnO synthesized by thermal method.

Materials and Methods

Preparation of ZnO nanoparticles

The materials used include: Zinc nitrate ($Zn(NO_3)_2{\cdot}6H_2O$) salt as metal precursors, native starch for controlling the agglomeration of the nanoparticles, and deionized water as solvent. The $Zn(NO_3)_2{\cdot}6H_2O$ (99%) was used as purchased from Sigma-Aldrich while the native starch was prepared in the laboratory using cassava tubers. 5g of native starch was dissolved in 100 mL of deionized water at 63 ^{o}C, before mixing 0.2 mmol of $Zn(NO_3)_2{\cdot}6H_2O$ into the starch solution. The solution was constantly stirred until a homogeneous solution was formed. The mixture was poured into Petri dishes and placed in an oven for 24 h. to dry at a temperature of 70 ^{o}C. The resulting solid was crushed into powder and calcined at temperatures at 450, 500, 550, 600, and 650 ^{o}C for 3 h.

Characterization of ZnO nanoparticles

The physical properties of the synthesized ZnO nanoparticles was carried out by Raman spectroscopy using Nd: YAG at 599 nm and the average particle size were also determined by Zeta Size Analyzer (ZEN1600). The absorbance spectra were recorded using the UV vis spectrometer (Shimadz-UV1800).

RESULTS AND DISCUSSION

Optical Properties of ZnO by UV-Visible Absorbance Spectroscopy.

The optical absorbance spectra of the synthesized ZnO nanoparticles have been recorded using a SHIMADZU (UV1800). **Figure 1** (a) - (f) shows the absorbance spectra of sample before calcination and the synthesized ZnO nanoparticles calcined at 450, 500, 550, 600, and 650 ^{o}C in the wavelength range of 200–800 nm. The presence of absorption peak at 250 nm in the sample before calcination in **Figure 1**(a) indicates the presence of organic sources form native starch and the incomplete formation of ZnO nanoparticles. The absorption peaks in the range of 290 to 380 nm were observed in **Figure 1** (b) to (f) which implies the formation of zinc oxide nanoparticles (Kortum and Lohr, 2012). A red shift in the absorption peak was also observed in the absorbance spectra of the synthesized ZnO nanoparticles as the calcination temperature increased, the wavelength increased from 299 nm at calcination temperature of 450 ^{o}C in **Figure 1**(b) to 370 nm at calcination temperature of 650 ^{o}C in **Figure 1**(f). These red shift in absorption peaks wavelength implies an increase in particle sizes as the calcination temperature increase.

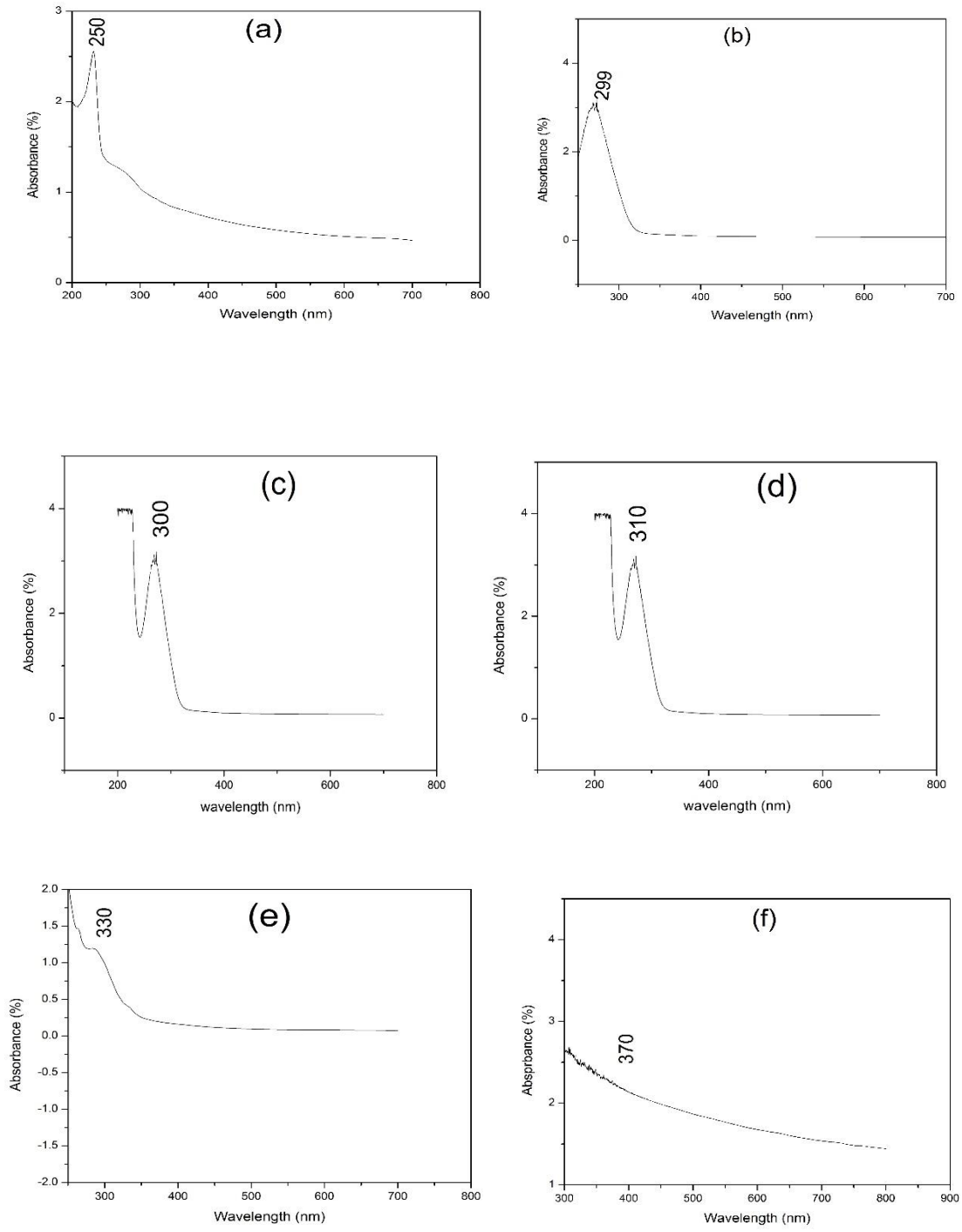


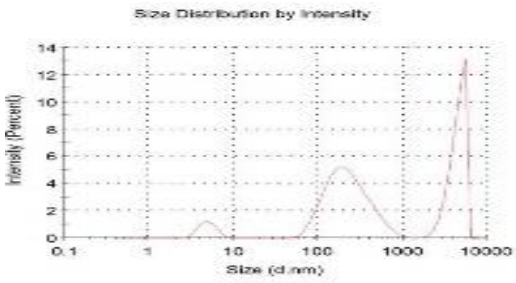
Fig. 1: The UV absorbance spectrum of the calcined samples (a) before calcination and after calcination at temperatures of (b) 450, (c) 500, (d) 550, (e) 600 and (f) 650 °C.

Particles size distribution of ZnO nanoparticles

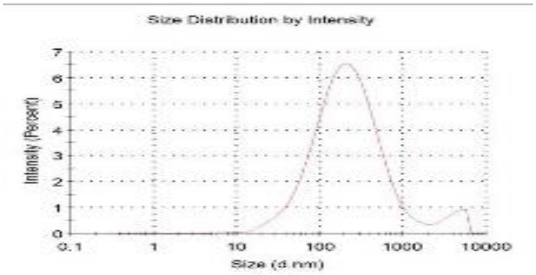
Figure 2 shows the size distribution of ZnO nanoparticles calcined at (a) 450, (b) 550 and (c) 650 ^{o}C. The result indicates that the particle size increased with increasing calcination temperature. **Table 1** shows results of the particle size distribution and it was observed that the particle size increased with the increase in calcination temperature. This suggested that as the temperature increases, several neighboring particles fused together to increase the particle size by the melting of their surfaces (Qu *et al.*, 2006). Although a poor particle size distribution was also observed from **Figure 2** which could be as a result of the native starch used as capping agent compared to previous studies which uses PVP or PVA as capping agents. Therefore, a further study is required in future to study the influence of native starch as capping agent on the particle size distribution of synthesized metal oxide nanoparticles by thermal method.

Table 1: The average particle size of zinc oxide measured by Zeta sizer for samples calcined at 450, 550, and 650^{O}C

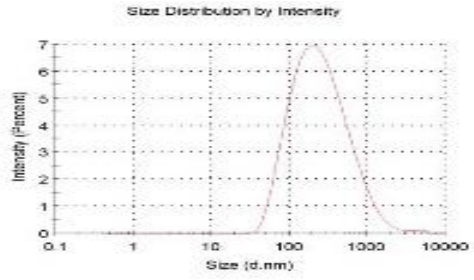
Specimens	Calcination temperature (^{o}C)	Zeta Size Analysis Average particle size (nm)		
		Peak 1	Peak 2	Peak 3
ZnO (1)	450	7	300	3000
ZnO (2)	550	0	300	5000
ZnO (3)	650	0	400	0



(a)



(b)



(c)

Fig. 2: Particle size distribution of samples calcined at (a) 450, (b) 550 and (c) 650 ^{o}C

Raman Spectrum Analysis

The Raman spectroscopy of samples calcined at different temperatures of 450, 550, and 650 °C are shown in **Figure 3**. The calcined samples show the diffraction peaks of 420, 872, and 2876 cm^{-1} respectively. The highest peak corresponding to the blue shift for all nanostructures in comparism with the previous research which confirms the presence of ZnO nanoparticles within all the samples at different calcination temperatures and all the spectra were found to be similar with previous works done by Qu *et al*. (2007), Parhi and Manivannan (2008), Stefanescu *et al*. (2011). The other peaks and their relative shifts are also present, these peaks could be due to organic particle still present in the sample, which is due to incomplete decomposition of the native starch during calcination and poor sample handling. The result showed that as the calcination temperature increased, the diffraction peaks became sharper and narrower and their intensity increased with increase in calcination temperature. This indicates intensification in crystallinity that originates from the increment of crystals size due to the size enlargement of the particles (Naseri *et al*., 2010).

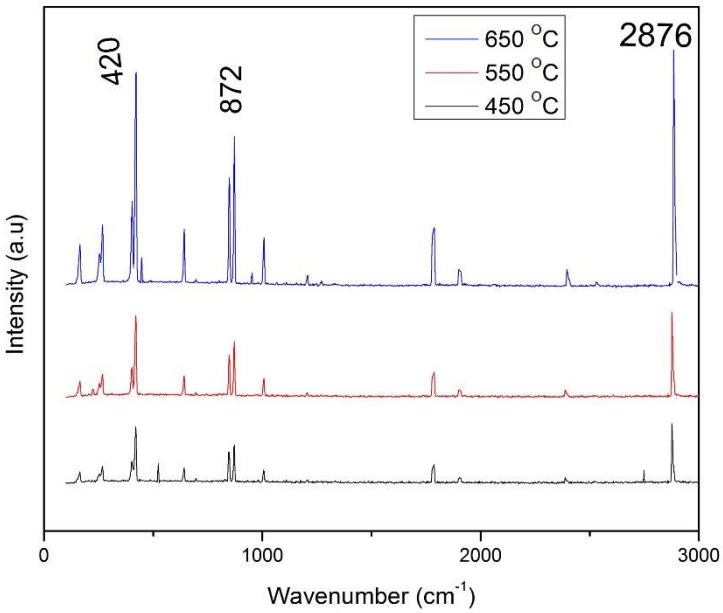


Fig. 3: Raman spectroscopy for ZnO nanoparticles calcined at temperatures of 450, 550 and 650 °C

CONCLUSION

ZnO nanoparticles were successfully synthesized using the thermal method with native starch as a capping agent. Average particle sizes of 300 nm were achieved with calcination temperatures of 450 to 650 °C. The particle sizes were observed to increase with increase in calcination temperature. UV-visible absorption spectroscopy confirmed the existence of zinc oxide at all calcination temperatures. The diffraction peaks of ZnO at 420, 872, and 2876 cm^{-1}…were observed in the Raman spectroscopy which confirms the formation of ZnO nanocrystals. The increase in the peaks were observed as the calcination temperature

increased from 450 °C to 650 °C, which indicates the increase in crystallinity of the samples as the particle size increased with increase in calcination temperature.

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Oreochromis niloticus Status and Fundamental Potential roles in Ajalomi, River Ethiope, Delta State

*[1]Iloba K. I., [2]Oyedokun S. I. & [3]Oderhohwo L.

[1'2'3]Department of Animal and Environmental Biology, Delta State University, Nigeria.

*Email: kisyiloba@gmail.com; +2348036761306

ABSTRACT

Between July 2018 and March 2019, the status and ecological importance of *Oreochromis niloticus*, a significant fish species in the Ajalomi axis of the River Ethiope, sandwiched between Abraka and Oria, were researched. The fish measured between 8.0cm and 34cm in length and weighed between 4.30g to 500.00g. The regression coefficient values (-1.79-2.24) found for *O. niloticus* in this river indicated a negative allometric growth tendency. However, the Condition factor k>1 throughout the sampling period signifies a healthy fish and optimum environmental status. There were weak positive associations (r^2>0.65) between all the morphometric parameters examined, except for the standard length and body weight (r^2=0.13). The gut analysis revealed that 10.71% of studied fish had an empty stomach, while 89.29% had food substances in their stomach. The diet composition of *O. niloticus* in Ethiope River identified the fish as a generalist feeder, selectively on 60% debris-sediment, 36% phytoplankton, 3% plant parts and 1% insect parts. The food spectrum shows that *O. niloticus* in Ethiope River exhibits diet shift and are primarily detritivore. They are thus, useful in the circulation of nutrient metabolites on which primary producer depends.

Keywords: *Allometric growth, debris-sediments, phytoplankton, stomach content*

INTRODUCTION

Oreochromis niloticus is a member of the family Cichlidae. The fish is African origin found in diverse aquatic habitats. Getso *et al.* (2017) had reported the fish to be present in brackish water. *O. niloticus is* frequently employed in aquaculture in tropical nations due to its hardy character, fast reproduction in captivity and capacity to strive well in semi-intensive farming on natural food alone (Samaradivakara *et al.,* 2012; Ayisi *et al.,* 2017; Francisco *et al.,* 2018). In Nigeria*, O. niloticus* is of nutritional value and forms the proper amount of fish protein supply due to its availability, flavour and wide appeal by various income classes. However, the physical and biological constitutions of the environment have considerably been indicated in the growth of *O. niloticus*, awarding them the term plastic organism (Azua *et al.,* 2017).

Fish morphometric features are the major parameters used to analyse fish development pattern, resilience and wellness in a given habitat. The relationship between the average weight of a given fish length is an essential regular task that explicitly provides valuable information on the fish stock condition (Pitcher

Iloba K. I., Oyedokun S. I. & Oderhohwo L. (2021). *Oreochromis niloticus* Status and Fundamental Potential roles in Ajalomi, River Ethiope, Delta State. 2nd Faculty of Natural Sciences Annual Conference. IBB University Lapai held between 30th August to 2nd September 2021. Pp 114-128

and Hart, 1982; Simon *et al.,* 2009; Flipos, 2014; Hamid *et al.,* 2015; Iloba *et al.,* 2020). Moreover, it has been employed traditionally applied in fisheries management. They are also necessary for determining disparities in intraspecific organisms from different geographical regions (Naeem *et al.,* 2012). Other morphometric correlations between the meristic features are also significant instruments in fisheries management. The link between an organism's mouth and total size, for example, can be used to estimate the size of prey and generalise the organism's place in the food chain (Momotaz, 2020). Furthermore, morphometric data such as the length of various body portions of fish as well as weight are important for species identification and categorization. It can also be used to analyse the general well-being and life history of a population from a distinct geographical region (Hossain *et al.,* 2016; Azad *et al.,* 2018). The length-weight relationship and condition factor are beneficial in fishery management because they are critical for estimating biomass, fish growth patterns, and fish general health (Iloba *et al.,* 2020; Zare-Shahraki *et al.,* 2020).

Apart from its food preferences, food spectrum overlap, and feeding methods, fish food appraisal and feeding habits reflect anthropogenic impacts and stressors on river systems (Chhaya and Sonawane, 2016). The presence or lack of food in the fish gut is an intrinsic component that affects the fish's physiological state (Rodriguez *et al.,* 2017). Thus, food availability (extrinsic) determines the condition factor (Rodriguez *et al.,* 2017). Thus, diet composition is a critical tool in community ecology because an organism's resource utilisation has a significant effect on population interactions within the community (Alhassan *et al.,* 2011: Kuebutorne *et al.,* 2019). Numerous researchers have collected and analysed data on the stomach contents of Tilapia from a variety of aquatic bodies. Artfiati *et al.* (2019) in Uganda's lakes Edward, George, and Kazinga channel in Uganda; Mohamed *et al.* (2019) in Egypt's fish ponds; Kuebutornye *et al.* (2019) in Ghana's Libga reservoir. Thus, information about a fish's diet can serve as an indicator of its functional role in the ecosystem and can also aid in the understanding and implementation of fish management and conservation. The morphometric characteristics of Tilapia, as well as its food and feeding habits, have been extensively studied in several water bodies, as revealed by the literature. Azua *et al.* (2017) studied the lower Benue River in Nigeria; Samaradivakara *et al.* (2012) studied selected reservoirs in Sri Lanka; and Kuebutornye *et al.* (2019) studied the Libga reservoir in Ghana. Despite the overwhelming amount of information on *O. niloticus*, a review of the literature revealed a dearth of information on the general status and functional potential role of *O. niloticus* in the Ethiope River up to this point. Thus, the purpose of this research is to ascertain *O. niloticus* general status and fundamental functional role in the Ajalomi section of the Ethiope River to supplement information on the Ethiope River's fisheries and fish management.

MATERIALS AND METHODOLOGY

Study Area

The River Ehiope is located in the southern part of Nigeria, within the Niger Delta basin (Figure 1). It was formed in Umuaja by a watershed. It flows through an evergreen forest zone and empties into Sapele Delta State's Benin River, between latitudes 6°31´ and 6°30´ N and longitudes 500-600 E, the River Ethiope flows. Its width varied between approximately 10m and 90m, and its

depth varied between 1m and 20m. The stream is continuous, peaking during the flood (Iloba, 2017).

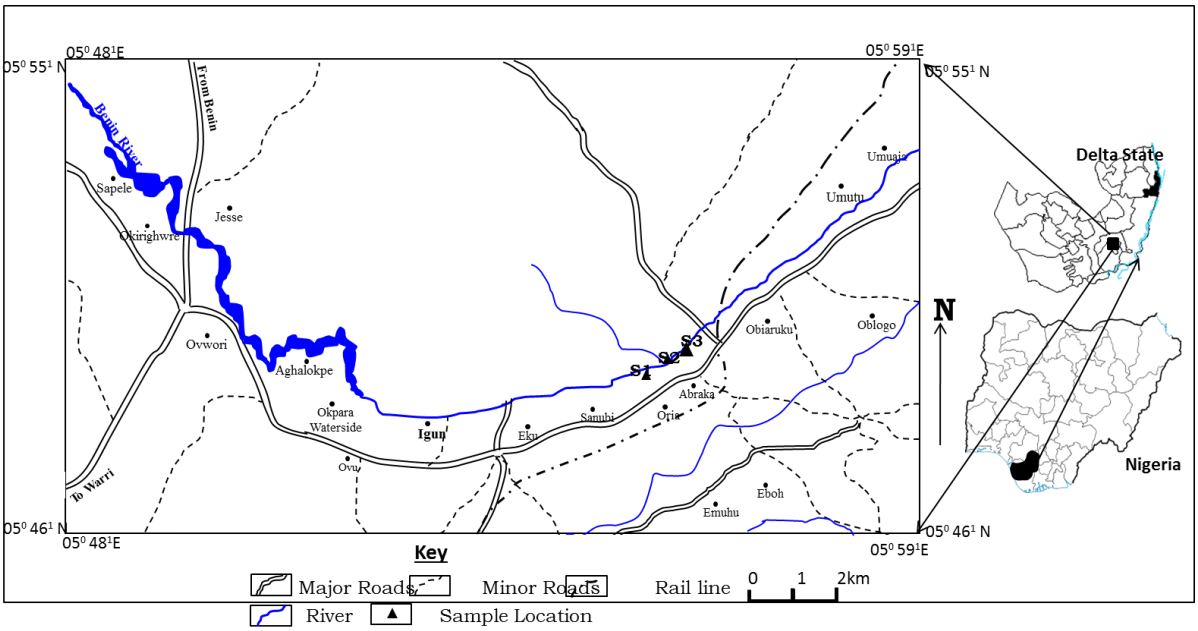

Figure 1. Location of River Ethiope in the Delta State of Nigeria,and sampled sites.

Sample Collection

Between July 2018 and March 2019, samples of *O.niloticus* were collected from artisanal fishermen in the Ethiope River. The fish were caught using a fishing dragnet with mesh widths of 60mm, 80mm, and 100mm, locally known as fingers 3, 4, and 5. On-site cooling of the samples with ice was followed by transmission to the laboratory for morphometric and gastric examination. After morphometric analysis, the fish were gutted and their stomach contents were examined.

Morphometric Analysis

Each sample was morphometrically analysed using an electric digital weighing balance calibrated to the nearest 0.10g. The Total Length (TL), Standards Length (SL), and Head Length (HL) were all measured to the nearest 0.1cm using a metre rule and measuring board.

Stomach Content Analysis

After dissecting the fish with a dissecting kit, the intestines were extracted for further analysis. After opening the gut, the contents were put onto a petric dish. These contents were amplified before being transferred to slides for accurate identification utilising pertinent graphical and descriptive keys under the compound microscope (Withford and Schumacher, 1973; Wehr and Sheath, 2003).

The following equation is used to compute the gut repletion index

Number of non-empty guts × 100 ÷ Total numbers of specimens

The equation below obtained the frequency of occurrence

$Fi = ni \times 100 \div n$

Where Fi is the frequency of occurrence of the i item in the sample

ni is the number of the gut in which the i food item is found

n is the total number of the gut with food sample

Length-weight Relationship

The length-weight relationship was assessed using the equation

$W = a+bL$ where

W is the weight (g)

a is the regression constant

b is the coefficient of regression

L is the length (cm)

This was log-transformed to linear regression as follows

$Log\ W = Log\ a + b Log\ L$

This was then plotted on the scattered diagram to show the length-weight relationships

Condition Factor

The Fulton's condition factor reveals the well-being of the fish, and it was calculated as

$K = W \times 100 \div L^3$

Where

K is the condition factor

W is the weight of fish (g)

L is the length of fish (cm)

Statistical Analysis

Coefficient of regression, regression constant, correlation and significant levels were analyzed using PAST 3 statistical software.

RESULTS

The frequency of occurrence, length range, percentage occupied, and mean weight of the various class intervals are summarised in Table 1. Fish measuring 13.00cm to 17.99cm in length were the most abundant, accounting for 40.46 percent of all fish examined.

Table 1. The Length range, mean weight ±SD, the frequency of occurrence of *O. niloticus*

Length range (cm)	Mean weight (g)	Frequency of Occurrence	Percentage (%)
8.00 – 12.99	29.54±35.87	24	18.32
13.00 – 17.99	124.19±28.26	53	40.46
18.00 – 22.99	161.67±38.16	47	35.88
23.00 – 27.99	201.05±69.22	2	1.53
28.00 – 32.99	374.85±135.43	4	3.05
33.00 – 37.99	244.70	1	0.76

The fish measured between 8.0cm and 34.0cm in length and weighed between 4.30g and 500.00g. The monthly length-weight relationship, correlation, level of significance, and condition factor is presented in Table 2. The condition factor varied between 1.76 and 3.63, the intercept a varied between 0.22 and 2.93, and the growth exponent b varied between 1.79 and 3.58. The p values were extremely significant (0.005), and the coefficient correlation coefficient r^2, varied between 0.50 and 0.94.

Table 2. Monthly Length-weight relationship, correlation and condition factor of *O. niloticus*

Month	LogW=Loga+bLogTL	r	r^2	k	P
July	LogW=0.64-1.79TL	0.93	0.87	1.76	0.000
November	LogW=2.93+1.40TL	0.70	0.50	3.63	0.001
December	LogW=0.01+3.58TL	0.94	0.94	3.38	0.000
February	LogW=1.50+1.54TL	0.82	0.71	2.11	0.001
March	LogW=0.22+2.24TL	0.94	0.88	2.44	0.000

Analysis of morphometric relationships throughout the study period was presented in Table 3. All parameters showed positive correlation except the standard length and the bodyweight, which showed no correlation (r=0.13). All morphometric parameters were highly significant ($p<0.005$). Total length and Bodyweight relationship revealed growth pattern (b value) was less than 3 ($b<3$) showing negative allometric growth.

Table 3. Morphometric Relationships of *O. niloticus* during the study period

Parameters	LogW=Loga+bLogTL	R	r^2	T	p
TL-BW	LogW=2.28+0.71TL	0.94	0.88	11.99	0.000
SL-HL	LogW=0.33+0.35TL	0.81	0.66	6.16	0.000
SL-BW	LogW=1.19+9.65TL	0.36	0.13	4.37	0.000
SL-TL	LogW=0.83+0.11TL	0.98	0.97	63.59	0.000
HL-BW	LogW=2.11+0.70TL	0.91	0.83	24.79	0.000
HL-TL	LogW=0.30-0.27TL	0.85	0.72	7.12	0.000

TL is Total Length, BW is Bodyweight, SL is Standard length, HL is Head Length, a is the regression's intercept while b is the coefficient of regression

Morphometric relationships were analyzed; the generalized linear model graph was generated as represented in Figure 1(a-f). All parameters were positively correlated except standard length and body weight. Pair with different units was transformed in logarithms to nullify the effect of the units on the result.

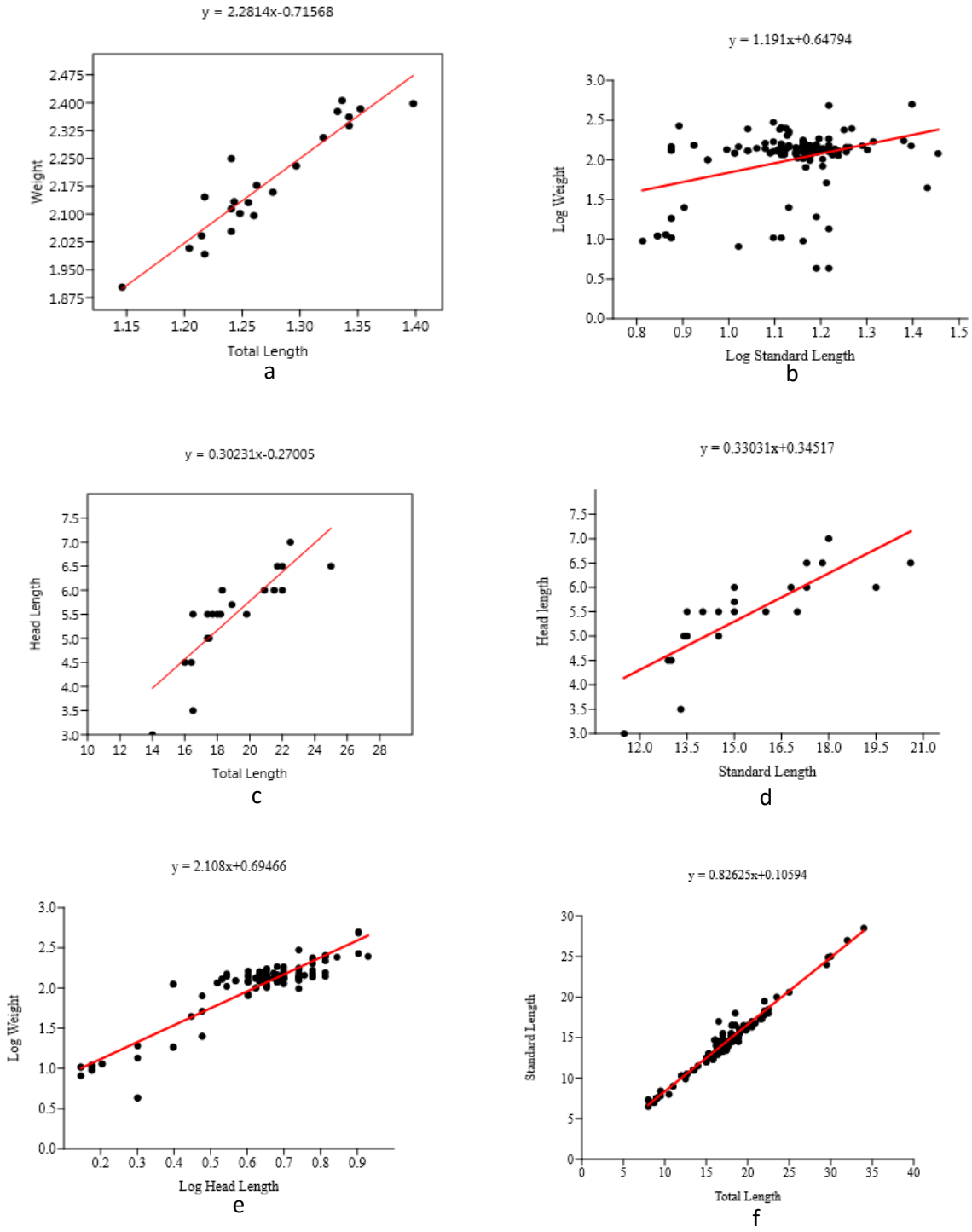

Figure 2: The morphometric relationships between the total length and the weight (a), standard length and weight (b), (c) the total length and the head length, (d) standard length and head length, (e) head length and body weight and (f) standard length and total length of *O. niloticus* in the Ajalomi section of Ethiope River.

The degree of the fish stomach fullness is presented in Table 4. 10.71% of the total fish stomachs examined were empty. 14.2% were found to have a full stomach, 7.1% had their stomach filled to about three quarter, 14.29% had stomach with half-filled. In comparison, 53.53% had only one-quarter of their stomach filled.

Table 4. Degree of stomach fullness of *O. niloticus*

Degree of Fullness	Percentage (%)
Full	14.29
Three quarter	7.14
Half	14.29
One quarter	53.57
Empty	10.71

The stomach content of the examined fish is presented in Figure 2. Debris were most abundant (60%) in the experimental fish's stomach content. Phytoplankton was 36%, plants 3% and insect parts 1%.

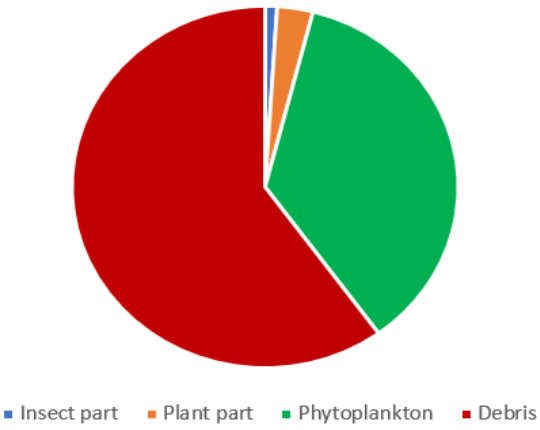

Figure 3. Stomach content of *O. niloticus* in Ethiope River

Phytoplankton diet component in the stomach content of the experimental fish is presented in Table 5. The fish fed primarily on diatoms, few desmids, Rhodophyta and Cyanophyta. 98.96% of the total phytoplankton belonged to Bacillariophyta division. 0.86% of the phytoplankton meal was Rhodophyta, 0.14% Cyanophyta and 0.02% Chlorophyta.

Table 5. Phytoplankton species found in the gut of *O. niloticus* of Ethiope River

Phytoplankton	Division	Amount	Percentage (%)
Selanastrum	Chlorophyta	1	0.01
Spirogyra dubia	Chlorophyta	1	0.01
Fischerella ambigua	Cyanophyta	10	0.14
Betrachospamum vagum	Rhodophyta	60	0.86
Frustulia rhomboides	Bacillariophyta	1	0.01
Diatomella balfouriana	Bacillariophyta	1	0.01
Actinallasp	Bacillariophyta	4	0.08
Eunotia sp	Bacillariophyta	9	0.13
Synedra ulna	Bacillariophyta	13	0.19
Asterionella Formosa	Bacillariophyta	28	0.40
Gomphonema sp	Bacillariophyta	33	0.48
Tabellaria flocculosa	Bacillariophyta	53	0.76
Fragillaria pinnata	Bacillariophyta	112	1.61
Navicula rhomboides	Bacillariophyta	515	7.41
Navicula lanceolata	Bacillariophyta	533	7.67
Navicula serians	Bacillariophyta	2230	32.10
Synedra acus	Bacillariophyta	3343	48.12
Total		**6947**	**100**

DISCUSSION

In the present study, the total length of *O. niloticus* in the river Ethiope varied between 8.0cm and 34.0cm. The greatest length (34.0cm) of *O. niloticus* in the Ethiope River was significantly greater than the length observed in several investigations. These include Arfiati *et al.* (2019) in Tagir (River Surabaya) (22cm), Adriana et al. (2015) in earthen ponds in Brazil (23cm), Azua *et al.* (2017) in the lower Benue River, Makurdi (10.26cm), and Kuebutornye *et al.* (2019) in the Libga Reservoir, Northern Region of Ghana. The greatest length, on the other hand, was shorter than that reported by Testafun 2018 (42.0cm). The difference in size range could be explained by the varying mesh sizes of fishing gear used on the Ethiope River. The alleged accelerated growth of *O. niloticus* in this system may be a result of the investigated fish's broad food spectrum and/or their ability to evade predators due to their dark colour fitting in with the dark sediments. The length range (13.00cm-17.00cm) in the length-frequency distribution chart reveals that undersized *O. niloticus* is overexploited. According to Shoko *et al.* (2015), *O. niloticus* typically begins reproducing at a total length of 20-30cm. However, the current study did not capture the reproductive biology of the investigated fish.

Nonetheless, more than half of the caught samples were shorter than the reproductive length reported by Shoko *et al.* (2015). It is worth noting, however, that the reproductive length range corresponds to that of the current study (18-22cm). 47 of the sampled fish were within Shoko *et al.* (2015)'s reproductive length range, indicating that the analysed fish possessed a considerable reproductive force in the river. In other words, the Ethiope River is capable of supporting a thriving commercial fishing.

Monthly regression coefficient (b value) fluctuations demonstrated negative allometry in *O. niloticus* growth in the Ethiope River implying that the fish become thinner as they expand in length. Laurat *et al.* (2019) arrived at a similar conclusion about the length-weight relationship and condition factor of the same fish species in Nigeria's Lower River Benue. *O. niloticus* grew with a negative allometric rate in all months except December in this study (Table 4). Numerous researchers have observed negative allometry (b<3) in *O. niloticus*. Getso *et al.* (2017) investigated the length-weight relationship and condition factor of *O. niloticus* in the Wudil River, Kano; and Fagbuaro et al. (2019) investigated the length-weight relationship and condition factor of *O. niloticus* in a tropical dam in southwestern Nigeria. Obasohan *et al.* (2012) examined the length-weight relationship and condition factor of *O. niloticus* collected in Ekpoma, Edo State, Nigeria. In contrast to the current report on *O. niloticus*, Berhan *et al.* (2019) described *O. niloticus* from Ethiopia's Koka reservoir. However, based on its morphometric characteristics in Thailand and biology in Ethiopia, Kosai *et al.* (2014) and Tsegay *et al.* (2018) demonstrated isomeric growth (b=3.0) on *O. niloticus*. The growth pattern may change as a result of changes in geographic location or ecological conditions. Additionally, the allometric growth pattern indicates that O. niloticus's specific gravity will not remain constant throughout its life in this river. Thus, a change in allometry is envisaged in tandem with a change in the shape of the fish (Khan *et al.,* 2012).

The significant positive correlation between *O. niloticus* length and weight in this study (Table 3) indicates that *O. niloticus* grew in a healthy and stable manner in this river. Apart from standard length and body weight (r^2=0.13), all

morphometric parameters and total length had a relatively strong correlation. Similarly, Azua *et al.* (2017) discovered no correlation between standard length and body weight of *O. niloticus* in the Benue River, Makurdi (r^2=0.0889). The weak link discovered may imply that a fish standard length is an imprecise tool for assessing fish growth and condition, but a useful tool in fisheries (Serife *et al.* 2014).

The relative condition factors (k) were more significant than one throughout the study period. The existence of multiple condition factors (k>1) in our study indicated that *O. niloticus* is physiologically sound (Alex *et al.,* 2012). The current study's stress-free condition factors revealed that the fish environment (Ethiope River) has an abundant food supply, resulting in lucrative fish growth supports and a healthy river (Famoofo and Abdul, 2020). Assefa et al. (2019) experiments' demonstrate a similar k>1 result. They discovered k>1 in the length-weight relationship and condition factor of *O. niloticus* in Lake Hayq Ethiopia. The condition factor of *O. niloticus* in this study corresponds to Obasohan *et al.* (2012) in the Ibiekuma stream.

According to the degree of stomach fullness, 10.71 percent of the fish had an empty stomach while 89.29 percent had varying amounts of food. The stomach voluminosity of *O. niloticus* in the current study indicates that the river's fish eating intensity compensated for the fish's exceptional condition (Famoofo and Abdul, 2020). *O. niloticus* consumes a diverse range of foods, as seen by the food variety. The composition or partitioning of *O. niloticus's* food indicated that it is primarily an omnivore but also thrives as a herbivore. The high food content indicates that there is sufficient food in the system. This fish's improved condition factor is a result of the availability of acceptable food in this river, as well as appropriate water with optimal aquaculture conditions. Regardless, some individuals were on an empty stomach. This stomach emptying could be a result of physiological stress, food regurgitation during the capture fight, or post-harvest digestion.

The stomach content analysis of *O. niloticus* revealed that the majority of the food in their stomachs was detritus and silt. The predominance of debris-eating fish in our study indicates that the fish is mostly a bottom-feeding omnivore. As a result, it may play a critical role in the system's nutrient recycling and regeneration, replenishing nutrients depleted in its specific water column and downstream (John and Paul, 2007). They were critical in sustaining phytoplankton development and connecting the river's trophic levels. Plants (herbivory) and insect parts (insectivory) bolstered *O. niloticus's* omnivory in the current study, accounting for 3% and 1% of the fish diet, respectively.

Oreochromis niloticus is mostly a Phytoplanktivore in this work. Athanasio *et al.* (2017), Azua *et al.* (2017), and Zakaria *et al.* (2017) have all witnessed *O. niloticus* exhibiting this feeding behaviour (2019). The fish consumed a variety of phytoplankton, as revealed by the stomach investigation. The diverse collection of 6947 phytoplankton counted was dominated by diatoms, accounting for 98.94 percent (6947), followed by Rhodophyta at 0.86 percent. Other small taxa include Cyanophyta (one species) at 0.14 percent and Chlorophyta at 0.02 percent (two species). The low abundance of these other phytoplankton types in *O. niloticus's* gut in this river indicates that *O. niloticus*has a low preference for them.

Apart from debris, *O. niloticus* preferred phytoplankton to other meals in the Ethiope River. According to Zakaria *et al.* (2019), phytoplankton cells have a high concentration of vitamin precursors and critical amino acids, which are believed to promote growth. *O. niloticus*, according to Azua *et al.* (2017), feeds mostly on phytoplankton and benthic algae. The findings of this study corroborate those of Athanasio *et al.* (2017), who reported that the majority of food consumed by *O. niloticus* in the stomach content of a Tanzanian pond is detritus and phytoplankton. The findings of this study corroborate those of Arfiati *et al.* (2019), who discovered that phytoplankton comprised 98.64 percent of *O. niloticus's* total stomach content in Surabaya's Jagir River. Cyanophyta were few in the experimental fish's intestines (ten individuals out of a total of 6947 phytoplankton). This could be because Cyanophyta impair the health of fish by building up poisons in their tissues (Mohamed 2016). Mohamed *et al.* (2013) discovered that when stressed *O. niloticus* is exposed to a pathogenic strain of Cyanobacteria, it exhibits delayed locomotion, increased metabolism, decreased total protein, and impaired reflexes. Cyanophyta comprised just 6.82 percent of the phytoplankton recovered from the stomach contents. On the other hand, Chrysophyta and Chlorophyta received 42.27 percent and 49.55 percent, respectively. This provides evidence for *O. niloticus'* preference for Cyanophya.

The different eating habits of *O. niloticus* show that the river has a diversity of niche regions. The occurrence of many niche areas demonstrates that *O. niloticus* possesses the eating flexibility necessary to adjust to shifting food constraints in its environment (Nandi and Saikai, 2015). Thus, *O. niloticus* contributes significantly to the food web of this river by bridging multiple trophic levels. Environmental variables such as stomach content, maturity, and age can all have an effect on the condition of fish (Obasohan *et al.,* 2012). The presence of food material in the guts of a significant proportion of the analysed fish may also contribute to *O. niloticus's* good condition in this river.

CONCLUSION

This study discovered that *Oreochromis niloticus* is overexploited in the Ethiope River, with a greater proportion of the fish examined being below reproductive length. *Oreochromis niloticus* in the Ethiope River are in good health, as evidenced by their condition. This study established that *Oreochromis niloticus* is being overexploited in the Ethiope River, as a greater proportion of the fish examined were below reproductive length. *Oreochromis niloticus* populations in the Ethiope River are healthy, as shown by the condition factor in this study, and they exhibit a negative allometric growth pattern. According to the food spectrum, *O. niloticus* in the Ethiope River undergoes diet shifts and is generally a bottom feeder omnivore. As a result, they will contribute to the circulation of nutrient metabolites by recycling nutrients and restoring nutrients depleted by primary producers. *O. niloticus* thus plays a critical role in the river's food cycle by bridging disparate trophic levels. Due to the selective feeding behaviour of *O. niloticus*, pond aquaculture may be advised, as detritus and phytoplankton are readily available.

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Phytochemical Composition and Antifungal Activity of Aqueous and *N*-Hexane Extracts of *Calotropis procera* Leaf

[*1]Madaki F. M., [2]Bilbis L. S., [2]Aliyu R. U. & [1]Kabiru A. Y.

[1]Department of Biochemistry School of Life Sciences, Federal University of Technology, Minna, Niger State.

[2]Department of Biochemistry School of Sciences, Usmanu Dan Fodiyo University Sokoto, Sokoto State.

*Corresponding author's Email**:** nmmadaki@gmail.com, Phone number: 08036482921

ABSTRACT

Natural products play important roles in drug discovery and development process, particularly in the field of infectious diseases. The aim of this study is to determine the antifungal activity of aqueous and n-hexane leaf extracts. The qualitative phytochemical study was performed according to standard methods. Antifungal activity of aqueous and n-hexane leaf extracts of *Calotropis procera* against dermatophyte (*Microsporum spp* and *Trichophyton spp*) at different concentrations (20mg/Ml, 30mg/mL, 40mg/mL, 50mg/mL and 60mg/mL) was carried out using agar incorporation method. The result of the qualitative phytochemical studies showed the presence of steroids, tannins, glycosides, phenols, terpenoids, flavonoids, alkaloids and saponins in aqueous extract of *C. procera*, while the n-hexane leaf extract showed absence of alkaloids, flavonoids and cardiac glycosides. There was complete inhibition of the growth of *Microsporum spp* and *Trichophyton spp*, after 14 days of culture with the aqueous extracts of *Calotropis procera* and at different concentrations (1.33mg/mL and 2.66mg/mL) of *n*-hexane leaf extract, there was complete inhibition of the growth. The result showed higher antifungal activity when compared to a conventional drug; fulcin.

KEYWORDS: *Calotropis procera*, Antifungal, *Microsporum spp* and *Trichophyton spp*

INTRODUCTION

The World Health Organization reported that 70–90% of the world's population depends chiefly on traditional medicine (WHO, 2004). Natural products play important roles in drug discovery and development process, particularly in the field of infectious diseases, where 75% of these drugs are of natural origin (Newman *et al*., 2003). The local use of natural plants as primary health remedies is due to their pharmacological properties. Many plant extracts owe their potency to the presence of metabolites (Kinghorn, 1994). Plants provide abundant resources of antimicrobial compounds and have been used for centuries to inhibit microbial growth (Zhang *et al*., 2006). The increasing incidences of fungal infections and gradual rise in azole resistance and available antibiotics had highlighted the need to find more alternative antifungal agents from other sources (Fostel and Lartey, 2000).

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For many years now, interest in new safer, cheaper and more effective antifungal agent has grown with the increasing incidences of fungal infections, in the science of natural products the antimycotic activity of higher plants remains largely unemployed compared with other microorganism. Fungal related diseases may not be common as other microbes but when present, they could be difficult to eradicate especially in immuno-suppressive situations (Bertram, 1984; Alexopoulos *et al*., 1994). Research on bioactive substances from plant sources has great scope and could lead to the provision of value - added economic return, and the establishment of natural plant provision (Adekunle, 2000). In Nigeria the crude extract of some local plants are used by the natives to cure fungal diseases in human. Some of these plants include *Brachystigia eurycomahams* (*Caesalpinaceae*), *Richardia Brasiliensis* (Gomez) *Rubiaceae* and *Calotropis procera* Sodom apple.

Calotropis procera (family *Asclepiadaceae*) is commonly called Sodom apple. It is un-branched with soft wooden trunk, yellowish brown stem bark and the slash exudes caustic latex that turns yellow on exposure to air (Aliero *et al*., 2001). In northern Nigeria the leaves, roots and stem barks of *Calotropis procera* are used in indigenous practice to treat fungal diseases (for topical application), convulsion, asthma, cough and inflammation. Aliero *et al*. (2001) reported the use of fresh follicles of the plant (soaked in cold water) for the treatment of asthma, the burnt dry stem for **wound healing** and the plant is also used in the treatment of several diseases of domestic animals.

The anti-fungal activity of *Calotropis procera* has been speculated by local people that it cures a number of contagious skin diseases caused by certain parasitic fungi e.g. ringworm infection and eczema (Joseph, 1970). Dermatophytes are fungi that require keratin for growth. These fungi can cause superficial infections of the skin, hair, nails skin and internal organs, respectively. Dermatophytes are spread by direct contact from other people (anthropophilic organisms), animals (zoophilic organisms), and soil (geophilic organisms), as well as indirectly from fomites. This research work will emphasis on the leaves of *C. procera* to ascertain the phytochemical constituents and antifungal properties as claim by traditional medicine practitioner against a fungal infection called ringworm.

METHODOLOGY

Collection of Plant Materials

The plant; *calotropis procera* were collected around new examination hall, Usmanu Danfodiyo University, Sokoto. This was done after confirmation from some traditional healers who claim that the plant is a cure for ringworm infection. The plant was identified in the herbarium of Biological Science Department. The plant leaves collected were room-dried and pulverized into powder and the powdered sample was subjected to aqueous and n- hexane solvent extraction (Matawalli *et al*., 2004).

Source of Infectious Fungi

Trichophyton rubrum (*Trichophyton spp.*) and *Microsporum gypseum* (*Microsporum spp,*) were clinical isolates obtained from Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria. They were maintained on Sabouraud Dextrose Agar (SDA) medium and re-identified by microscopic examination of a portion at mycology laboratory of Biological Sciences Department, Usman Dan Fodiyo University of Sokoto.

Materials and Reagents

Materials

Beakers(250 mL and 500 mL), Separating funnel, Volumetric flask(250 mL,500 mL and 1000 mL), Funnel, Measuring cylinder,(10 mL and 250 mL) and Petri dishes are made by Pyrex England, Weighing balance (Sartarius 2351), Metlee Balance-p163, Hot plate (Precision scientific U.S.A.), Autoclave (potable) (Arnold and sons ltd), Incubator (Gallenkamp,England).

Reagents

Malt extract agar, Ethanol, Formaldehyde, Methylated spirit, n-hexane BDH chemical, England. All reagents were of analytical grade. Distilled water, Antibiotic (streptomycin), Dana pharmaceuticals India and Fulcin tablet M& B Ltd.

Phytochemical Screening

Plants extracts were qualitatively screened using standard techniques for the detection of alkaloid, flavonoids, glycosides, steroids, tannins and saponins (Sofowora, 1993; Evans, 1995).

PREPARATION OF PLANT EXTRACTS AND MEDIA

Extraction and Fractionation of Sample (n-hexane) Extract

Five different concentrations (20 mg/mL, 30 mg/mL, 40 mg/mL, 50 mg/mL and 60 mg/mL) were prepared by dissolving 10g of the *Calotropis procera* (*C. procera*) leaves in 500 mL, 333 mL, 250 mL, and 166.5 mL of distilled water respectively to obtain aqueous extract.

Fifty grams (50 g) of dried sample was weighed and dissolved into 500 mL distilled water in a conical flask for 24 h. The solution was then filtered to obtain aqueous extract. Equal volume of n-hexane was then mixed with aqueous extract and then fractionated using separating funnel. The n-hexane extract were evaporated and the dried sample weighed. Two different concentrations (1.33 mg/mL and 2.66 mg/mL) were prepared from the dried samples according to modified method of Hassan *et al*. (2006).

PREPARATION OF MALT EXTRACT MEDIA

Thirty grams (30 g) of malt extract powder was dissolved in 1000 mL of distilled water in a conical flask. The solution was shaken gently to allow complete dissolution. The solution was then sterilized using an autoclave at 121^{o}C for 30 minutes and was allowed to cool for 45 ^{o}C. Fifteen milliliters (15 mL) of the malt extract agar was dispense into 10 conical flask and autoclaved using the same procedure described above.

BIOLOGICAL SCREENING BY AGAR INCOPORATION METHOD

Agar and Plant Extract Incorporation and Subsequent Inoculation of the Organism

Five milliliters (5 mL) of the water extract was added to conical flasks containing malt extract agar. The solution was shaken and transferred into the petri dishes in the incubator room. Five milliliters (5 mL) of the last water extract, obtained after fractionation was added to a conical flasks of leaf extract. Five milliliters (5 mL) of n-hexane extract were also added to the conical flasks and transferred into the petri dishes in the incubator.

Pure culture of *Trichophyton spp* and *Microsporum spp* were inoculated into different petri dishes at the middle using 2 × 2 mm inoculum i.e., by diffusion on a solid medium (agar incorporation method) fulcin was used as a standard and two petri dishes that do not

contain the plant extract were used as mycological control. The growth of the organisms were observed daily for two weeks and their diameter measured.

RESULTS

Percentage Yield of Extracts

The extracts yield obtained for the different solvent extracts evaluated (Table 1). *C. procera* aqueous extracts had the highest extract yield (%), while *C. procera* n- hexane extracts had the least extract yield (%).

Table 1: Percentage (%) Yield of *C. procera* Extracts

***C. procera* Extracts**	**Leaf powder (g)**	**Extract yield (%)**
Aqueous	50.00	0.21
n-Hexane	50.00	0.50

Qualitative Phytochemical Screening of *C. procera* Leaf Extracts

The qualitative phytochemical constituents of *calotropis procera* leaf extract revealed the presence of tannins, alkaloids, flavonoids, phenols and glycosides in aqueous extract while flavonoids and glycosides were absent in n-hexane fraction of *calotropis procera*.

Table 2: Qualitative Constituents of Aqueous and n-hexane *C. procera* Leaf Extracts

Phytochemicals	***C. procera* Aqueous leaf extracts**	***C. procera* n-hexane leaf extracts**
Alkaloids	**+**	**-**
Flavonoids	**+**	**-**
Saponins	**+**	**+**
Steroids	**+**	**+**
Phenols	**+**	**+**
Terpenoids	**+**	**+**
Tannins	**+**	**+**
Glycosides	**+**	**-**

Note: - = not dictated, += dictated

ANTIFUNGAL SCREENING RESULTS

Effect of *C. procera* Aqueous Leaf Extracts on Diameter of Growth of *Microsporum spp*

Table 3 shows the results of effect of *C. procera* aqueous leaf extract on the diameter of growth of *Microsporum spp* for 14 days (2 weeks). The growth of *Microsporum spp* was completely inhibited by the extract at all the concentrations when compared with the negative control (inoculated with *Microsporum spp* but not treated), while the positive control (inoculated with *Microsporum spp* treated with standard drug; fulcin) was only able to suppress *Microsporum spp* till day 5 where growth was observed up to day 14.

Table 3: Effect of *C. procera* Aqueous Leaf Extracts on Diameter of Growth of *Microsporum spp*

Concentration	***Days***													
(Mg/ml)	1	2	3	4	5	6	7	8	9	10	11	12	13	14
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	-	-	-	-	-	-	-	-	-	-	-	-	-	-
50	-	-	-	-	-	-	-	-	-	-	-	-	-	-
60	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-ve control	5	9	15	18	22	27	30	35	40	44	50	65	79	90
60 Fulcin	-	-	-	-	5	10	15	20	32	40	35	30	24	12

Effect of *C. procera* Aqueous Leaf Extracts on Diameter of Growth of *Trichophyton spp*

Table 4 shows the results of effect of *C. procera* aqueous leaf extract on the diameter of growth of *Trichophyton spp* for 14 days (2 weeks). The growth of *Trichophyton spp* was completely inhibited by the extract at all the concentrations studied when compared with the negative control (inoculated with *Trichophyton spp* but not treated), while the positive control (inoculated with *Trichophyton spp* treated with standard drug; fulcin) was able to inhibit the growth *Trichophyton spp* up to day 14.

Table 4: Effect of *C. procera* Aqueous Leaf Extracts on Diameter of Growth of *Trichophyton spp*

Concentration	***Days***													
(Mg/ml)	1	2	3	4	5	6	7	8	9	10	11	12	13	14
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	-	-	-	-	-	-	-	-	-	-	-	-	-	-
50	-	-	-	-	-	-	-	-	-	-	-	-	-	-
60	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-ve control	5	9	15	18	22	27	30	35	40	44	50	65	79	90
60 Fulcin	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Effect of *C. procera* n-Hexane Leaf Extracts on Diameter of Growth of *Microsporum spp*

Table 5 shows the results of effect of *C. procera* n- hexane leaf extract on the diameter of growth of *Microsporum spp* for 14 days (2 weeks). The growth of *Microsporum spp* was completely inhibited by the extract at all the concentrations tested when compared with the negative control (inoculated with *Microsporum spp* but not treated), while the positive control (inoculated with *Microsporum spp* treated and with standard drug; fulcin) was only able to suppress *Microsporum spp* till day 5 where growth was observed up to day 14.

Table 5: Effect of *C. procera* n-Hexane Leaf Extracts on Diameter of Growth of *Microsporum spp*

Concentration	***Days***													
(Mg/ml)	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2.66	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-ve control	5	9	15	18	22	27	30	35	40	44	50	65	79	90
30 Fulcin	-	-	-	-	5	10	15	20	32	40	35	30	24	12

Effect of *C. procera* n-Hexane Leaf Extracts on Diameter of Growth of *Trichophyton spp*

Table 6 shows the results of effect of *C. procera* aqueous leaf extract on the diameter of growth of *Trichophyton spp* for 14 days (2 weeks). The growth of *Trichophyton spp* was completely inhibited by the extract at all the concentrations tested when compared with the negative control (inoculated with *Trichophyton spp* but not treated), while the positive control (inoculated with *Trichophyton spp* treated with standard drug; fulcin) was also able to inhibit the growth *Trichophyton spp* up to day 14.

Table 6: Effect of *C. procera* n-Hexane Leaf Extracts on Diameter of Growth of *Trichophyton spp*

Concentration	***Days***													
(Mg/ml)	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2.66	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-ve control	5	9	15	18	22	27	30	35	40	44	50	65	79	90
30 Fulcin	-	-	-	-	-	-	-	-	-	-	-	-	-	-

DISCUSSION

In this study difference in extract yield was obtained for the plant extracts, which could be due to solvent difference, polarity and affinity. The phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, steroids, triterpenoids, saponins and glycosides in *C. procera* extracts with the exception of flavonoids and glycosides which were absent in the n-hexane extract. This could be due to difference in solvent used in the analysis. Solvent polarity is an important parameter that affects the yield of a plant material. Thus the higher the polarity, the better the solubility of compounds such as phenols. This result is similar to the study reported by Hassan *et al*. (2006). The presence of bioactive compounds could be the reason for the antifungal activity and the use of this plant for medicinal purposes.

Medicinal plants are potential source of new drugs. Since they contain a enormous quantity of molecules with a great variety of structures and pharmacological activities. Several well established human antiprotozoal drugs have their origins in nature, such as quinine an alkaloid from *Cinchona* sp. (Rubiaceae) and artemisinin, a sesquiterpene lactone from *Artemisia annua* (Asteraceae) used to treat malaria or emetine, an alkaloid from *Cephaelis*n *ipecacuanha* (Rubiaceae) used to treat amoebiasis. Additionally, these antiprotozoal plant-derived compounds have been used as leads to develop other semi-synthetic or synthetic drugs with better efficacy, safety or pharmacokinetic profiles (Tagboto and Townson, 2001).

The biological screening carried out on *C. procera* is a preliminary study to confirm the antifungal property on *Microsporum spp* and *Trichophyton spp.* Results revealed that aqueous extract of *Calotropis procera* had complete inhibitory effect on the growth of *Microsporum spp* at all the various concentration tested. While 60 mg/mL of Fulcin did not show complete inhibitory effect on the growth of *Microsporum spp*.at the 5thday, growth of 5mm was noticed which increased to 40mm at the 10th day and later decreased to 12mm at the 14thday.

The n-hexane leaf extracts, showed complete inhibitory effect on the growth of *Microsporum spp* and *Trichophyton spp*. when compared with the control, it shows that the crude extracts are more effective that the conventional drug (Fulcin), since 1.33 mg/mL of n-hexane completely inhibited the growth of *Microsporum spp* and *Trichophyton spp*. while about 30 mg/mL of Fulcin was required to inhibit the growth of *Microsporum spp*. The extract completely inhibited the growth of *Trichophyton spp*. while 1.33 mg/mL growth in diameter was observed at the 6thday which increased to 54.5mm at the 14thday. This shows that 2.66 mg/mL of root n-hexane extract is more effective on *Trichophyton spp*. than *Microsporum spp.* Therefore, it can be deduced that *C. procera* has a dose dependent activity on the organisms.

At this point it can be suggested that all these phytochemicals observed had contributed to the antifungal activity of the plant. Tannins have been reported to inhibit growth of microorganisms by precipitating microbial protein and making nutritional protein unavailable to them (Idu, 2007); while the antimicrobial effects of flavonoids have been attributed to their ability to form complex with extra cellular, soluble protein and with bacterial cell wall proteins (Musa *et al*., 2008). The flavonoids have been known to be synthesized

by plants in response to microbial infection (Al-Bayati and Al-Mola, 2008).

CONCLUSION

This research work has been able to establish some medicinal fact about the plant used. Therefore, the medicinal uses and claims of these plants in the treatment of ringworm infection by traditional practitioners should not be discouraged. Therefore, the inhibitory activity of the *C. procera* extract was verified in this study and thus established the medicinal use of the plant by local folks to treat fungal infection.

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Mycological Evaluation of Smoked Dried *Clarias gariepinus* and *Tilapia zilli* sold in Minna Metropolis

***Oyero J. O., Adejola E. O. & Olatunji P. O.**

Department of Water Resources, Aquaculture and Fisheries Technology, Federal University of Technology, Minna, Niger State

*Corresponding author: hadassahadejola@gmail.com; 09015178591

ABSTRACT

The study was conducted to evaluate the mycological assessment on smoked dried *Clarias gariepinus* and *Tilapia zilli* sold in open market of Minna metropolis using traditional smoking kiln and improved smoking kiln. The fish samples were obtained from the Kure, Mobil market and Larepo concept farm in Minna, Niger state. The mycological load on each sample was determined using the standard serial dilution method procedure. The colony forming unit on the sample obtained from the two smoking kiln was significantly different ($p<0.05$) in *Tilapia zilli* but there was no significant difference ($p>0.05$) in the *Clarias gariepinus* sample from the two smoking kiln. All the samples collected were contaminated with fungi growth except fish from sample improved smoking kiln ($ITSKT_3$) which revealed no fungi growth. However, improved traditional smoking shows low level of fungi contamination varying from $2.0\times10^5$to 3.0×10^5 cfu/g in ($ITSKC_1$ and $ITSCK_3$). While traditional smoked *Clarias gariepinus* range from 3.0×10^5 to 7.0×10^5 cfu/g in $ITSCK_2$ and $ITSCK_1$ respectively. Similarly, the level of contamination of fungi *T.zilli* in traditional smoking kiln was observed to be higher which ranges between $7.0\times10^5$to 6.8×10^6 cfu/g in $TSKT_1$ and $TSKT_2$ respectively while (ITSKT3 shows no growth) as against $3.0\times10^5$to $4.0\times10^5$of $ITSKT_1$ and $ITSK_2$ respectively.

Keywords: mycological evaluation, fungi, *Clarias gariepinus,Tilapia zilli,* kiln

INTRODUCTION

Fish constitutes an important source of protein intake of many people in the world which is very cheap compared to other sources of protein (Fawole *et al*., 2018). Despite the value of fish, fish is a highly perishable material that deteriorates soon after harvest at high ambient temperature therefore requiring immediate preservation methods. Oyero (2006) reported that the deterioration leads to spoilage which causes change in organoleptic qualities such as appearance, texture, smell, taste and flavor due to presence of pathogenic microorganisms and their toxins. The action starts immediately after the death of fish which is usually accompanied with enzymatic digestion, oxidation of fat, microbial action and bacterial decomposition which plays a huge role in the spoilage of fish (Eyo, 2001).

Fish are usually hawked without taking cognizance of the microbial contamination from the environment. In Nigeria, smoked fish products could be contaminated with

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microorganisms from the processing units and market place before reaching end consumers owing to the fact that processors and hawkers display them in an open manner that could be possible sources of microbial contamination (Fawole *et al*.2018). Mycological action has been playing a huge role in the spoilage of fish and microbial contamination of food is an obstacle of food safety that must be tackled. The effect of mycology infestation on fish and fisheries product is usually caused by *Aspergillus, Penicillium, yeast* and mould (Eyo,2001). In view of the above an experiment was conducted to evaluate the mycological assessment on smoked dried *Clarias gariepinus* and *Tilapia zilli* sold in open market of Minna metropolis using traditional smoking kiln and improved smoking kiln.

METHODOLOGY

Sample collection

The fish samples *Clarias gariepinus* and *Tilapia zilli* with total weight of 200g and 110g respectively were obtained from Kure and Mobil market for traditional smoking sample while the improved traditional smoking kiln samples were obtained from Lapero concept farm.

Determination of mycological load of smoked dried fish

To determine the level of fungi contamination, 1g of the sample was taken to estimate the number of living organisms per gram. Standard serial dilution method as described by Onwuka (2005) was employed in the determination of the total mycological count. All apparatus used were autoclaved for 30 minutes at 121^0C.

Experimental design

The data obtained were subjected to independent variable (T-test) to test the data variable using SPSS version 20.

RESULTS AND DISCUSSION

Table 1. Shows the fungi load of smoked dried *Clarias gariepinus and Tilapia zilli*

Sample	Fungi count (cfu/g)
$ITSKC_1$	2.3 ± 1.20^a
$ITSKC_2$	5.0 ± 1.15^a
ITSKT	1.50 ± 0.28^b
TSKT	6.00 ± 1.22^a

Mean with different superscript on a column are significantly different ($p<0.05$)

ITSKC-Improved traditional smoking kiln *Clarias gariepinus*, TSKC-Traditional smoking kiln *Clarias gariepinus*, ITSKT-Improved traditional smoking kiln *Tilapia zilli* and TKST Traditional smoking kiln *Tilapia zilli.*

Table 2. Shows the fungi load of smoke dried *Clarias gariepinus* and *Tilapia zilli*

Sample	Fungi count (cfu/g)
$ITSKC_1$	3.0×10^5
$ITSKC_2$	2.0×10^5
$ITSKC_3$	3.0×10^5
$TSKC_1$	7.0×10^5
$TSKC_2$	3.0×10^5
$TSKC_3$	5.0×10^5
$ITSKT_1$	3.0×10^5
$ITSKT_2$	4.0×10^5
$ITSKT_3$	NG (no growth)
$TSKT_1$	1.0×10^5
$TSKT_2$	6.8×10^6
$TSKT_3$	3.4×10^6

ITSKC-Improved traditional smoking kiln *Clarias gariepinus*, TSKC-Traditional smoking kiln *Clarias gariepinus*, ITSKT-Improved traditional smoking kiln *Tilapia zilli* and TKST Traditional smoking kiln *Tilapia zilli.*

The results obtained from the table shows varying levels of fungi count in smoked dried fish using two different smoking kilns.

The fungi count as presented in table 1shows a varying level of significant difference ($p<0.05$) across the entire sample. However, *Tilapia zilli* smoked with TSK was observed to have a higher value of 6.00cfu/g as compared to 1.50cfu/g recorded from *Tilapia zilli* smoked with ITSK with difference of 4.50cfu/g. Similarly, the *Clarias gariepinus* smoked with TSK was observed to have higher fungi load with difference of 2.33cfu/g when compared with 5.000cfu/g of *Clarias gariepinus* smoked with ITSK. This agrees with Ayeloja *et al.* 2018 whose studies established that smoking kiln used in artisanal fishery, poor processing method, handling and mode of storage is mostly responsible for the load of fungi in smoke dried fishery products.

The results obtained from table 2 shows that the fungi load of the smoke dried *Clarias gariepinus* and *Tilapia zilli* shows level of contamination with the exemption of $ITSK_3$ which revealed no fungi growth varying from 2.0×10^5 to3.0×10^5 in ($ITSKC_1$ and $ITSCKC_3$). While traditional smoked *Clarias gariepinus* range from 3.0×10^5 to7.0×10^5 in $TSKC_2$ and $TSKC_1$ respectively. Similarly, the level of contamination of fungi *T.zilli* in

traditional smoking kiln was observed to be higher which ranges between 7.0×10^5 to 6.8×10^6 cfu/g in $TSKT_1$ and $TSKT_2$ respectively while ($ITSKT_3$) which shows no growth as against 3.0×10^5 to $4.0\times10^5$of $ITSKT_1$ and $ITSKT_2$ respectively. This can be due to improper proper processing practices, facilities and unhygienic post processing handling as reported by Akinola *et al*. (2006).

CONCLUSION

Clarias gariepinus and *Tilapia zilli* purchased from Mobil and Kure market smoked with improved traditional smoking kiln technique revealed a lesser level of fungi load than samples subjected to traditional smoking kiln. This is a result of poor processing and handling process which is a major source of contamination.

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Nutritional Compositions of Three Commonly Consumed Powder Vegetables Sold in Lapai, Niger State, Nigeria

***[1]Uthman A., [2]Anigo K. M., [1]Musa A., [3]Abdulazeez A., [4]Garba Y. & [5]Joseph P. S.**

[1]Department of Biochemistry, Ibrahim Badamasi Babangida University, Lapai–Niger state.
[2]Department of Biochemistry, Ahmadu Bello University, Zaria – Kaduna State
[3]Department of Chemistry, Ibrahim Badamasi Babangida University, Lapai – Niger State
[4]Department of Crop production, Ibrahim Badamasi Babangida University, Lapai–Niger State
[5]Department of Chemistry, Air Force Institute of Technology, Kaduna – Kaduna State

*Corresponding author's E-mail: jelilibch@gmail.com. Tel: 07033002211

ABSTRACT

Vegetables form an important part of diets in any given locality as they are major sources of micronutrients for maintenance of good health and prevention of some diseases. Indigenous powder vegetables sold in Lapai market was investigated for their nutritional benefits to the populace that commonly consumed them as part of their major meal. It is against this background that three commonly consumed indigenous powder vegetables were obtained from three different markets in Lapai, Niger state, Nigeria and analyzed for proximate and mineral compositions in *Adansonia digitata* (Baobab leaves), *Abelmoschus esculentus* (Okra) and *Annona senegalensis* (Wild sour sop) using standard analytical procedures. The results obtained showed that the vegetables had moisture contents in the range of 5.44±0.20 – 7.67±0.34 mg/100g, ash content (5.00±0.00 – 16.00±0.00 mg/100g), crude fat (2.33±0.34 – 15.00±0.67 mg/100g), crude fibre (0.24±0.02 – 1.28±0.22 mg/100g), crude protein (10.12±3.90 – 16.46±4.60 mg/100g) and total carbohydrate ranges between 50.21±3.16 – 64.20±3.30 mg/100g. The mineral compositions of the vegetables showed that calcium concentration was between 0.40±0.18 – 1.95±2.64 mg/Kg, Mg (1.07±0.08 – 1.13±0.03 mg/Kg), Cu (0.27±0.00 – 0.49±0.19 mg/Kg), Fe (0.49±0.03 – 0.63±0.05 mg/Kg), Zn (0.28±0.06 – 0.40±0.05 mg/Kg), Na (4.78±0.41 – 8.57±0.39 mg/Kg) and K (68.73±1.96 – 153.20 ±15.29 mg/Kg). The study showed that indigenous powder vegetables contain appreciable amount of both macro and micronutrients (mineral) that could contribute to the nutritional needs of the people.

Keywords: Vegetables, Nutritional composition, Minerals, Baobab leaf and *Annona senegalensis*

INTRODUCTION

Nature has made plants useful throughout the existence of man. Man uses plants as food, clothing, fuel, shelter and in management of different diseases. Green leafy vegetables are very nutrient-dense and incredibly healthy. They are vital sources of antioxidants and are very useful in providing weight loss and maintenance, because it keeps one feeling full and helps

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control hunger. Leafy vegetables contain fiber, vitamins; minerals and antioxidants that protect against free radicals that cause disease (Park *et al.,* 2013; Enker, 2013).

Vegetables are very vital to human, both economically and nutritionally. Economically, they are relatively cheap to grow and act as a quick source of income to many rural women. Nutritionally, they are good source of vitamins, minerals and dietary fibre; and water to aid digestion. Vegetables are rich in minerals such as potassium, sodium, calcium, iron, zinc and phosphorus. They also contain high amount of thiamine, ascorbic acid, riboflavin and β-carotene. The water content of vegetables is about 70% or more and leafy vegetable also contain many phytochemicals which are needed for health-promotion and disease prevention (Fayeme, 1999). They are consumed in very small quantities, and are used in almost every meal or used alone as salad or as a side dish with main meal. They are known to be important sources of protective foods (Nnamani *et al.,* 2009; Sheela *et al.*, 2004). They have also been reported to be good sources of oil, carbohydrates, minerals as well as vitamins (Adenipekun & Oyetunji 2010).

According to George (2003), the potassium content of leafy vegetable is good in the control of diuretic and hypertensive complications. Vegetable fats and oils are known to lower blood lipids thereby reducing the occurrences of diseases associated with the damage of the coronary artery (Adenipekun & Oyetunji 2010). The food value of vegetable is low owing to the large amount of water present (79 – 96 %) (Sharma, 2004). The nutritive value of vegetable is increased greatly because of the presence of minerals and vitamins. Vegetables also serve as roughages that help in digestion (Sharma, 2004). They constitute essential diet components by contributing proteins, vitamins, iron, calcium, and other nutrients that are in short supply.

The most commonly consumed indigenous vegetables in Lapai include *Adansonia digitata* commonly called baobab in English and kuka in Hausa and Nupe; *Abelmoschus esculentus* commonly called okra in English and kpanmi or tsuku in Nupe and *Annona senegalensis* known as wild soursop or wild custard apple in English and nungbere in Nupe. Lapai is one of the Nupeland in Niger state, Nigeria, the leaves/pods of these vegetables are eaten both fresh as well as dry powder form, mostly in making a popular local soup called kuka soup (Eni kuka), okra soup (Eni kpanmi or Tsuku) and Nungbere soup (Eni nungbere).

Adansonia digitata is one of eight species of baobab and is widespread throughout the hot dried region of tropical Africa, mostly in Sudan, Namibia, Ethiopia, Malawi, Zimbabwe and Nigeria (Danthu *et al*., 1995). The fresh tender leaves are usually harvested during rainy season and up to last month of the rainy season into the dry season when part of it is sun-dried for future use (Nordeida *et al.*, 1996). The dried leaves are pounded and sieved into flour for future use as green vegetable in soup preparation. Apart from the leaves as vegetable, the bark and root are used in many traditional medicines (FAO, 1988). Seeds are used as a thickening agent in soups, but they can be fermented and used as a flavouring agent, or roasted and eaten as snacks (Addy & Eteshola, 1984). Dry leaf powders are also used to prepare sauces (Chadare *et al.,* 2009). Young leaves are widely used, cooked as spinach, or frequently dried, often powdered and used for sauces (Sibibe & Williams, 2002).

Annona senegalensis is known in different languages in English (wild sour sop, wild custard

apple); French (pommecannelle du senegalannone); Nupe (Nungbere); usually known as Gwándàndààjìiin in Hausa and dukuu-hi (Fulani). *Annona senegalensis* (soursop) locally known as nungbere in Nupe land is a species of seed vegetable which grow both on dry and raining seasons. It is a savanna plant which is widely spread from Senegal to Nigeria and also in Central African Republic (Abdullahi *et al.*, 2003). *Annona senegalensis* is a shrub or small tree 2-6 m tall but may reach 11m under favourable conditions; bark smooth to roughish, silvery grey or grey-brown, with leaf scars and roughly circular flakes exposing patches of the bark. *Annona senegalensis* had been reported as vegetables used in soup, fodder (livestock feeding), as timber used for making poles and tool handles, in tannin (dyestuff), essential oil (car-3-ene) and effective insecticide (Orwa *et al.,* 2009). The bark is used for treating guinea worms and other worms, diarrhoea, gastroenteritis, snakebite, tooth ache and respiratory infections. Gum from the bark is used in sealing cuts and wounds. The leaves are used for treating pneumonia and as a tonic to promote general well-being. The roots are used for stomach-ache, venereal diseases, chest colds and dizziness. Various plant parts are combined for treating dermatological diseases and ophthalmic disorders. (Orwa *et al.,* 2009).

Okra (*Abelmoschus esculentus*) is an important vegetable crop, the fresh fruit is known as "kpanmi" while the dry powder form is known as "tsuku" in Nupe language. Okra is a multipurpose crop due to its various uses of the pods, fresh leaves, buds, flowers, stems, and seeds. Okra immature fruits (pods), which are consumed as vegetables, can be used in salads, soups and stews, fried or boiled (Habtamu *et al.*, 2014). Despite its nutritional compositions, okra pod is a powerhouse of valuable nutrients (Adetuyi *et al*. 2011) and affordable source of protein, carbohydrates, minerals, vitamins, and dietary fiber (Habtamu *et al.*, 2014). Therefore, promoting the consumption of okra pods could provide cheap sources of nutrients that can improve the nutritional status and reducing the prevalence of malnutrition especially among resource-constrained households and can also be used as a means of dietary diversification. This study is therefore aimed at evaluating the nutritional constituents of the dry powder vegetables usually consumed by the people of the study area.

METHODOLOGY

Study Area

The study was conducted in Lapai town, Niger State, Nigeria. Lapai is situated between the latitudes 09°04' N and 09°05' N and longitudes 06° 34' E and 06°35'E of the equator. The major economic activity of the people of Lapai is farming with specialization in crops, animal husbandry and fishing.

Sources of Samples

The powder vegetables of *Adansonia digitata* (kuka), *Abelmoschus esculentus* (tsuku) and *Annona senegalensis* (nungbere) were bought from three different markets in Lapai township, Lapai Local Government Area of Niger state. The powders were sieved and used for the analysis.

Chemical Analysis

Triplicate sample of each vegetable was used to analyze for proximate composition using the standard methods of the Association of official analytical chemistry (AOAC) (2000). Moisture content was calculated from a weighed sample after heating at 105^{o}C for 4hrs. Total nitrogen

was determined by the Micro-Kjeldahl method, and crude protein estimated by multiplying the total nitrogen (N) by 6.25, a conversion factor. Total crude fats were estimated by petroleum ether extraction using soxhlet extractor apparatus and crude fibre was determined by digesting the sample with 1.25% H_2SO_4 and 1.25% NaOH. Total ash was estimated after incinerating sample in muffle furnace (SXL-1008) for 6hrs at 550^oC and carbohydrate content was estimated by difference. Mineral elements were determined according to AOAC (2000). Exactly 1.0g of each sample was ash in a muffle furnace (SXL-1008) at the temperature of 550^oC and the ashes of various samples were dissolved in 10ml of 0.1M HCl, filtered and made up with distilled water to the mark in a 100ml volumetric flask. The mineral elements Mg, Ca, Zn, Fe, Cu, were determined using Atomic Absorption Spectrophotometer (AAS) (AA500 spectrophotometer, Pg Instrument) while Na and K were determined using Flame photometer (Jenway, PFP 7).

Statistical Analysis

The data obtained were subjected to Oneway ANOVA using IBM SPSS statistics 23 package. Means were separated using Duncan's Multiple Range Test (DMRT). Significant difference was accepted at $p<0.05$. Values are given as Mean ± S.D.

RESULTS

Proximate composition of powder vegetables

Proximate composition of the vegetables is presented in Table 1. It shows that the moisture content of the vegetables varies between 5.44±0.20 and 7.67±0.34 g/100g. *Adansonia digitata* (7.67±0.34 g/100g) had the highest value while *Annona senegalensis* (5.44±0.20 g/100g) had the least moisture content. The ash content ranged from 5.00±0.00 to 16.00±0.00 g/100g, with *Adansonia digitata* (16.00±0.00 g/100g) having the highest ash content amongst the other vegetables. The ash content for *Adansonia digitata* was significantly ($p<0.05$) higher when compared to the other vegetables. The crude fat of the vegetables shows that *Annona senegalensis* (15.00±0.67 g/100g) had the highest mean value, followed by *Adansonia digitata* (10.44±1.02 g/100g) while *Abelmoschus esculentus* (2.33±0.34 g/100g) recorded the least value. There was significant ($p<0.05$) difference between the mean values for fat content of the three vegetables. The range of crude fibre of the samples is between 0.24±0.02 to 1.28±0.22 g/100g, with *Annona senegalensis* and *Adansonia digitata* having the lowest and highest contents respectively. Crude protein of the samples was from 10.12±3.90 to 16.46±4.60 g/100g with *Annona senegalensis* (10.12±3.90 g/100g) having the lowest content while *Abelmoschus esculentus* had the highest crude protein content (16.46±4.60 g/100g). The table also revealed that the total carbohydrate of the three vegetables were high. It ranges between 50.21±3.16 to 64.20±3.30 g/100g, with *Annona senegalensis* (64.20±3.30 g/100g) recording the highest value, followed by *Abelmoschus esculentus* (63.71±4.77 g/100g) while *Adansonia digitata* (50.21±3.16 g/100g) had the least value. The mean value of *Adansonia digitata* was significantly ($p< 0.05$) different from the two other samples.

Table 1: Proximate Composition of Powder Vegetables (g/100g dry matter)

Vegetables	Moisture Content	Ash Content	Crude Fat	Crude Fibre	Crude Protein	Total Carbohydrate
Adansonia digitate	7.67±0.34[b]	16.00±0.00[c]	10.44±1.02[b]	1.28±0.22[c]	14.40±2.05[a]	50.21±3.16[a]
Abelmoschus esculentus	7.56±0.20[b]	9.17±0.76[b]	2.33±0.34[a]	0.77±0.32[b]	16.46±4.60[a]	63.71±4.77[b]
Annona enegalensis	5.44±0.20[a]	5.00±0.00[a]	15.00±0.67[c]	0.24±0.02[a]	10.12±3.90[a]	64.20±3.30[b]

Values are Mean ± S.D of triplicate determinations. Value with different superscripts down the column are significantly different ($p<0.05$).

Mineral Contents of Powder Vegetables

The calcium concentration of the powder vegetables shows that *Adansonia digitata* had the highest concentration (1.95±2.64 mg/Kg), followed by *Annona senegalensis* (0.50±0.08 mg/Kg) whereas *Abelmoschus esculentus* had the lowest concentration (0.40±0.18 mg/Kg). There was no significant ($p>0.05$) difference in the calcium content amongst the vegetables. The magnesium contents ranged from 1.07±0.08 - 1.13±0.03 mg/Kg, with *Abelmoschus esculentus* recording the highest value while *Annona senegalensis* had the lowest content. Copper content indicates *Abelmoschus esculentus* (0.49±0.19 mg/Kg) had the highest value, followed by *Annona senegalensis* (0.41±0.04 mg/Kg) while *Adansonia digitata* (0.27±0.00 mg/Kg) had the least value. There was no significant ($p>0.05$) difference in the copper concentration amongst the three samples. Level of iron content in the samples revealed that *Abelmoschus esculentus* (0.63±0.05 mg/Kg) had the highest values while *Adansonia digitata* (0.49±0.03 mg/kg) recorded the least mean value. The iron content in *Abelmoschus esculentus* was significantly ($p<0.05$) higher when compared to the two other samples. The highest concentration of zinc was found in *Abelmoschus esculentus* (0.40±0.05 mg/Kg), followed by *Annona senegalensis* (0.36±0.07 mg/Kg) while the lowest concentration was found in *Adansonia digitata* (0.28±0.06 mg/kg). Among the studied powder vegetables, *Abelmoschus esculentus* (8.57±0.39 mg/Kg) had the highest concentration of sodium while *Adansonia digitata* (4.78±0.41 mg/kg) recorded the least value. Similarly, the same trend was observed for potassium content with *Abelmoschus esculentus* (153.20±15.29 mg/Kg) recording the highest mean value and *Adansonia digitata* (68.73±1.96 mg/kg) had the lowest concentration. There was significant ($p<0.05$) difference in potassium content amongst the samples (Table 2).

Table 2: Mineral Contents of Powder Vegetables (mg/Kg)

Vegetables	Ca	Mg	Cu	Fe	Zn	Na	K
Adansonia digitata	1.95±2.64[a]	1.10±0.09[a]	0.27±0.00[a]	0.49±0.03[a]	0.28±0.06[a]	4.78±0.41[a]	68.73±1.96[a]
Abelmoschus esculentus	0.40±0.18[a]	1.13±0.03[a]	0.49±0.19[a]	0.63±0.05[b]	0.40±0.05[a]	8.57±0.39[b]	153.20±15.29[c]
Annona senegalensis	0.50±0.08[a]	1.07±0.08[a]	0.41±0.04[a]	0.55±0.03[a]	0.36±0.07[a]	7.72±0.74[b]	121.00±17.65[b]

Values are Mean ± S.D of triplicate determinations. Value with different superscripts down the column are significantly different ($p<0.05$).

DISCUSSION

The nutrient compositions of three commonly consumed indigenous powder vegetables sold in Lapai markets were assessed for their proximate and mineral analyses and the result indicates that the moisture contents were low. Ijeomah *et al.,* (2012) reported high moisture content for *Adasonia digitata,* (9.52 mg/100g), 11.7 mg/100g for *C. sesamoides* and 26.0 mg/100g for *A. esculentus.* The moisture content of *A. senegalensi* in this study was 5.44±0.20 g/100g, which is lower than the value of 12.2% reported by Yisa *et al.*, (2010), 5.98% value was observed for *Hibiscus sabdariffa* seeds (Anhwange *et al.*, 2006) and value of 9.69 – 13.33 g/100g for *Abelmoschus esculentus* pod (Habtanu *et al.*, 2016). More so, 11.02% was recorded for *Acalypha hispida* leaves (Abdulazeez *et al.*, 2020), which was also higher when compared to the moisture content in this present study. The low moisture content of the three vegetables shows that it can be preserved for long time without microbial spoilage. However, low moisture content of any food products, means the nutrients are more concentrated and the longer the shelf life of the product (Zakpea *et al.,* 2010).

The ash contents of *Adansonia digitata* (16 g/100g) had the highest value amongst the other two vegetables. This means that *A. digitata* is a better source of mineral deposit than the other two vegetables. This value was higher than the value reported for *Vernonia amygdalina* (9%) and *Corchorus olitorius* (10%) (Oguntona, 1998). Similarly, the ash content recorded for *Abelmoschus esculentus* pod (5.37-11.30 g/100g) was lower than the value for *A. digitata* but was in agreement with the value for *Abelmoschus esculentus* (Habtanu *et al.*, 2016) and that of Adetuyi *et al.,* (2011) (7.19-9.63%) for okra varieties. However, the ash content of *A. senegalensis* in this study was lower than 12.1% reported for the same plant by Yisa *et al.,* (2010). *A. senegalensis* had the highest crude fat content amongst the vegetables, which indicates that the vegetable is a good source of fat/lipid and the crude fat values of the three powder vegetables were significantly ($p<0.05$) different from each other. However, Yisa *et al,* (2010) reported higher value (24%) for *A. senegalensis*. However, low crude fat contents were observed for *A.digitata* (2.12 mg/g), 1.74 mg/g in *C. sesamoides* and a high content in *A. esculentus* (17.22 mg/g) (Ijeomah, *et al.,* 2012). The crude

fat content of dried wild leafy vegetables reported by Raymond *et al.* (2015) were lower when compared to the present study; *Emilia coccinea* (0.57%), *Ficus thonningii* (2.57 %), *Annona senegalensis* (2.94%) and *Hibiscus sabdariffa* (3.43%). Fat is necessary in diet because it serves several vital functions (Levetin, 2008).

Crude fibre was highest in *A. digitata* and lowest in *A. senegalensis*. However, these values were comparatively lower to the value of 8 – 11.96% reported for baobab leaves subjected to different drying methods (Adejuyitan *et al.,* 2014), crude fibre for dried wild leafy vegetables in Benue state (10.51-36.86%) (Raymond *et al.,* 2015) and Yisa *et al.,* (2010) reported higher value of 17.60% for crude fibre in *A. senegalensis*. Vegetable leaves with high crude fibre may contribute to a reduction in the incidence of certain diseases like colon cancer, coronary heart disease, diabetes, high blood pressure, obesity and other digestive disorders (Ogunlade *et al.,* 2011). The crude protein of the vegetables shows that *A. esculentus* had the highest value even though there was no significant ($p>0.05$) difference in mean value of each vegetable. But this value was lower than 20.12% of *Gnetum africanum* (Okerulu & Onyema, 2015) and this compared favourably with 17.5% recorded by Ekop (2007) for the seeds of *Gnetum africanum* and Adetuyi *et al*. (2011) reported 13.61-16.27% of protein for varieties of okra. Plant protein still remains a major source of food nutrient for the less privileged population in developing countries like Nigeria such that the protein content of the leaves makes it suitable for consumption and a rich source of vegetable protein (Emedu & Anyika, 2011). The relatively high protein content suggests high amount of essential amino acids which, serve as an alternative source of energy when the carbohydrate metabolism is impaired via gluconeogenesis (Iheanacho & Udebuani, 2009).

The carbohydrate contents of the vegetables revealed that *A. senegalensis* had the highest content though not significantly ($p>0.05$) different from *A. esculentus* while *A. digitata* recorded the lowest value. These values were higher than the value reported for *A. esculentus* (21.66 mg/100g), *A. digitata* (23.02 mg/100g) and 35.72 mg/100g for *C. sesamoides* (Ijeomah *et al.,* 2012). However, the results were in agreement with 52.39% for *Gnetum africanum* leaves (Okerulu & Onyema, 2015), 52.32% reported for *Pachiraglabra* and 45.92% for *A. Africana* seed flowers (Ogungbele, 2006) and 52.18% for *A. hydridus* (Akubugwo *et al.,* 2007). Most vegetables are generally not good sources of carbohydrates (Emebu & Anyika, 2011) but these vegetables could serve as good sources. As far as vegetables are concerned, some of them are rich sources of fibre while others contain traces of some nutrients. Carbohydrate provides the body with a source of fuel and energy for daily activities (Yisa *et al.,* 2010).

Mineral Compositions

The mineral compositions of the three powder vegetables indicate that *Adansonia digitata* had the highest calcium content and this indicates that it is a better source of calcium than the two other vegetables. Although, they were relatively lower when compare to 18.2 mg/100g in *A. digitata*, *C. sesamoides* (50.05 mg/100g) and 170.60 mg/100g in *A. esculentus* (Ijeomah *et al.,* 2012). *A. senegalensis* was reported to be 1.35 mg/g of calcium content (Yisa *et al.,* 2010) and 11.20 mg/kg for *Gnetum africanum* leaves (Okerulu & Onyema, 2015) which were much higher than the value obtained in this present

study. Calcium is a vital element that helps in bone formation and blood coagulation whose deficiency may contribute to rickets, curvature of the spine, pelvic and thoracic deformities (Wardlaw & Smith, 2006). Magnesium concentration in the vegetables revealed that *A. esculentus* had the highest value and *A. senegalensis* recorded the lowest content. Okerulu & Onyema (2015) reported a higher value of 12.00 mg/kg for *Gnetum africanum* leaves and a value of 240 mg/kg for *A. senegalensis* (Yisa *et al.*, 2010). The presence of magnesium in these leaves may prevent cardiomyopathy, impaired spermatogenesis and bleeding disorders (Chaturvedi *et al.,* 2004). *A. esculentus* power leaf had the highest copper concentration followed by *A. sengalensis* and the lowest was observed in *A. digitata*, this may suggests that *A. esculentus* could provides more copper contents to the body than the two other vegetables. Similar result had been reported for *G. africanum* leaves (Okerulu & Onyema, 2015) but a higher value of 0.78 mg/kg of copper had been reported for *Acalpha hispida* leaf (AbdulAzeez *et al.,* 2020). Copper is essential for red blood cell formation and an enzymatic element for normal plant growth and development but can be toxic at excess dose (Levetin, 2008). Diagnosed deficiency is rare but when it become deficient, it may lead to anemia, impaired immunity and bone diseases (Levetin, 2008). Iron is an essential component of human heamoglobin and helps to facilitates the oxidation of carbohydrates, protein and fat and hence help to regulate body weight, which is a very important factor in diabetes.

The result also revealed that *A. esculentus* had the highest iron concentration than the other two vegetables. But much higher iron content had been reported for *G. africanum* leaves (7.23 mg/kg) (Okerulu & Onyema, 2015) and 1,800 mg/kg for *A. senegalensis* (Yisa *et al.*, 2010). The zinc contents in this study are far below those reported for local okra variety of 12.9 – 13.7 mg/kg (Adetuyi et al., 2011) and 2.40 mg/kg for *G. africanum* leaves (Okerulu & Onyema, 2015). Zinc is an essential trace element needed for healthy plant growth and plays an important role in biochemical and physiological processes including normal growth, brain development, behavioural response, bone formation and wound healing. Zinc is involved in RNA and DNA synthesis, which influences cell division, repair and growth. Zinc may enhance cell growth and multiplication (Rosenkranz *et al.*, 2017). Zinc deficiency in diabetic patient could impair power to perceive odour and loss of sensation to the skin. Deficiency of zinc can lead to recurrent infections, lack of immunity and poor growth, male hypogonadism, skin changes, poor appetite and metal lethargy (Prasad, 2020).

The sodium concentration of the dry powder vegetables showed that *A.esculentus* had the highest content but not significantly ($p>0.05$) different from each other. Similar trend was observed for potassium, where *A. esculentus* recorded the highest concentration and followed by *A. senegalensis*. But the concentrations are significantly ($p<0.05$) different from each other. Potassium and sodium are essential electrolytes found in biological fluids and are central to the maintenance of cellular membrane potential, osmoregulation as well as transmit nerve impulse and muscle cells. Their deficiency has been associated with impaired renal function, alterations of gastric secretions and intestinal motility (Princewill-Obgonna *et al*., 2019). Potassium also influence glucose and lipid metabolism. Increase intake of potassium can lower blood pressure and may help prevent strokes. However excess potassium intake (hyperkalamia) may lead to heart failure and

death. Similarly, high sodium intake has been associated with high fluid retention, leading to hypertension and heart failure.

CONCLUSION

The nutrient compositions in the investigated dry powder vegetables mostly consumed in Lapai township are adequate in nutrients and can therefore help to reduce or prevent diseases.

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Investigation of the Diversity of Keratinophilic Fungi of the Animal House of Ibrahim Badamasi Babangida University Lapai, Niger State, Nigeria

***Hamza U. I., Tafida A. M., Yahaya I., Ndayako H. H., Gabi A. U., Salihu I. M., & Aliyu A. D.**

Department of Biological Sciences, Ibrahim Badamasi Babangida University, P. M. B. 11, Lapai, Niger State, Nigeria.

*Corresponding author's email: uihamza@yahoo.com

ABSTRACT

The dominant group of organisms known to utilize keratin as the sole source of carbon and nitrogen are keratinophilic fungi. The main thrust of this research was to investigate the occurrence of keratinophilic fungal species of Ibrahim Badamasi Babangida University Lapai Animal house. Hair-baiting technique of Vanbreuseghem was used for the isolation of the fungi from three sites (cattle house; goat house, and sheep house). Diversity indexes such as Shannon-Wiener, Simpson dominance index and Evenness were used to ascertain fungi diversity. A total of ninety two (92) isolates were identified into eight (8) different genera, viz: *Aspergillus, Chrysosporium, Curvularia, Microsporum, Mucor, Penicilium, Trichoderma*, and *Trichophyton.* Maximum percentage of contribution was observed with *Aspergillus species* in all the three sites. While minimum percentage of contribution was observed with *Microsporum species* in all the three sites. *Aspergillus species* were the common species in all the sites while *Trichoderma species* were the rare species in all the sites. Dominance and Evenness were high in Sheep house with the values 2.403 and 0.057 respectively and low in Goat house with the values 0.441 and 0.014 for Dominance and evenness respectively. According to Shannon's diversity index, sheep house had the highest value (2.566) while Goat house had the lowest value (1.496). Similarly, Simpson's diversity index was found to be highest in Goat house (1.266) and lowest in Cow house (0.070). A very high diversity of keratinophilic fungi observed in this study is a noteworthy finding for public health significance.

Keywords: isolates, carbon, nitrogen, hair-baiting, sites, public health

INTRODUCTION

Fungi are a group of microorganisms with a large distribution in soil (Malek *et al.*, 2013). These organisms play a crucial role within the soil system and soil-borne plant life diseases (Ziaee *et al.*, 2016). Variety of soil fungi have been identified as potential pathogens of humans and animals (Zarrin & Haghgoo, 2011). Keratinophilic fungi are types of fungi that live within the soil and colonize numerous keratinous substrates, turn out keratinases, and decompose them into parts with lower mass (Jain & Sharma, 2012).

The flexibility of fungi to invade and colonize scleroproteinous tissues is closely related to their ability to use keratin (Kwon-Chung & Bennett, 1992). Dermatophytes are

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keratinophilic fungi that are typically anthropophilic or zoophilic in their natural surroundings. However, a number of these fungi occur within the soil as saprophytes that are termed as geophilic dermatophytes (Gugnani, 2000). On the other hand, the non-dermatophyte fungi, like *Aspergillus flavus, Fusarium Oxysporum,* and *Chrysosporium* species have the flexibility to colonize around hair and are isolated from the cutaneal lesions of humans and animals as timeserving agents (Kotwal & Sumbali, 2016). Some of these fungi are motive agents of cutaneal plant life infections named dermatomycosis. The prevalence of these fungi depends on various factors, like the presence of creatinine within the soil, pH, and geographical location (Deshmukh & Verekar, 2006). A number of these fungi, like dermatophytes, are known to cause tinea infections that may well be transmitted from soil to humans (Kimanya, 2013). In general, soil may be thought-about as a reservoir for human infection (Pereira *et al.*, 2019). Forests, farmyards, park soils, and sediments of the rivers and oceans containing humus and organic materials are the most effective candidates for growth of keratinolytic and saprophytic fungi (Ali & Jamous, 2000).

The presence of keratinophilic fungi in animal house soils has serious consequences as these animals bear dermatophytes on their skin without showing any sign of infection (Jangid & Begum, 2018). Depending on the nature of soil and climate, keratinophilic fungi species can differ from country to country (Irum *et al*., 2007).

In recent years, several researchers have reported the isolation of keratinophilic fungi from different parts of the world (Gupta *et al.,* 2012; Deshmukh *et al*., 2008; Deshmukh & Verekar, 2011; Anbu *et al.,* 2004 & Vidyasagar *et al.,* 2005). Similar studies have also been carried out in Nigeria (Nwokeoma *et al.*, 2017; Hamza *et al.*, 2018) however, there is no documented report on the diversity of keratinophilic fungi from the soil of IBBU Lapai, Animal house. Thus, the objective of this study was aimed at the isolation and identification of keratinophilic fungi from the soil of IBBU Lapai Animal house.

METHODOLOGY

Study Area

This study was carried out at Ibrahim Badamasi Babangida University Lapai School farm in Niger State

Samples Collection

Sixty (60) soil samples were collected from five different sites in the school farm. These sites were mapped out into four regions each *viz,* North, South, West, and East respectively. The soil samples were collected from the topmost part of the soil (depth of 5 cm with a hand trowel and were transferred into a sterilized polyethylene bag and labeled accordingly. The soil samples were then transported to the laboratory for further analysis Human hair was also obtained and defatted by soaking in diethyl ether for twenty four hour.

Isolation of Fungi Using Hair Baiting Method

Hair baiting technique of Vanbreuseghem (1952) was employed for the isolation of keratinophilic fungi. 50g of each soil sample was measured using weighing balance into sterile petri dishes with a replicate of three (3) petri dishes each. Human hair was then spread over the soil samples in the petri dishes and was moistened with distilled water and incubated at room temperature. Fungal growths observed were

sub-cultured severally in order to obtain pure cultures.

Identification of Fungi

A single drop of lacto phenol cotton blue was placed on a sterilized microscopic slide and an inoculum from fungus culture was picked and transferred onto the slide. The fungal inoculum was mixed with the stain using a teasing needle. Cover slip was placed on the slide to avoid air entrapment and was examined under a microscope. The morphology of each of the observed structures were described and identified based on their characteristics. Micrographs of Watanabe (2002) David *et al*. (2007) were used as atlas for comparison.

Statistical analyses

Percentage of Contribution and Frequency

Percentage of contribution was calculated as well as percentage of frequency using the following formulas.

$$\text{Percentage of contribution} = \frac{Number\ of\ colonies\ of\ fungus\ in\ a\ sample}{total\ number\ of\ all\ colonies\ of\ all\ species\ in\ a\ sample} \times 100$$

$$\text{Percentage of Frequency} = \frac{Number\ of\ samples\ in\ which\ a\ particular\ fungus\ occurred}{total\ number\ of\ samples\ examined} \times 100$$

Based on the frequency occurrences the fungi were grouped as: rare, R= (0-25% frequency), occasional, O = (26-50% frequency), frequent, F= (51-75% frequency) and common, C= (76-100% frequency) species.

Diversity Indexes

Diversity indexes were determined using Shannon's index and Simpson's index

Shannon's index = -Σ*Pi* **ln** (*Pi*). (Shannon, 1948)

Where *Pi* is the proportion of individuals found in species *i*. For a well-sample community, we can estimate this proportion as *Pi* = n*i*/N, where n*i* is the number of individuals in species *I*, N is the total number of individuals in the community **ln** is the natural log and Σ is the sum of the calculations.

Simpson's index (D1) = 1- ΣPi^2 (Simpson, 1949)

Dominance and evenness

Species dominance and evenness were determined using Simpson's equations:

Dominance (D2) = 1/ ΣPi^2

In the Simpson equations, **p** is the proportion (n/N) of individuals of one particular species found (n) divided by the total number of individuals found (N), Σ is still the sum of the calculations, and **s** is the number of species.

Evenness = D2/S

Where D2 is the dominance and S is the number of species.

RESULTS

Table 1 shows the percentage contribution of fungal species isolated from the study sites. *Aspergillus species* had the highest percentage of contribution in all the three sites (Cow house, Goat house and Sheep house) while *Microsporum species* had the least percentage of contribution in all the sites.

Table 1. The Percentage of Contribution of Fungal Isolates from IBBU Lapai Animal House

Fungal species	Cow house		Goat house		Sheep house	
	Colonies	%	Colonies	%	Colonies	%
Aspergillus species	11	57.90	19	61.29	26	61.90
Chrysosporium species	2	10.50	4	12.90	6	14.30
Microsporum species	1	5.30	1	3.23	1	2.40
Mucor species	1	5.30	-	-	-	-
Trichoderma species	2	10.50	3	9.68	2	4.80
Trichophyton species	2	10.50	2	6.45	3	7.10
Penicilium species	-	-	2	6.45	2	4.80
Curvularia species	-	-	-	-	2	4.80
Total	19	100.00	31	100.00	42	100.00

Table 2 Shows Percentage of Frequency of Fungal isolates from IBBU Lapai Animal House. *Aspergillus species* were the common species in all the three sites examined, while *Trichoderma species* were rare in all the three sites examined.

Table 2. Percentge of Frequency of Fungal Isolates from IBBU Lapai Animal House

Fungal species	Cow House	Goat House	Sheep House
Aspergillus species	**C**	**C**	**C**
Chrysosporium species	**R**	**O**	**O**
Microsporum species	**O**	**R**	**R**
Mucor species	**R**	**-**	**-**
Trichoderma species	**R**	**R**	**R**
Trichophyton species	**O**	**R**	**R**
Penicilium species	**-**	**R**	**O**
Curvularia species	**-**	**-**	**R**

Rare, R= (0-25% frequency), occasional, O = (26-50% frequency), frequent, F= (51-75% frequency) and common, C= (76- 100% frequency) species.

Table 3 shows the dominance and evenness of fungal isolates from soil samples of IBBU Lapai Animal House. Based on the dominance, Goat house had the least value (0.441), while Sheep house had the highest value (2.403). Similarly, based on the Evenness, Goat house had the least value (0.014), while Sheep house had the highest value (0.057).

Table 3. Simpson's Dominance and Evenness of Fungal Isolates from the Soil samples of IBBU Lapai Animal House

Index	Cow House	Goat House	Sheep House
Simpson's Dominance	**1.075**	**0.441**	**2.403**
Simpson's Evenness	**0.056**	**0.014**	**0.057**

Shannon's diversity index indicated that sheep house had the highest value (2.566) while Goat house had the least value (1.496). Similarly, Simpson's diversity index was found to be highest in Goat house (1.266) and least in Cow house (0.070) (Table 4).

Table 4. Diversity Index of Fungal Isolates found in Soil samples of IBBU Lapai Animal House

Diversity Index	Cow House	Goat House	Sheep House
Shannon's Index	1.695	1.496	2.566
Simpson's Index	0.070	1.266	0.584

DISCUSSION

The results of the study showed that Keratinophilic fungi are present in thirty-one soil samples of the animal house. This finding support the facts that consider soils to be a major field for fungi and a reservoir for human infection as well as the best media for the growth of keratinophilic and saprophytic fungi, particularly when their humus and organic matter content is high (Ali-Shtayeh and Jamous, 2000; Pakshir *et al*., 2013). The isolation of fungal species such as *Aspergillus* sp., *Chrysosporium* sp., *Curvularia* sp., *Microsporum* sp., *Mucor* sp., *Penicillium* sp., *Trichoderma* sp and *Trichophyton* sp in a relatively high number in this study suggests their dominance in the soil and agrees with findings from related studies on fungi isolation from different soil types around the world. (Ogbonna and Pugh, 1987; Moallaei *et al*., 2006; Ganaie *et al*., 2010; Pakshir *et al*., 2013 ; Bisen & Tiwari 2015). The isolation of *Aspergillus* sp with the highest percentage of contribution in all the three sites (Cow house, Goat house and Sheep house) is of significant health concern since it is reported to be and can cause serious health issues such as opportunistic Aspergillosis when inhaled by people with immunosuppression in particular (Latge, 1999). The fungi are also known to produce a secondary metabolite called aflatoxin. (Hicks *et al*., 1997; Bhosale *et al*., 1999). Aflatoxin instigates strong reactions from its wounds at very low levels. Aflatoxicosis is a condition that is characterized by nausea, abdominal pain, pulmonary edema, seizures, coma, and death (Prescott *et al*., 2005, Kimanya, 2013). Therefore, *Aspergillus* poses a risk to people living or working in and around soils that it inhabits either by causing disease to them, causing food spoilage or causing food poisoning from its secretion of aflatoxin on stored food products (Tournas, 2005).

While *Microsporum species* with the lowest percentage of contribution in all the sites can cause spoilage of foods. This is in line with findings of Hong *et al*., (2010); Jasuja *et al*.,

(2013); Soni & Sharma (2014) and Hamza *et al*., (2018).

The species of *Microsporum* and *Trichophyton* are isolated from all locations. This is similar to the work of Ogbonna & Pugh (1987) and Agu *et al*. (2013), who isolated various species from Nigerian soils of *Microsporum* and *Trichophyton*. Mancianti and Papini (1996), Moallaei *et al*. (2006), Singh *et al*. (2009), Ganaie *et al*. (2010), Gupta *et al*. (2012) and Bisen & Tiwari (2015) also isolated *Microsporum* and *Trichophyton* species, among other fungi, in their respective keratinophilic soil fungi studies. The presence and abundance of soil isolates of the species *Microsporum* and *Trichophyton* is a threat to the public because they cause dermatomycoses, also known as ringworms or tinea (Prescott *et al*., 2005). These cutaneous fungal diseases are the most common fungal diseases of humans worldwide, including *Tinea barbae*-beard hair ringworm, *Tinea capitis*-scalp ringworm, *Tinea cruris*-groin ringworm, *Tinea pedis*-athlete foot and *Tinea unguium*-nail bed ringworm (Woodfolk, 2005). Several researchers have also reported the occurrence of *Mucor* species in soil samples (Kumar *et al*., 2013; Altayyar *et al*., 2016; Zarrin & Haghgoo, 2011). This fungus is known to be among other fungi causing a gaggle of infections known as zygomycosis (mucormycosis). *Curvalaria* and *Trichoderma* species were isolated in this study.

The result of the Percentage of frequency shows that Aspergillus was the common genus in the three sites. This support similar findings of Mini *et al.,* (2011); Olajubu and Folurunso (2014); Ashok *et al.,* (2015); Agu *et al.,* (2013). The dominance of the *Aspergillus* in the soil could be attributed to its spores which are greatly dispersed with high resistance to environmental conditions, and the suitability of the spores to grow in different soil pH concentration. In addition, the genus *Aspergillus* is known to produce some toxins such as aflotoxins, achrotoxins. These toxins, if secreted may inhibit the growth of other fungi.

According to Simpson's Dominance, Goat house had the least value (0.441), while Sheep house had the highest value (2.403). Similarly, according to Simpson's Evenness, Goat house had the least value (0.014), while Sheep house had the highest value (0.057). (Table 3). Dominance is more concern with dominant species. In this case, a few rare species with a few representatives has no effect upon diversity. Evenness on the other hand is the degree to which individual are split among species.

Shannon's diversity index was high in sheep house while Simpson's diversity index was high in Goat house. The higher the values the higher the diversity would be. Hence, it is perfectly logical to say that diversity indices are high in sheep and goat house. Shannon's index is an information statistic index, it assumes all species that are represented in a sample and they are randomly sampled. Simpson's index is the probability that two randomly chosen individuals belong to different species

CONCLUSION

This study showed the diversity of keratinophilic fungi in the soils of Animal house at IBBU Lapai. Their presence in a relatively high number shows that the animal house are contaminated with keratinophilic fungi and dermatophytes such as *Aspergillus*, *Chrysosporium*, *Curvularia*, *Microsporum*, *Mucor*, *Penicillium*, *Trichoderma*, and *Trichophyton* species. And in view of their significant health concern, there may be a likely huge potential of fungal disease outbreak in the farm area which may be injurious to both the animals and the human population.

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Bacteriological Assessment of Tiger Nut Milk

***Olusola L. F., Agholor K., Ahmed A., Mustapha A. & Mohammed A. S.**

Department of Biological Science, Niger State Polytechnic, Zungeru, Niger State, Nigeria.

Email: *lucyemma366@gmail.com*, sola2015.oe@gmail.com Phone no. +2348131593008

ABSTRACT

Tiger-nut milk, popularly known as Kunu-Aya is a traditional non-alcoholic beverage. This study was conducted to ascertain bacteriological purity of tiger nut milk Sold in minimarket of Niger State Polytechnic Zungeru, Niger State. Nine samples were randomly collected from three different shops and they were evaluated for bacterial load and presence of bacteria using standard microbiological Methods. The result obtained from this study revealed that sample A_1 from shop 1 has the highest total bacterial count (2.0×10^5 cfu/mL), followed by sample B2 from shop 2 (2.2×10^4 cfu/mL). The total coliform count ranged from 1.0×10^2 to 7.0×10^2 cfu/mL the bacteria isolates includes: Bacillus subtilis, Salmonella species, Staphylococcus aureus, Pseudomonas species, Escherichia coli, and Klebsiella species. The Staphylococcus aureus has the highest frequency of occurrence (78%) followed by Bacillus Species while Klebsiella Species had the lowest. The high bacteria count and the presence of potential coliform bacteria in some of the samples is an indication of contamination and this can potentially pose a health hazard to consumers. Hence, there is need to establish a safety standard and regulations for producers and food vendors of kunun-aya and other locally processed beverages made for the public consumption.

***Keywords**: Tiger-nut milk, safety, non-alcoholic, beverage, coliforms, contamination.*

INTRODUCTION

Tiger nut which is scientifically called *Cyperus esculentus* belong to the family of *Cyperaceae,* which produces rhizomes from the base of the tuber that is somewhat spherical. It is a tuber that grow freely and is widely consumed in West Africa, Nigeria inclusive, East Africa and parts of Europe particularly Spain as well as in the Arabian Peninsula. In Nigeria, Tiger nut is locally called by Hausa tribe as "Aya" Yoruba tribe as "Ofio" and Igbo language as "Akiausa"

Tiger nut (*Cyperus esculentus*) is naturally different in colour (black, brown and yellow). However, only two varieties, (yellow and brown) are readily available in the markets. The yellow variety is preferred over others due to its inherent properties such as large size, attractive colour and fleshier nature. It also yield more milk upon extraction, contains lower fat and higher protein and less anti nutritional factors especially polyphone.

Tiger nut (*Cyperus esculentus*) can be prepared and consumed in different form, eaten as snack, roasted, dried, baked (it has a unique sweet that is found to be ideal for use in the baking industry) and can be made into a refreshing beverage called tiger nut milk (Udeozor and Awonorin,

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2016 Okafor *et al*., 2013) It is a good alternative to wheat flour, as it is gluten free and good for people who cannot take gluten in their diet. (Kayode, *et al.,* 2017 Borges, *et al.,* 2014). It is considered a good flour or additive for the baking industry, as its natural sugar (good option for diabetics). The high fiber content of its raw material (tiger nut) makes the product very healthy. Tiger nut flour does not lose any of its nutritional properties in the milling process, (Akoma *et al.,*2016; Aletor *et al.,* 2018; Abodunrin, 2019).

Tiger-nut milk is refreshing and have many health benefits. Report indicates that it contributes to the reduction in Low Density Lipoprotein (LDL), and increases, High Density Lipoprotein (HDL) cholesterol. Gambo and Da'u (2017). Reported that tiger-nut milk are found to be good in preventing arteriosclerosis, and can help prevent heart problems, thrombosis as well as activate blood circulation. Tiger nut milk (*kunun-aya)* is also found to be rich in phosphorus, calcium, magnesium, iron as well as in vitamin C and E and free from lactose and gluten (Belewu and Belewu 2017).

According to Ogodo *et al.,* (2018) Tiger nut milk (Kunun-aya) is rich in vitamins, minerals, digestive enzymes such as amylase, catalase and lipase as well as in phytochemicals and at nutritional factors such as tannins, phytic acids, saponins, glycosides, steroids etc. However, tiger-nut lack sodium, lactose, gluten and cholesterol, a property that makes it suitable for lactose and gluten intolerance patients as well as hypertensive individuals (Adyemi and Umar, 2016; Adekanmi *et al.,* 2018; Adejuyitan *et al.,* 2013). Report also indicates that tiger-nut contain higher essential amino acids than those proposed in the protein to satisfy adult needs Also, tiger nuts are reported to be aphrodisiac and carminative, promoting urine production and menstruation (Gyar, *et al*., 2014)

Tiger-nut is also an excellent source of some useful minerals such as iron and calcium which are essential for body growth and development (Oladele & Aina, 2016). They also contain other mineral elements such as phosphorus, potassium sodium, magnesium, zinc and traces of copper and vitamins E and C (Oladele and Aina, 2016). Its energetic value (100 cal/100g) makes it a very good energetic drink. A very important point is that it does not contain lactose or gluten (Adejuyitan *et al*., 2013; Bibek, 2018).

It is an established fact that bacterial infection has been one of the biggest challenges to human environments particularly where proper hygiene is lacking. One of the simplest way to get assess to human internal body is through consumption of contaminated food thereby posing a threat to human life (Gambo and Da'u, 2017). Even though Tiger nut milk (Kunu-aya) possesses different useful properties which can be of great benefit to human health, however, its production within Niger State Polytechnic Zungeru, Niger state has been affected by the presence of microorganisms that deteriorate the milk and cause spoilage as a result of unhygienic preparation due to the use of contaminated utensils, Poor quality tiger-nuts and impure water. The presence of some pathogenic bacterial in some of the samples have been a matter of serious public health concern. Therefore, this study was designed to look into the bacteriological assessment of Tiger nut milk (Kunu-aya) sold at the School Minimarket, Niger State Polytechnic, Zungeru.

Moreover, this study will be of immense benefit to the consumers and producers of Tiger nut milk

(Kunun-aya), health sector, and government, as the results will educate the general public on the likely bacterial that can contaminate or be associated with Tiger nut milk (Kunun-aya) if exposed or not hygienically prepared and need to keep the environment hygienic. This research will also serve as a resource base to other scholars and researchers interested in carrying out further research in this field of study, if applied will go to an extent to provide new explanation to the topic.

METHODOLOGY

Sample Collection

Tiger nut milk were bought from School minimarket Niger State polytechnic, Zungeru. Three bottles of tiger nut milk were obtained randomly from three different shops and then transferred to the microbiological laboratory of Niger state polytechnic Zungeru for analysis. Serial dilutions were prepared up to 10^{-6} for total bacteria count. Standard bacteriological method were employed for the isolation of bacteria as recommended by (Cheesbrough, 2006)

Preparation of Media

5.6g of nutrient Agar and MacConkey agar were weighed and added to a 200mL of distilled water in a 300mL medical flat bottle and thoroughly mixed by inverting the bottle about 3-4 times to dissolve the powder. The media was then sterilized by autoclaving for 15minutes at 121^0C and subsequently stored at 50^0C incubator to prevent the agar from setting. 20mL of the sterilized nutrient Agar and MacConkey were dispensed into Petri dishes in a sterile cabinet and allowed to cool and set for at least 30-35 minutes. Subsequently, the agar plates were placed in sterile bags and stored in an inverted position at 4^oC pending further use for up to five days.

Serial Dilution

The tiger nut milk were mixed manually by shaking and serially diluted. 1mL of each sample of tiger nut milk was transferred into a test tube containing 9mL of distilled water using sterile pipette and mixed to obtain dilution10^{-1}. One milliliter (1mL) of dilution (10^{-1}) was then transferred into another test tube (10^{-2}) containing 9mL of distilled water using separate 1mL pipette, these transfers were repeated until dilution 10^{-6} was achieved. 1mL was obtained from test tube labeled 10^{-4} and spread evenly on agar plates.

Isolation and Identification of Bacterial Isolate from Tiger Nut Milk

Nutrient agar was used as a general medium for total viable count. MacConkey agar was used for enumerating total coliform count and to isolate lactose fermenting gram negative bacteria MacConkey agar was used to isolate lactose fermenting gram negative bacteria. All plates were incubated at 37^0C for 24hour for microbial growth. Identification of the isolates was based on cultural, morphological and biochemical characteristics.

Gram Staining:

Morphological features of pathogenic bacteria were determined by carrying out the Gram staining technique which was done by smearing a slide with 24 hours bacterial culture and heat fixed. This was then placed on a staining tray; and the smear was flooded with Crystal Violet solution as the primary stain and allowed to stand for 1 minute. The slide was rinsed with distilled water and then flooded with Gram's

iodine for 1min which binds the Crystal Violet with the cells, thereafter; the slides were decolorized with 70% alcohol and counterstained with Safranin for 2 minutes. The slides were then rinsed and air dried before viewing via light microscope using x100 objectives with oil immersion lens.

Biochemical Identification

Indole Test

Procedure – The test organism (isolate) was inoculated in a test tube containing 3mL of sterile tryptone water. Incubation was done at 37^0C for 24hrs. 0.5mL of kovac's reagent was added and shacked gently. The examination for a red ring like colour on the surface of the layer within 10 minute was done.

Catalase Test

Procedure – This was performed by dropping a loopful of hydrogen peroxide on a clean grease free slide followed by the mixing of the loopful of isolate with the hydrogen peroxide on the slide. The production of gas bubbles from the mixture which occurred almost immediately, is a positive reaction

Coagulase test

A loopful of the test isolate is smeared on a slide, mixed with normal saline and treated with a drop of serum which was then mixed together. Agglutination occurs within 5-10 second which shows positive

RESULT AND DISCUSSION

Result

Table 1. Bacterial load of Tiger Nut Milk samples

Shops	Samples	Total bacterial count (cfu/mL)	Total coliform count (cfu/mL)	Total *staphylococcal* count (cfu/mL)
	Sample A_1	2.0×10^5	1.1×10^2	8.0×10^3
	Sample A_2	1.2×10^4	5.0×10^2	5.0×10^3
Shop1	Sample A_3	1.5×10^4	6.0×10^2	5.0×10^3
	Sample B_1	2.1×10^4	4.1×10^2	7.0×10^3
	Sample B_2	2.2×10^4	7.0×10^2	6.0×10^3
Shop2	Sample B_3	1.3×10^4	4.0×10^2	4.0×10^3
	Sample C_1	1.5×10^4	1.0×10^2	6.0×10^3
	Sample C_2	1.9×10^4	6.0×10^2	5.0×10^3
Shop 3	Sample C_3	1.5×10^4	5.0×10^2	4.0×10^3

Table 2. Distribution of the Isolates in the Samples

	Shop 1			Shop 2			Shop 3		
	Sample A_1	Sample A_2	Sample A_3	Sample B_1	Sample B_2	Sample B_3	Sample C_1	Sample C_2	Sample C_3
Bacillus species	+	+	-	+	+	-	+	+	-
Salmonella species	+	-	+	+	+	-	+	+	-
Staphylococcus	+	-	+	-	+	+	+	+	+
Pseudomonas species	+	-	+	-	+	-	-	-	-
Escherichia coli	+	+	+	+	-	-	+	-	-
Klebsiella species	-	-	+	+	-	-	-	-	-

Table 3: Percentage occurrence of the isolates in the samples.

Bacteria	Frequency	Percentage (%)
Bacillus Species	6	67
Salmonella Species	6	67
Staphylococcus aureus	7	78
Pseudomonas Species	3	33
Escherichia Coli	5	56
Klebsiella Species	2	22

Table 3: presents the percentage occurrence of the isolated bacteria from Tiger nut milk (Kunun-aya). The table showed that *Staphylococcu* has the highest value of 7 representing 78%, followed by *Bacillus Species* and *Salmonella Species* having occurrence of 6 accounting for 67%, *Escherichia coli,* 5 given 56%, *Pseudomonas Species*, 3 representing 33% and while *Klebsiella* Species having the lowest value, 2 representing 22%.

DISCUSSION

Table 1: presents the bacteria enumeration in the various samples. It was revealed that sampleA_1 from shop 1 has the highest total bacterial count (2.0 x 10^5 cfu/mL), followed by sample2 from shop2 (2.2 × 10^4 cfu/mL), sampleB_1 from shop2 (2.1 × 10^4 cfu/mL), sampleC_2 from shop3 (1.9 × 10^4 cfu/mL), sampleA_3 from shop1, sampleC_1 from shop3 and sample C_3 from shop3 having (1.5×10^4 cfu/mL) each. Sample A_2 from shop1 and sample B_2 from shop2 showed a count of 1.3 x 10^4 cfu/mL and 1.2 x 10^4 cfu/mL respectively, while the laboratory prepared sample presented the least count of 3.0 x 10^2 cfu/mL. The total coliform count ranged from 1.0 x 10^2 to 7.0×10^2 cfu/mL while the staphylococcal count ranged from 4.0×10^3 to 8.0×10^2 cfu/mL. From the analysis

carried out it could be observed that most of the samples of tiger nut milk bought contained high bacterial count ranging from 3.0×10^2 cfu/mL to 2.2×10^4 cfu/mL sample 2 from shop2 which conformed with the findings of Adyemi, (2016) who reported a total bacterial count ranging from 1.0×10^2 cfu/mL to 8.9×10^4 cfu/mL in tiger nut milk.

The *coliform* count and *staphylococcal* counts in the present study ranged from 1.0×10^2 colonies to 7.0×10^2 cfu/mL and 4.0×10^3 to 8.0×10^3 cfu/mL respectively. The high bacterial count observed in the samples could be attributed to environmental factors (exposure of the samples to soil, air etc.), type of water used in processing as well as personal hygiene of the handlers. It could also be due to microorganisms inherent to the Tiger nut from which the tiger nut milk was obtained which was later multiplied due the milling method and milling machine used. The low counts observed in the laboratory prepared tiger nut milk is an indication that it was aseptically and hygienically prepared and showed a mark of quality product.

Table 2 presents the bacteria isolated from the various samples and their distributions. The result revealed that *Bacillus* species was present in samples A_1 and A_2 from shop 1, samples B_1 and B_2 from shop 2, sampleC_1 and C_2 from shop 3. *Salmonella* species was present in sampleA_1 and sampleA_3 from shop 1, sampleB_1 and sampleB_2 from shop 2, sampleC_1 and sampleC_2 from shop3. *Staphylococcus aureus* was isolated from all the samples except samplesA_2 and B_1. *Pseudomonas* species was present in sampleA_1 from shop 1, sampleB_3 from shop 2 and sample B_2 from shop 2. *Escherichia coli* was presents in sampleA_1, sampleA_2 and sampleA_3 from shop 1, sampleB_1 from shop 2 and sampleC_1 from shop3. *Klebsiella* species was present only in sampleA_3 from shop 1 and sampleB_1 from shop 2.

Furthermore, the finding of the study revealed that the bacteria isolated were *Klebsiella* species, *Bacillus* species, *Staphylococcus aureus*, *Salmonella* species, *Escherichia coli, Pseudomonas* species which agreed with the study of Hubert, *et al*., (2017) who also isolated *E. coli* and *Bacillus* spp, *Enterococcus* spp, *S. aureus* and *P*. *aeruginosa, Streptococcus* spp from tiger-nut, however, with a little deference in the percentage of these bacteria. Similarly, Musa and Hamza (2015) isolated *Staphylococcus aureus*, *E. coli* and *Streptococcus* species from Tiger nut milk (Kunun-aya).

CONCLUSION

In conclusion the bacteriological assessment of Tiger nut milk (Kunun-aya) obtained from Niger State Polytechnic Zungeru School minimarket revealed that tiger-nut milk sampled for the study possesses a high bacteria load which revealed that *Staphylococcus* was the highest bacteria isolated with about 78%, followed by *Bacillus Species* with 67% while *Klebsiella* Species was the least with 22%. It also shown that most of the samples were contaminated with pathogenic microorganisms which are of public health importance. Hence, the need to employ standard hygienic measures during preparation and storage of tiger nut milk, to avoid outbreak of diseases associated with some of the organisms isolated in this study.

Recommendations

Based on the findings, the study recommended that;

i. There should be proper hygiene during the processing and production of tiger nut milk (Kunun-aya).
ii. The environment where tiger nut milk (Kunun-aya) are produced should be properly maintained to avoid contamination from pathogenic bacterial.
iii. Sellers of Tiger nut milk (Kunu Aya) should make sure that the container or bottle used in selling the milk are washed properly.

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Prevalence of Toxigenic Mycoflora in Groundnut Cake (Kulikuli) Sold in Niger State

***[1]Musa M. L., [1]Adebola M. O., [1,2]Aremu M. B., [1]Zainab M. B. & [1]Habib M. B.**

*Correspondence: Musaliman25@gmail.com, 07060788623

[1]Department of Plant Biology, Federal University of Technology, Minna, Nigeria

[2]Nigerian Stored Products Research Institute, Km 3. Asa Dam Road, Ilorin Kwara State

ABSTRACT

Deterioration of groundnut cake ('kuli kuli') a bi-product of processed groundnut oil consumed widely in Nigeria is of great concern. Therefore, this study investigated the toxigenic mycoflora commonly present in kuliKuli sold in the markets in Niger State, Nigeria. A total of Eighteen (18) samples were collected from 10 markets across each of the three agricultural zones of Niger State, namely; Bida, Mokwa (zone 1), Minna, Shiroro (Zone 2), Kotongora, and Kagara (zone 3) respectively. Isolation of associated fungi was done on PDA inoculated with 10^4 dilution factors and incubated at room temperature. A total of 166 fungal species were identified which include *Aspergillus*, *Penicillium*, *Rhizopus,* and *Fussarium.* The order of percentage occurrence (*A. niger* 27.11% being the highest and *F. oxysporum* 4.82% the least) was *A. niger* (27.11%)> *A. flavus* (19.88%)>*P. chrysogenum* (16.87%)> *A. parasiticus* (11.45%)> *Rhizopus spp.* (10.84%)> *A. fumigatus* .(9.03%)> *F. oxysporum* (4.82%. The result indicates that majority of fungi isolated from KuliKuli sold in the markets in Niger State are toxigenic fungi. Therefore, improved management of these oil-rich products from the farm, postharvest storage, and processing will enhance the high-quality product to access a larger market and reduce the risk of health challenges that go with consuming contaminated Kuli Kuli.

Keywords: Toxigenic Mycoflora, Prevalence, Groundnut, Distribution, KuliKuli

INTRODUCTION

Groundnut cake (kulikuli) is one of the products obtained from groundnut seeds after extraction of the oil (Honfo *et al*., 2010). Groundnut cake is one of the most important staple food supplements for the majority of the Nigerian population (Honfo *et al*., 2010; Jolly *et al*., 2009). in Nigeria, it serves as an important source of food and constitutes an inexpensive source of protein, fat, minerals, and vitamins in the diets of rural populations, especially children ('kulikuli) (Adjou *et al*., 2012).

The chemical composition of groundnut seeds per 100g edible portion as reported by USDA (2010) is moisture (6.5g), carbohydrate (16.1g), lipids (49.2g), protein (25.8g), dietary fibre (8.5g), magnesium (168mg), phosphorus (376mg) and iron (4.6mg). Groundnut kernels contain 40-50% fat, 20-50% protein, and 10-20% carbohydrate and are rich in vitamin E, niacin, riboflavin, thiamine, folacin, calcium, phosphorus, magnesium, zinc, iron, and potassium (USDA 2010). Groundnut cake is used

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as a feed supplement for livestock, as fertilizer, and for the preparation of kuli-kuli and (traditional recipe in Nigeria) (Olayinka *et al*., 2013). Kuli-Kuli is a groundnut-based snack that is consumed by all age range and social class among Nigerian populace. It is also used as a major ingredient in poultry feed formulation (Olayinka *et al*., 2013).

The qualitative loss of groundnut cake (kulikuli) can be attributed to biochemical changes in protein, carbohydrates, fatty acids, and vitamins due to fungal contaminations (Waliyar *et al*., 2015). Mostly, kulikuli is vulnerable to microbial contaminants such as fungi (*Aspergillus*, *Fussarium*, and *Penicillium*) that produced mycotoxins which leads to significant economic loss (Sultan and Magan, 2010; Makun *et al*., 2010).

Over the year, poor handling of groundnut cake has been responsible for contaminations leading to varying health challenges such as cancer and liver cirrhosis, weakened immune systems (Wu and Khlangwiset, 2010). Hence, this research studied the prevalence of toxigenic mycoflora in groundnut products (KuliKuli) sold in some markets in Niger State.

MATERIALS AND METHODS

Collection of samples

A total of Eighteen (18) samples of groundnut cake 50g each from four major producing areas collected from 10 markets across each of the three agricultural zones in Niger State, namely Bida, Mokwa (zone 1), Minna, Shiroro (Zone 2), Kotongora, and Kagara (zone 3) respectively were individually homogenized. The samples were collected based on the rate of KuliKuli utilization by the residents and the population.

Preparation of Potato Dextrose Agar (PDA)

Thirty-nine (39) grams of PDA (Hi-media) was suspended in 1000ml distilled water and heated to dissolve the powder completely, the medium was sterilized by autoclaving at 121^{o}C for 15minutes (Manufacturer's guide).

Isolation and Identification of fungi contaminating Kulikuili

One gram(1g) of homogenized kuli-kuli was aseptically suspended into 9ml of sterile distilled water in a test tube and vortexed properly. 1ml was serially diluted up to fourth fold 10^4. 1ml from the fourth dilution fold test tube (10^4) was transferred into a sterile Petri plate. Twenty (20ml) of Potato Dextrose Agar (PDA), to which 1ml of streptomycin was added, and then poured into the petri dish incubated at 28±2^{C} for 3 days. After the third day, a single conidium was picked up with a sterile needle and viewed under microscopic observation, transferred individually to PDA plates, and incubated at ambient temperature (Subramanian *et al.,* 2013). The monoculture was prepared and stored on PDA slants at 40±2^{C}. Subculture was made at regular intervals. The fungal isolate was identified using the fungal family of the world mycological monograph (Cannon and Kirk 2007, Adebola and Amadi 2012) under microscopic observation.

RESULTS AND DISCUSSION

RESULTS

Isolated and Identified toxigenic mycoflora

A total of 166 fungal species were isolated and identified belonging to four genera. *Aspergillus niger* had the highest percentage of occurrence (27.11%) and the least was *F. oxysporum*(4.82%). The general order of occurrence was *A. niger*

(27.11%)> *A. flavus* (19.88%)>*P. chrysogenum* (16.87%)> *A. parasiticus* (11.45%)> *Rhizopus spp.* (10.84%)> *A. fumigatus*. (9.03%)> *F. oxysporum* (4.82%), respectively as shown in table 1 below.

Table 1: Percentage, frequency of fungi isolated groundnut cakes (kuikuli) samples collected in Niger state

S/N	Fungus isolated	Frequency	Percentage %
1	*Aspergillus flavus*	33	19.88
2	*A. parasiticus*	19	11.45
3	*A.niger*	45	27.11
4	*A. fumigatus*	15	9.03
5	*Fussarium species*	8	4.82
6	*Penicillium species*	28	16.87
7	*Rhizopus species*	18	10.84
8	Total	166	100

Characteristics Features of isolated fungi

Aspergillus niger: Rapidly growth colonies on PDA with abundant submerged mycelium, Carbon black/ Deep brownish-black conidial heads. Non-Branched conidiophore with bulb end carries conidia like sun rays. Pin like black growth to pale yellow conidial on reverse Petri dish plate with at initial globose and then radiate well-defined columns.

Aspergillus flavus: Moderate to rapid growth colonies on PDA with Pin-like green growth, Yellow/greyish green. Non-Branched conidiophore with bulb end carries conidia.

***Fussarium spp*:** Colonies appear brown in the center & with white edges, a White cottony colony with dense growth on PDA short crescent conidiophores, septate hyphae with abundant micro-conidia, Spindle-like conidia, and multi-cellular.

Penicillium chrysogenum: Colonies are usually gradually to fast-growing, Green or Green-greyish color colonies with a white ring at the margin, sometimes white, mostly consisting of dense conidiophores. Brush-like conidiophore carries conidia, Conidiophores is hyaline, erect, branched, and penicillate at the apexes with 2-3 metula, 3-4 verticilatephialides, and catenulate conidia in each phialide, forming rather compact cylindrical (Plate 1).

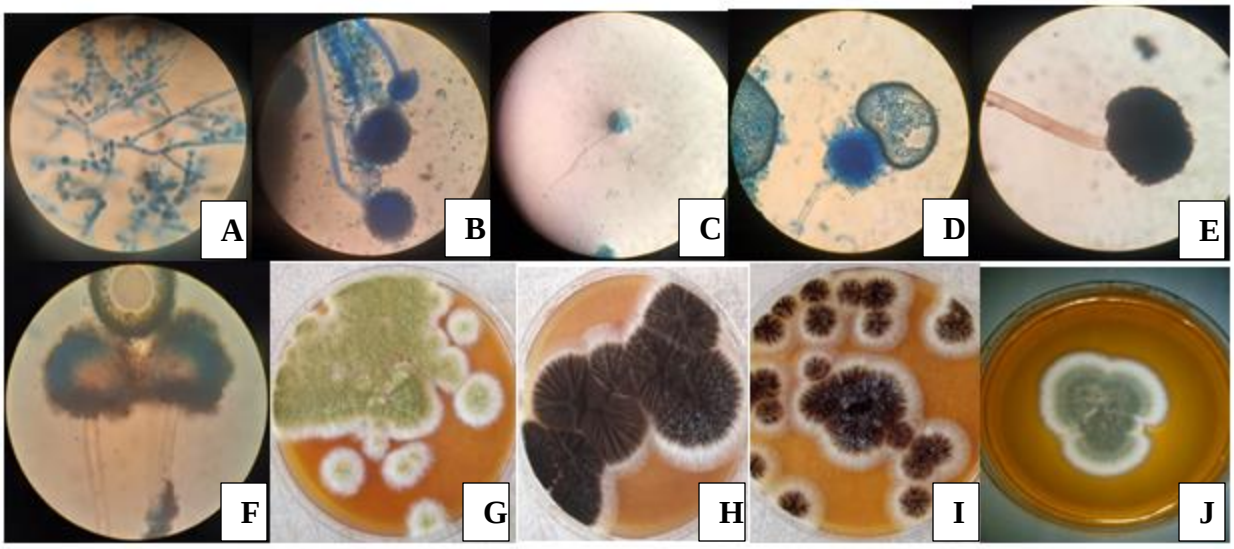

Plate 1. Photomicropha of Isolated and Identified toxigenic mycoflora

A. Fussarium species: B. *A. parasiticus.* C. *P. chrysogenum.* D. *A. flavus.* E. *A. niger.* F. *A. fumigatus.* G. Pure culture of *A. flavus.* H. Pure culture of *A. fumigatus.* I. Pure culture of *A. niger* J. Pure culture of *P. chrysogenum*

DISCUSSION

The five fungal genera(*Aspergillus, Fusarium, Penicillium, and Rhizopus*) isolated from kuli-kuli collected from different agricultural zones in Niger State revealed that this commodity is highly contaminated which comfirm the earlier work of Vikas and Mishra (2010) who isolates nine species of fungi from the seeds of different varieties of groundnut during one storage year. The finding in this work is also in line with Chavan (2011) who reported the species of *Aspergillus, Penicillium, Fussarium*, *Rhizopus*, and *Alternaria* were commonly occurring postharvest molds in storage conditions.

The result from this study corroborates the work of Odeniyi *et al*,. (2019) who isolated *Aspergillus*, *Fussarium*, *Penicillium*, and *Rhizopus* species from Kulikuli and other groundnut based products in Niger state. The mycoflora commonly associated with groundnut cake (Kuli kuli) samples belonging to the genera *Aspergillus*, *Penicillium*, *Rhizopus*, and *Fussarium* were found present in all samples studied. Oftentimes, *Rhizopus* species may also occur as a common saprophyte of many foods both at pre-harvest and post-harvest stages. Boli *et al*., (2013) recorded the occurrence of these species of fungi among others in their work on groundnut butter from Benin. These fungal species are reported to be commonly associated with the raw material (groundnut), amongst other materials, in their spore forms either in the field during plant propagation, nut storage, product preparation, packaging, or storage of finished product (Mupunga *et al*., 2017). They may also end up initiating deterioration of the food material and/or produce spores that assist their survival (Mupunga *et al*., 2017).

The presence of *Aspergillus* species such as *A. flavus* and *A. niger*, *Fussarium* species, *Penicillium* species, and *Rhizopus* in the Kulikuli samples pose a toxicological threat to the consumers since the majority of the strains of

these fungal species have been reported to be toxigenic (Jimoh and Kolapo 2008; Makun *et al*., 2010). *Rhizopus* is known to liberate a metabolite rhizonin A (Ezekiel *et al*., 2011) while aflatoxins, ochratoxins, fumonisins, trichothecenes, citrinin, and patulin are well produced during metabolism by the other above mentioned fungi. In 1990, Akano and Atanda reported the presence of these fungi and aflatoxins in Kulikuli from Ibadan, Oyo State, Nigeria after the incidence of deaths resulting from consumption of aflatoxin-contaminated foods in Nigeria.

CONCLUSION AND RECOMMENDATIONS

The result from this study shows a high incidence of fungal contamination from groundnut cake (KuliKuli) sold in major markets from Bida, Mokwa, Chanchaga, Shiroro, Kotongora, and Rafi in Niger State mostly toxigenic molds, *Aspergillus niger, A. flavus* and *P. chrysogenum* having the highest occurrence and their percentage occurrence has a direct effect on food values. Therefore, improved management of this high protein source and oil-rich products from the farm, postharvest storage, and processing will enhance the high-quality products to access a larger market and reduce the risk of health challenges that go with consuming contaminated Kuli Kuli.

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Gastroprotective Activity of N-hexane and Chloroform Fractions of *Sesamum radiatum* Leaf Extract on Aspirin Induced Ulceration in Rat

[*1]Hamzah R. U., [1]Busari M. B., [2]Agboola A. R., [3]Mohammed H. A., [1]Sayyadi A. [1]Momoh O. L. & [1]Ejiro O.

[1]Department of Biochemistry, Federal University of Technology Minna, Niger State, Nigeria.

[2]Department of Biochemistry, College of Medicine, University of Calabar, Calabar, Crossriver State., Nigeria

[3]Department of Biological Oceanography, Nigerian Institute for Oceanography and Marine Research (NIOMR)Victoria Island Lagos, Nigeria.

*Correspondence Email: rabiola@futminna.edu.ng (+23408035911608)

ABSTRACT

Gastric ulcer is one of the most common gastrointestinal disorders, which causes a high rate of morbidity all over the world. Available drugs for ulcer treatment are associated with several side effects, thus the need to search for alternative treatment using plants and vegetable which are readily available and safer. The effect of n-hexane and chloroform fractions of *Sesamum radiatum* leaf extract on aspirin induced-ulceration in albino rats were investigated. Ulcerative index, alkaline phosphatase (ALP), aspartate amino transferase (AST), alanine amino transferase (ALT), superoxide dismutase (SOD), catalase (CAT), Malondialdehyde (MDA) and the extent of inhibition of lipid peroxidation were determined using standard methods. Acute toxicity study revealed that the fractions were nontoxic up to a dose of 5000mg/kg bodyweight. Animals administered with 100 and 500mg/kg bodyweight of n-hexane fraction exhibit inhibition of ulceration of 33.33% and 50.00% while those of chloroform fractions showed 50.00% and 66.0% inhibition respectively compared to the 0% of the untreated group. AST, ALT, ALP and MDA Level of the ulcer induced rats treated with both fractions were significantly ($p<0.05$) reduced in a dose dependent manner. The SOD and CAT activities of *Sesamum radiatum* fractions treated groups showed significant increase as compared with untreated and toxic group. It can therefore be suggested that the n-hexane and chloroform fractions of *S. radiatum* may possess considerable therapeutic potential in the management of gastric ulcer.

Keywords: *Sesasum radiatum*, aspirin-induced ulcer, n-hexane, chloroform

INTRODUCTION

Ulcer is an open sore or lesion that occurs on the skin or membranous areas of the body. It could be external or internal (Mcquaid, 2007). Ulceration of the gastric mucosa due to contact with gastric juice is called gastric ulcer. Where it occurs in the lining of the stomach or duodenum,

Hamzah R. U., Busari M. B., Agboola A. R., Mohammed H. A., Sayyadi A. Momoh O. L. & Ejiro O. (2021). Gastroprotective Activity of N-hexane and Chloroform Fractions of *Sesamum radiatum* Leaf Extract on Aspirin Induced Ulceration in Rat. 2nd Faculty of Natural Sciences Annual Conference. IBB University Lapai held between 30th August to 2nd September 2021. Pp 178-186

it is referred to as peptic ulcer. Peptic ulcer is a heterogeneous group of disorder involving the upper gastrointestinal tract that shows incidences of relapse, drug interactions and side effects such as arrhythmias, impotence, gynaecomastia, haematopoietic changes, etc. (Mcquaid, 2007).

Peptic ulcer is a gastro intestinal disorder due to an imbalance between the aggressive factors like acid, pepsin, *Helicobacter pylori* and defensive factors like bicarbonate secretion, prostaglandins, gastric mucus and innate resistance of the mucosal cell factors (Ramakrishnan, 2007). Normally peptic ulcer develops when aggressive factors overcome the defensive factors (Mcquaid, 2007). The major factors that disrupt the equilibrium between aggressive factors and defensive factors are Helicobacter pylori, acid–pepsin hyper secretion, non-steroidal anti-inflammatory drugs, sometimes idiopathic due to usage of tobacco, psychological stress and rapid gastric emptying (Atawodi, 2003).

Herbal medicines are considered as better alternatives for the treatment of peptic ulcer (Chukwurah, 2000). For instance, proton pump inhibitors (Omeprazole, lansoprazole) may cause nausea, abdominal pain, constipation, diarrhoea and H2 receptor antagonists (cimetidine) may cause gynaecomastia, loss of libido. Due to the occurrence of these side effects from the use of synthesized drugs for many diseases, Herbal medicines are considered safe for the treatment of ulcers with lesser adverse effects, economical, effectiveness and less toxic (Oyetayo, 2007).

Sesamum radiatum belongs to the family of Pedaliaceae. It is a leafy vegetable locally called *ekukugogoro* oe *Ewe atura* in Yoruba language, *beni* or gingelly in English, *ridi* in Hausa, and in Igala, *Oro dudu* (Gills, 1992). It is an indigenous vegetable that grows in small quantity in the rural areas. The plant occurs throughout the tropical Africa mainly as weed, where it is gathered in the wild and used as a potherb (Auwalu *et al*., 2007). It is one of the many neglected leafy vegetables of the tropics due to lack of knowledge of its medicinal importance.

In South - Western Nigeria the leaves are used to bring relaxation and health to the body, possibly because they relieve constipation and cure other ailments on ingestion (Odugbemi, 2008). The leaves, seeds and oil serve as food especially in farming communities in Nigeria (Akpan-Iwo *et al*., 2006). The leaves are also used for treating various stomach ailments. While the use of non-steroidal anti-inflammatory drugs (NSAID) may be effective, they are sometimes associated with relapse and adverse effects (Dharmani & Palit, 2006). This has led to renewed interest in the search for new anti-ulcer drugs from natural sources. This study was therefore carried out to determine the effects of the chloroform and n-hexane fractions of *Sesamum radiatum* on aspirin-induced ulceration in rats.

MATERIALS AND METHODS

Sample Collection, Chemicals and Control drug

Sesamum radiatum leaves were obtained from a private garden in Fiidi, Makurdi local Government of Benue in January of 2014.The plant was identified at the Department of Biological Science, University of Agriculture, Makurdi, Benue State.

Chemicals of analytic grade such as: methanol, chloroform, linoleic acid and Tween 20 produced by Amazon Chemical Limited, n-hexane, hydrogen peroxide, hydrochloric acid (Sigma Chemical Co. USA), ascorbic acid (Sigma Aldrich, USA) and others were used.

Aspirin (Greenfield Pharmaceutical Limited (JIANG S.U,China) and Omeprazole (Ranivan 20mg) produced by CIPLA Limited, India) were used.

Extraction and Fractionation of Plant Sample

The leaves were thoroughly washed under running tap water and were air dried at room temperature for seven (7) days, grounded into powder using mortal and pestle. Fifty grams of the dried powdered leaves was weighed into a distillation flask and 400 ml of methanol solution was added to the flask. The mixture was refluxed for two hours, filtered hot using muslin cloth and subsequently evaporated using a rotary evaporator. The fractionation of the crude extract of the sample was carried out using chloroform and n-hexane to obtain the fractions. The fractions were subsequently concentrated using a rotary evaporator (Bibby Sterling Ltd, Stone Staffordshire ST 15OSA, UK) and water bath (45^0C). The semi-dry extracts were weighed placed in a sterile sample bottles and stored in a refrigerator until require for further analysis (Ogbadoyi *et al.,* 2007).

Experimental Animals

A total of forty (40) Wister albino Rats (weighing about 120 - 200 g) of both sexes were used in this experimental study. Animals were procured from the animal house of Department of Pharmacology and Clinical Pharmacy ABU, Zaria. They were transported in well ventilated plastic cages to Department of Biochemistry, Federal University of Technology Minna, Niger State. The Animals were allowed to acclimatize for a period of 3 weeks and fed with grower feed throughout this period having free access to tap water. Animal handling and experimentations complied with the principles governing the use of laboratory animals as laid out by the international standard set by the UK Animals (Scientific Procedures) Act, 1986 and associated guidelines, the European Communities' council directive of 24 November 1986 (86/609/EEC), and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 8023, revised 1978).

Determination of Acute Toxicity LD_{50}

Acute toxicity studies were performed according to organization for economic co-operation and development (OECD) guidelines. Animals were observed for 14days to check for behavioral changes in animals and mortality rate (Wannang & Bichi,2004).

Induction of Ulcer and Experimental Design

Aspirin (25mg/kg body weight) was administered orally to all animals in the toxic and treatment groups. The animals were made to fast for 24 hours and administered with aspirin of 200 mg/ kg bw. Orally (Das & Banerjee, 1993). Animal were randomly grouped into seven groups of four animals each as follow; **Group 1:** Normal control group; **Group 2**: Positive Control (Aspirin + Omeprazole 20 mg/kg bw.); **Group 3**: Negative control (Induced with Aspirin and not treated); **Group 4:** Induced with Aspirin + 100 mg/kg bw. of chloroform fraction (CF); **Group 5:** Induced with Aspirin + 500 mg/kg bw. of chloroform fraction. (CF), Group 6: Induced + treated with 100mg/kg bw. of n-hexane fraction (NHF); Group 7: Induced + treated with 100mg/kg bw. of n-hexane fraction (NHF). The experiment lasted for 14 days.

Collection of blood and Determination of Biochemical parameters

Collection of blood samples was done according to the method described previously by Yakubu *et*

al. (2006). Rats were euthanized and plasma were collected for biochemical assays.

Assessment of Gastric Mucosal Lesion

The stomachs of the rats were removed and open along a greater curvature and were washed with ice-cold saline and examined for microscopic mucosal lesions. The gastric mucosal lesions were expressed in terms of ulcer index (UI) according to method described by Peskar *et al.,* (2002).

Preparation of Stomach Tissue Homogenate

The stomach of animals was washed in ice cold normal saline (0.9% NaCl) solution, blotted and weighed. The grounded tissues (each 0.5g) were then treated with 4.5mL of appropriate buffers, at different pH for each biochemical assay. The resulting mixture was then homogenized and centrifuged at 1000 rpm for 15 minutes then it was removed from the centrifuge and the supernatant was decanted and stored at 10^0C for further analysis.

Determination of liver Enzymes and Antioxidant Activities

Liver enzyme activities of the rat's plasma were determined using the methods as specified by the Randox Kit used. Superoxide dismutase and catalase activities of tissue homogenate of experimental animals were determined following the method described by (Mishra and Fridovich, 1972) and (Sinha *et al*., 1972) while lipid peroxidation was determined by measuring Malondialdehyde using the method of (Armstrong & Browne, 1994).

Statistical Analysis

The results are given as mean ± Standard error using the SPSS 16.0 software. Group comparisons were statistically analyzed using one- way analysis of variance (ANOVA) with multiple comparisons versus control group. Values of P<0.05 were taken as significant.

RESULTS

The ulcerative index, liver enzyme assay (AST, ALT, and ALP) and antioxidant assay results of the chloroform fraction and n-hexane fraction of *Sesamum radiatum* leaf extract are presented in figure 1-7.

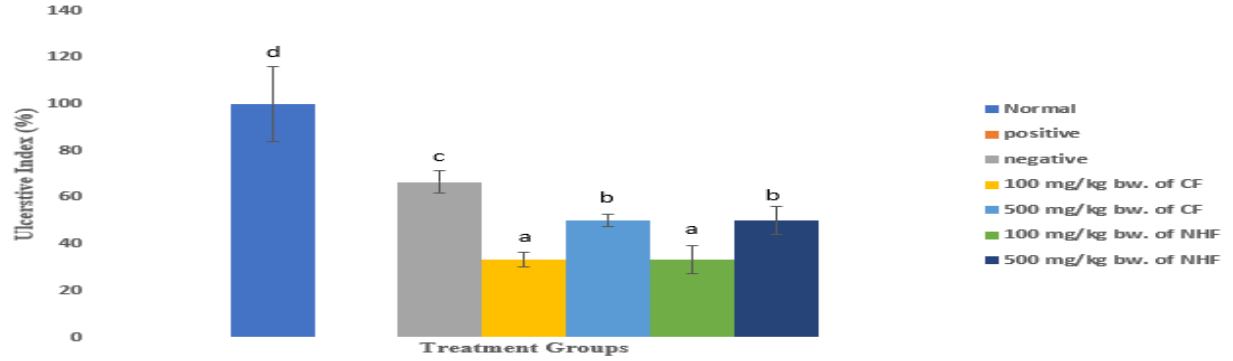

Figure 1: Effect of Chloroform and n-Hexane *Sesamum radiatum* Fractions of Leaf Extract on Ulcerative Index in Aspirin-Induced Ulcered Rats.

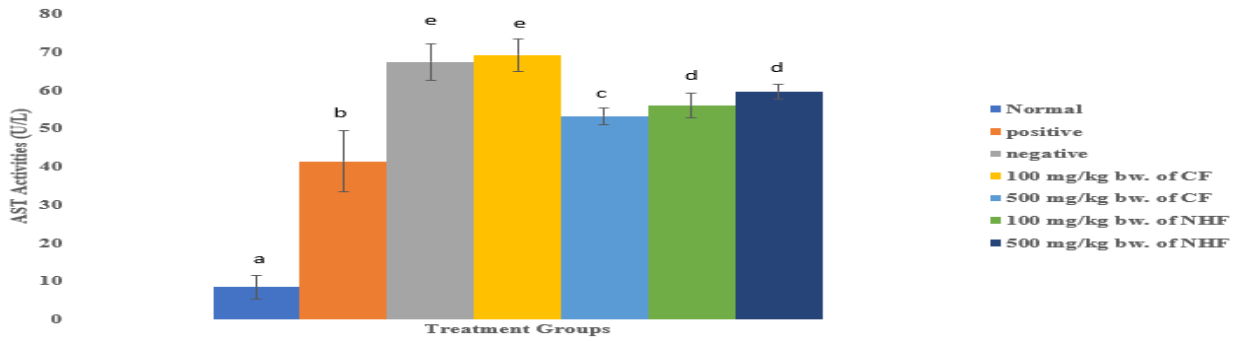

Figure 2: Effect of Chloroform and n-Hexane *Sesamum radiatum* Fractions of Leaf Extract on AST Activity in Aspirin-Induced Ulcered Rats.

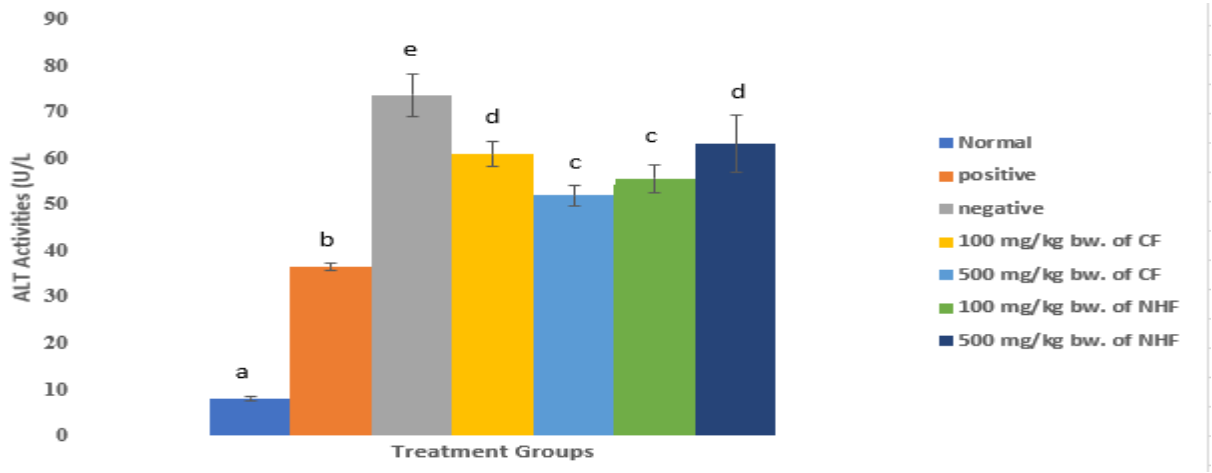

Figure 3: Effect of Chloroform and n-Hexane *Sesamum radiatum* Fractions of Leaf Extract on ALT Activity in Aspirin-Induced Ulcered Rats

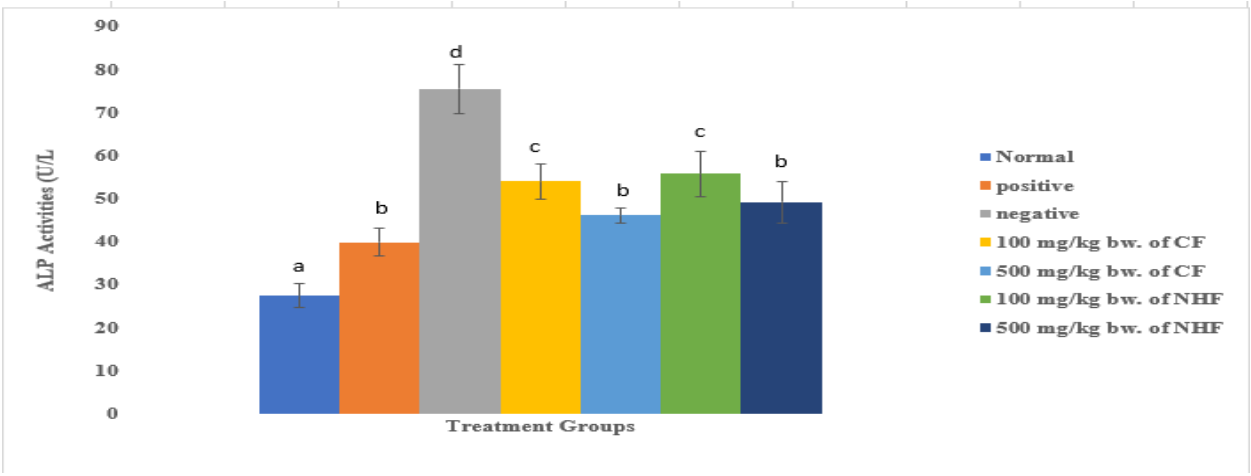

Figure 4: Effect of Chloroform and n-Hexane *Sesamum radiatum* Fractions of Leaf Extract on ALP Activity in Aspirin-Induced Ulcered Rats

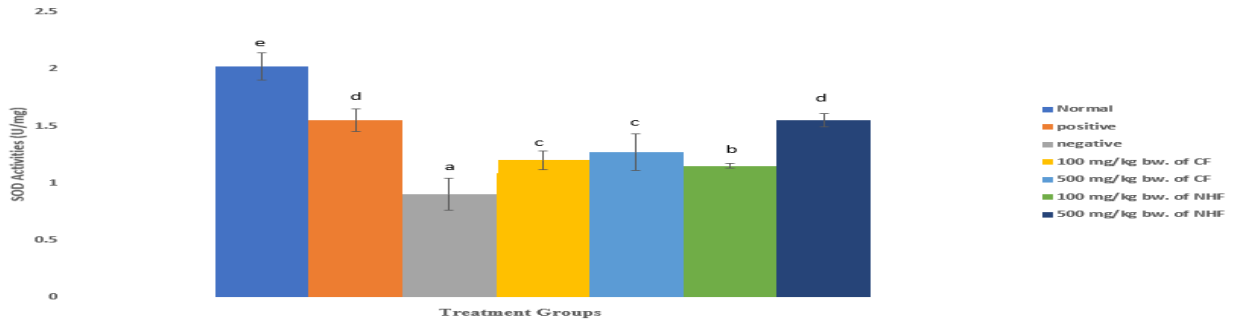

Figure 5: Effect of Chloroform and n-Hexane *Sesamum radiatum* Fractions of Leaf Extract on Superoxide Dismutase (SOD)Activity in Aspirin-Induced Ulcered Rats

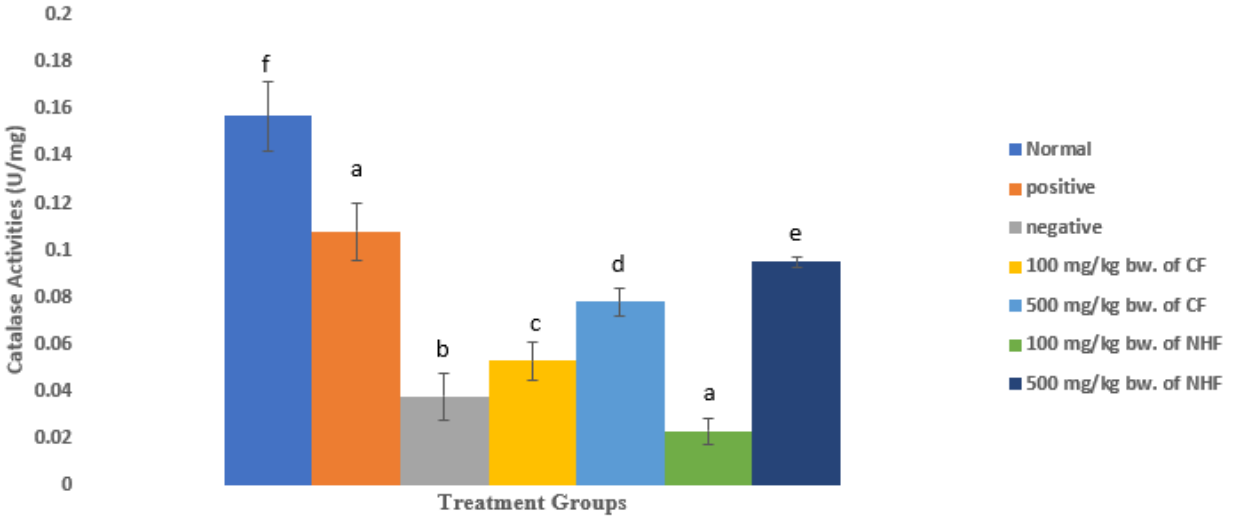

Figure 6: Effect of Chloroform and n-Hexane *Sesamum radiatum* Fractions of Leaf Extract on Catalase (CAT)Activity in Aspirin-Induced Ulcered Rats

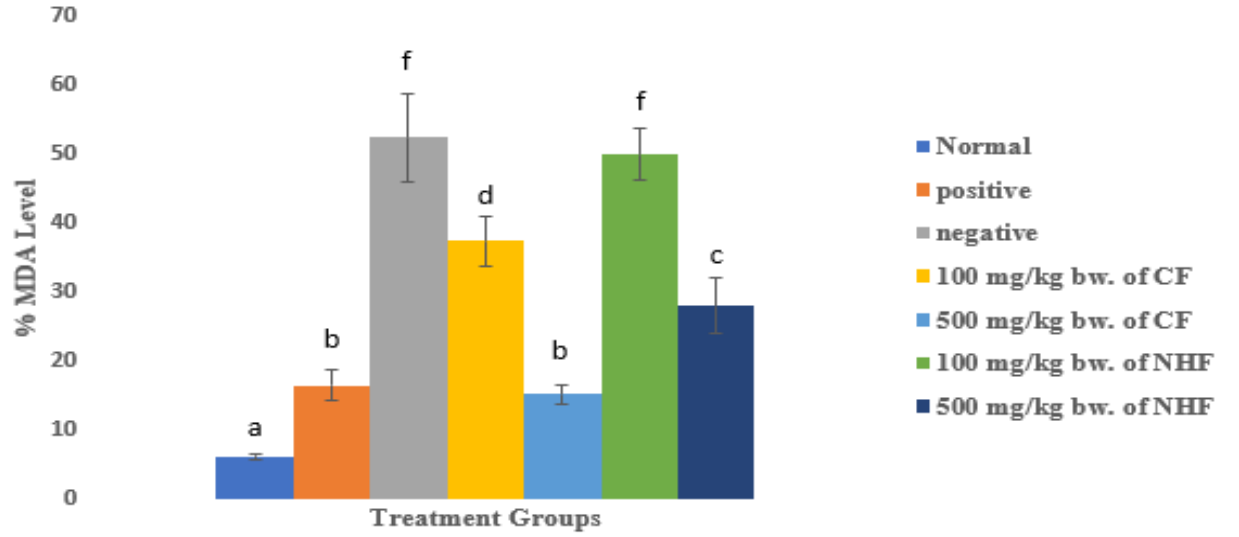

Figure 7: Effect of Chloroform and n-Hexane *Sesamum radiatum* Fractions of Leaf Extract on Malondialdehyde (MDA) Level in Aspirin-Induced Ulcered Rats

DISCUSSIONS

The result of this study revealed that the chloroform and n-hexane fractions at 100 and 500mg/kg body weight led to the rapid healing in aspirin-induced gastric ulcer therefore significantly reducing the ulcerative index when compared to the untreated group. This result is compatible with a previous research result in which *Bauhinia racemosa*, *Moringa pterygosperma* and *Trianthema pentandra* were reported to cause a significant reduction in the ulcerative index of experimental rats (Akhtar & Ahmad, 1995), but is not in agreement with the report of Akhtar and Ahmad 1995, that showed that *Cordia latifolia* did not cause a reduction in ulcerative index. (Mozafar & Hossein, 2006) also reported a similar result in their experiment where they concluded that extract of *Falcaria vulgaris* reduced the ulcerative index in ethanol induced gastric ulcer in rats. Other works that agree with this result include that of Omojola *et al.* (2019), which reported that coconut milk reduced the ulcerative index in ethanol induced rats, Manowar *et al.* (2015) also showed the reduction of ulcerative index by *Sesamum indicum* in rats induced with ulcer

An increase in the liver enzymes were observed in the negative group and this indicate hepatic injury occurred due to aspirin-induction causing leakage of the enzymes into the blood (Myagmar *et al.,* 2004). *S. radiatum* administration significantly decreased the levels of AST, ALT and ALP that shows its tissue damage preventing action and this may be attributed to the presence of phytochemicals capable of healing hepatic injury.

Catalase activity (CAT) and Superoxide dismutase activity (SOD) level were significantly decreased whereas there was significant increase in Malondialdehyde (MDA) activity in the in the negative group when compared with treated and control group. This decrease could be due to generation of free radical leading to lipid peroxidation. Administration of chloroform and extracts of *S. radiatum* at 100 and 500mg/kg bw were able to improve the antioxidant parameters. This result is in agreement with previously published work by Vinodhini *et al*., (2007) which show bale leaf extracts showed positive effect on the status of antioxidant parameters.

CONCLUSION

In conclusion, the results shows that chloroform and n-hexane fractions of *Sesamum radiatum* leaf extract possess gastro protective activity .and provides support for the traditional use of this plant in the treatment of gastric ulcer. Further studies are ongoing to isolate the active components and to elucidate their mechanism of action.

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Preparation, Characterization and Applications of Activated Carbon from Agricultural Wastes in Adsorption of Heavy Metals

***Gene A. S., Gomina M., Akanbi J. F., & Ibrahim H. A.**

Department of Physics, Ibrahim Badamasi Babangida University Lapai, Nigeria.

*Corresponding author: genesalahudeen@gmail.com

ABSTRACT

Activated carbons (ACs) play an important role in many fields of science and technology such as purification of liquids and gases, separation of mixtures and catalysis. Due to advances in science and technology, various chemicals and substances are used to improve the quality of human daily life as well as consumer products. This study is aimed at preparing activated carbon using groundnut shell and corn cobs with high efficiency for the uptake heavy metals in contaminated water. Thermo-gravimetric analysis (TGA) was carried out for the optimal temperature of carbonization for each sample of the ACs. Characterizations were also done for the observation of surface area, pore volume and pore size distribution using Brunauer Emmett and Teller (BET) method, where the surface areas of the prepared Groundnut shell activated carbons (GSACs) and Corn cob activated carbons (CCACs) were found to be 950.046 m^2/g and 514.113 m^2/g respectively. The application of the ACs for the removal of heavy metals was carried out through dispersion of the prepared ACs onto contaminated water for removal of selected heavy metals at different reaction time and masses, followed by characterizing the treated water using Atomic Absorbance Spectroscopic (AAS). It was observed that the adsorption of heavy metals by GSACs was more efficient than the CCACs, due to the high surface area exhibited by GSACs. This research will pave way for the recycle of agricultural waste products for useful applications in water/wastewater treatment.

Keywords: Activated carbon, agricultural wastes, heavy metals, adsorption

INTRODUCTION

Activated carbon popularly known as activated charcoal or activated coal, is a common term for carbon materials, which comprises charcoal. The fine structure possessed by AC increased the surface area of pores that result in the possession of powerful adsorptive properties. Carbon is available in three main forms; these are; powder, granular and pellet. Nonetheless, the most frequently used are granular and powdered AC (Dabrowski *et al*., 2005; Zarifah 2010; Mohammed *et al*., 2014). Agricultural wastes and by-products can be used for the production of AC with a high adsorption capacity, considerable mechanical strength, and low ash content (Savova *et al*., 2001). A literature survey reveals a huge amount of information on the potential of agricultural wastes as raw materials for the production of commercial

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activated carbon (Ioannidou and Zabaniotou, 2007). Various types of agricultural wastes and by-products have been studied depending on their local availability. Materials that have drawn much interest include nut shells and stones, seed hulls/husks, plant straws/stalks, sugar cane bagasse, agro-forestry residues such as saw dust, etc. which is why this study focus on production of activated carbon from agricultural waste (Corn cobs and Groundnut shells) which will have the potential of removing heavy metals from contaminated water. The presence of heavy metals in water makes it unsuitable for drinking as well as recreational activities. Therefore, the necessity of clean drinking water calls for immediate attention and focus on the production of activated carbon from agricultural wastes and by-products for use in water treatment. There are usually a few preparatory stages before the actual production of activated carbon, with raw materials washed, sun dry, crushed, carbonized at certain temperature and pounded into powder form before activating with chemicals. After activation, the surface area (m^2/g) of the produced ACs was determined using Brunauer, Emmett and Teller (BET) method where the surface areas of GSACs and CCACs were 950.046 m^2/g and 514.113 m^2/g, respectively. Also, the adsorption capacity was studied with the help of Atomic Absorption Spectrophotometry (AAS) analysis in which the adsorption was better for the GSACs due to its large surface than CCACs.

Lua and Guo (2001) prepared activated carbon from oil palm stones following carbonization at 600 ^oC for 2 h and an activation step with CO_2 in a range of temperatures between 500 and 900 ^oC for retention times 10–60 min. The highest surface area (1366 m^2g^{-1}) was obtained at 900 ^oC and 30 min retention time. The lowest surface area (356 m^2g^{-1}) was observed at the lowest temperature, 600 ^oC. Ng *et al*., (2003) produced activated carbon from pecan shells by steam activation at 850 ^oC for 2 h after carbonization at 700 ^oC for 1 h. Surface areas were in the range, 750–850 m^2g^{-1}. Wartelle *et al*., (2000) examined the production of activated carbon from macadamia nut and hazel nut in a two-stage pyrolysis activation process. The first stage took place at 700 ^oC under N_2 gas for 1 h, followed by activation at 800 ^oC under a stream of 70 % N_2 and 30 % CO_2 for 6 h.

Mallick (2001), used Mahogany sawdust to develop an effective carbon adsorbent. This adsorbent was employed for the removal of dyes from spent textile dyeing wastewater. The experimental data were fitted to Langmuir and Freundlich models of adsorption. There are many cheap, easily available materials such as wheat husk, straw, palm fiber, rubber wood saw dust, bamboo dust, date pits, palm fiber, coconut shell, groundnut shell, oil cake etc which have been used as the source for the synthesis of activated carbon. Halandemiral *et al*., (2008), prepared activated carbon from Hazelnut bagasse through chemical activation technique. The surface area developed was significant, 1489 m^2/g. It was employed to remove Sandolan blue from the water bodies. In all these studies the most important parameters examined were activating agent, mass of the AC and retention time.

METHODOLOGY

Materials

For this study, groundnut shells and corn cobs were obtained as local raw material in Lapai town.

Chemicals and Reagents

Hydrochloric acid (HCl) was used as activating agent which was collected from Chemistry Department, Ibrahim Badamasi Babangida University, Lapai, Niger state. Also, distilled water was used.

Instrumentation

Muffle furnace, oven, weighing machine, crucible, beaker, measuring cylinder, test-tube, filter paper, funnel, petri dish, and sample container.

Preparation of Groundnut Shell Activated Carbon/Carbonization Process

The groundnut shell obtained from local market was washed with distilled water to remove water soluble impurities and then, sun-dried to reduce the moisture content, then the initial mass was recorded so as to know the percentage loss of the ash content after carbonization. It was later placed in the muffle furnace at 220 ^{o}C for 50 min., after it turn to charcoal, it was allowed to cool. The weight was noted before it was ground into fine powder.

Activation process of groundnut shell charcoal

The groundnut ACs was impregnated with HCl for 2 h at room temperature to create large surface/pore size, which was later filtered and the filtrate was then washed thoroughly with warm deionized water until the washed water was test neutral to pH paper. The residue was placed in an oven for 1 h at 80 ^{o}C. After this period, the sample was removed and stored in airtight plastic container for further analysis.

Preparation of corn-cobs activated carbon

The corn-cobs were washed with distilled water to remove impurities and then sun-dried to reduce the moisture content. The dry sample was crushed into smaller size and placed in the muffle furnace at 230 ^{o}C for 30 min. After carbonization, it was allowed to cool. The sample was then pounded using mortar and pestle into fine powder. The corn-cobs ACs was soaked in a solution of HCl for 3 h at room temperature and filtered. The residue was rinsed repeatedly with deionized water until the filtrate was neutral against a pH paper. The acid-free ACs was dried in an oven for 1 h at 80 ^{o}C and stored in plastic sample container for further analysis.

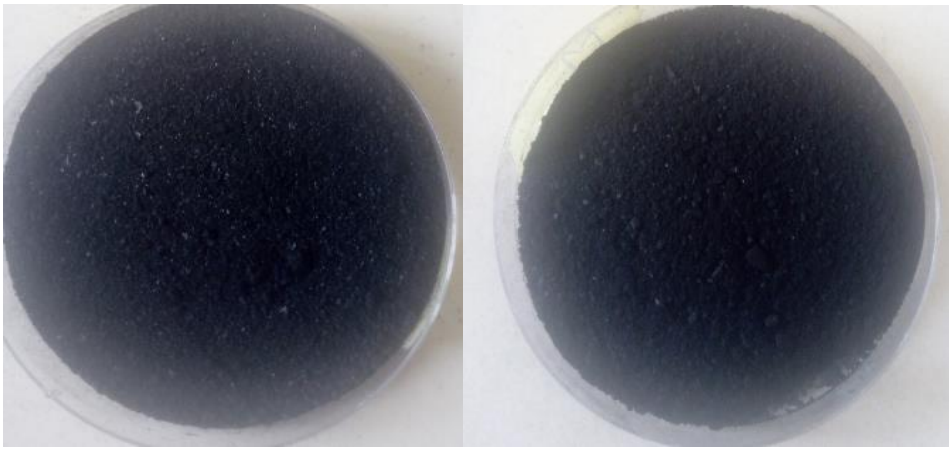


Figure 1: Prepared activated carbon from (a) Groundnut shell (b) Corn cobs.

RESULTS AND DISCUSSION

Thermo Gravimetric Analysis (TGA)

The thermogravimetric analysis (TGA) for groundnut shells and corn cobs are shown in **Figure** 2a and 2b respectively. It was observed that in **Figure** 2a, the groundnut shells started degrading at about 250 ^{o}C and continues to lose weight up to 500 ^{o}C. With this observation, 200 ^{o}C was used for the carbonization of the groundnut shell so as to have little loss to ashes, so as to obtain quality charcoal with appreciably good functional properties. However, from **Figure** 2b it showed that the corn cobs started degrading at about 300 ^{o}C and continues to lose weight up to 490 ^{o}C where large mass loss was recorded. This observation, 230 ^{o}C was used for the carbonization of the corn cobs so as to have little loss to ashes and get quality charcoal.

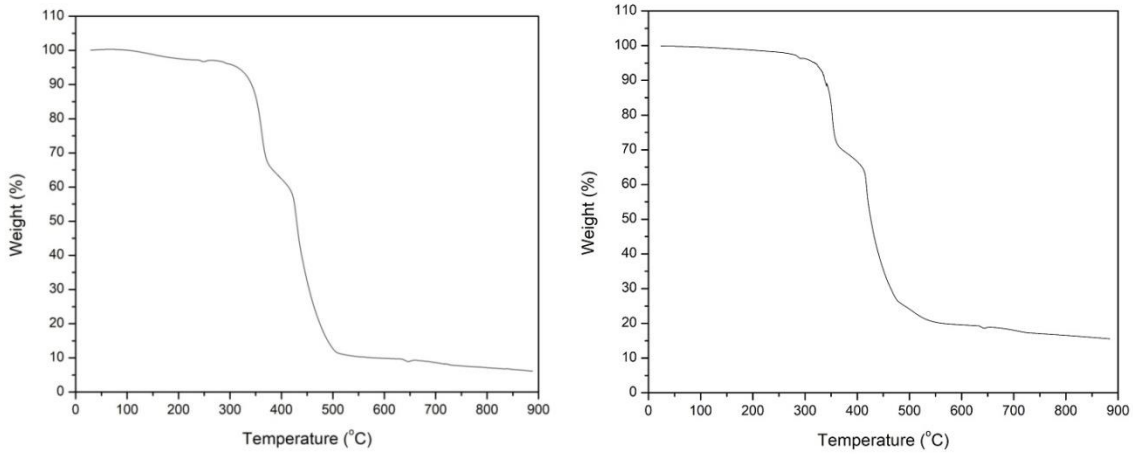


Figure 2: The thermogravimetric analysis (TGA) of (a) Groundnut shell and (b) Corn cobs.

Brunauer, Emmett and Teller (BET) analysis

Brunauer, Emmett and Teller (BET) method is a technique employed for finding the specific surface area (m^2/g) of nanostructure materials. The specific surface area, pore size and volume distribution of the groundnut shells activated carbon and corn cobs activated carbon were studied using the BET method in nitrogen adsorption. The surface areas of GSACs and CCACs were 950.046 m^2/g and 514.113 m^2/g respectively. It clearly showed that the obtained surface area and pore size depend on the quantity of chemical introduced, the mass of the ACs and activation time. Also, the smaller the size of a material, the larger the surface area it will exhibit, and the larger the size of the material, the smaller the surface area. With the BET result, it was clear that the particles of GSAC had larger surface area than the particles of CCAC.

Atomic Absorption Spectrophometer (AAS) Analysis

Atomic absorption spectrophometry (AAS) is a procedure for the quantitative determination of chemical elements using the absorption of optical radiation (light) by free atoms in the gaseous state. This technique was used for determining the concentration of selected elements being analyzed in the water samples.

Table 1: Concentrations of the heavy metal in water sample before treatment

Heavy metals	Lead	Cadmium	Mercury	Copper	Nickel
Concentrations (ppm)	2.514	1.844	0.321	1.712	0.219

Table 1 shows the concentrations of each selected heavy metal in water sample being treated with the prepared activated carbon from groundnut shells and corn cobs. However, it was observed that Lead was the heavy metal with higher concentration followed by cadmium, copper, mercury and nickel.

Table 2: Concentrations of the heavy metal in water sample after treatment with GSAC at different time and equal mass

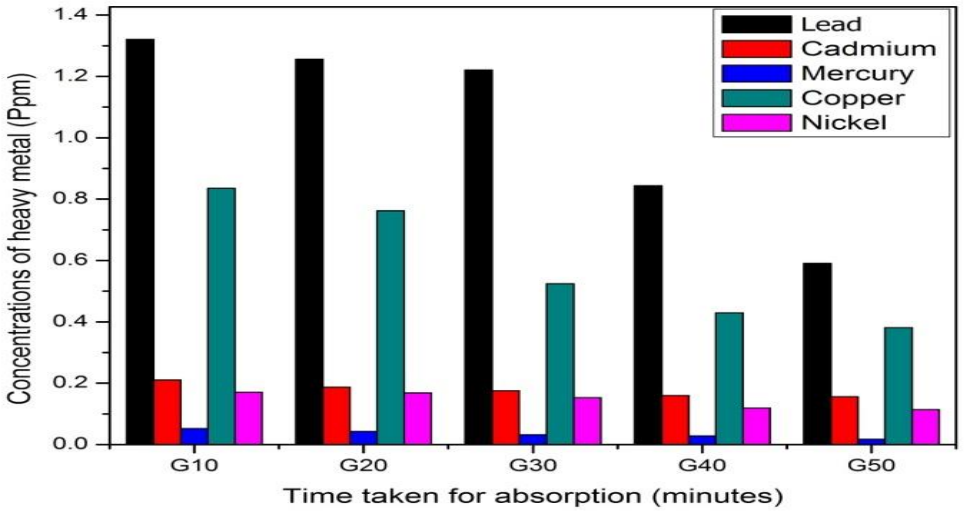
S/N	**Time Interval (Min)**	**Heavy metals Concentration (ppm)**				
		Lead	**Cadmium**	**Mercury**	**Copper**	**Nickel**
1	10	1.321	0.210	0.052	0.835	0.171
2	20	1.256	0.187	0.043	0.762	0.168
3	30	1.221	0.175	0.032	0.524	0.153
4	40	0.844	0.160	0.028	0.429	0.119
5	50	0.591	0.156	0.017	0.381	0.114

Table 2 shows the concentrations of the heavy metal in water samples after treatment with GSAC at different time and equal mass (0.7 g). It was observed that the concentrations of each heavy metal start decreasing as the retention time of the ACs in the water sample increases after treatment with GSAC, even though, the mass was constant. This clearly indicates that increase in retention time will of course decrease the concentrations of the heavy metals in the water sample.

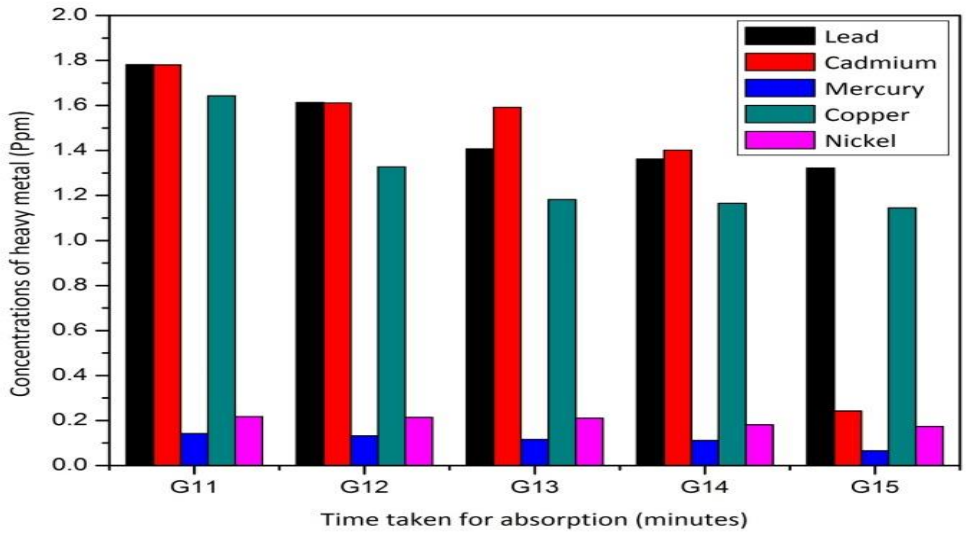
Table 3: Concentrations of the heavy metal in water sample after treatment with GSAC at different mass and equal time

S/N	**Mass (g)**	**Heavy metals concentration (pm)**				
		Lead	**Cadmium**	**Mercury**	**Copper**	**Nickel**
1	0.5	1.781	1.780	0.142	1.643	0.217
2	1.0	1.613	1.611	0.132	1.327	0.214
3	1.5	1.407	1.592	0.116	1.182	0.211
4	2.0	1.362	1.401	0.111	1.166	0.181
5	2.5	1.322	0.242	0.065	1.145	0.174

Table 3 shows the concentrations of the heavy metals in water sample after treatment with GSAC at different masses and equal time (10 min). Although, the decrease in the concentrations was not as significant as that of the **Table 2** because of different retention time. Therefore, an increase in the mass of the AC applied at constant retention time shows a less significant decrease in the concentrations of the heavy metals in the water sample compared to an increase in retention time at constant mass. The implication of this is that, small adsorbent mass is required for best performance.



a



b

Figure 3: Concentrations of the heavy metal in water sample after treatment with GSAC at (a) different retention time and equal mass (b) different mass and equal retention time

Figure 3 (a) and (b) represents the bar chart of various concentrations of heavy metals in the sample of equal mass and different time of GSACs and the bar chart of various concentrations of heavy metals in the water sample of different mass and equal time of GSACs respectively. It was observed that in **Figure** 3(a) the concentration gradually reduced

as the retention time increased while in **Figure** 3(b) it was also observed that an increase in mass of the GSAC applied to the water samples brought about a decreased in concentrations of the heavy metals as well. Though, the decrease was not as significant compared to that observed in **Figure** 3(a), due to the short retention time of the ACs in the water sample (10 min.).

Table 4: Concentrations of the heavy metal in water sample after treatment with CCAC at different time and equal mass

S/N	Time Interval (Minutes)	Heavy metals concentration (ppm)				
		Lead	Cadmium	Mercury	Copper	Nickel
1	10	2.161	1.431	0.221	1.211	0.218
2	20	1.903	1.332	0.161	1.004	0.175
3	30	1.844	1.201	0.154	0.197	0.114
4	40	1.416	0.983	0.066	0.185	0.045
5	50	0.915	0.895	0.032	0.118	0.033

Table 4 shows the concentrations of heavy metals in water samples after treatment with CCAC at different time and equal masses of 0.7 g. It was observed that the concentrations of each heavy metal decreased as the retention time of the ACs in the water sample was increased after treatment with CCAC, even though the mass was constant. But the decrease was not as significant compared to GSAC as seen in **Table 2**. These observations was attributed to the fact that groundnut shells are richer in carbon than corn cobs.

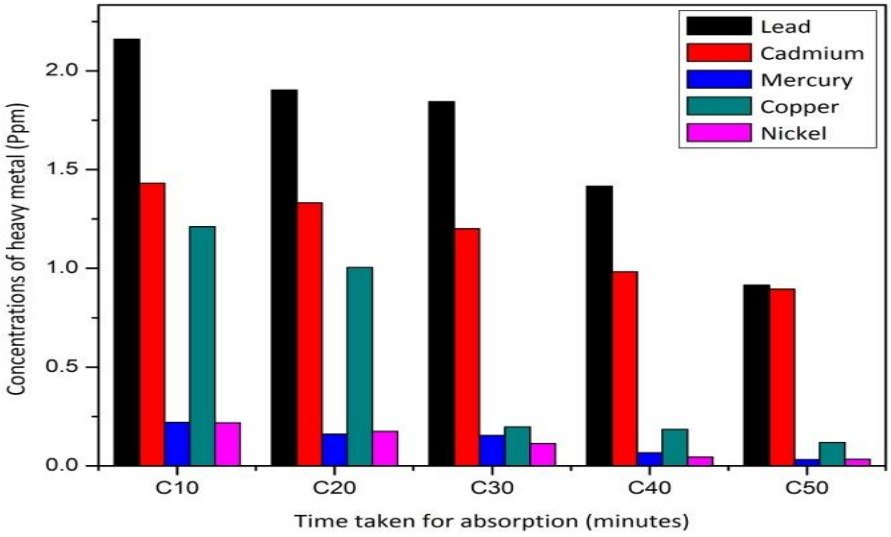


Figure 4: Bar chart of concentrations of the heavy metal in water sample after treatment with CCAC at different time and equal mass

Figure 4 shows the bar chart of various concentration of heavy metals in the water samples after treatment with equal mass of CCACs and at different retention time. As the retention time increased, the concentrations of the heavy metals also decreased. Here, the increase in mass of the AC applied was responsible for the observed decreased in metal concentrations.

CONCLUSION

The presence and concentration of the selected heavy metals were successfully determined using the Atomic Absorption Spectrophometer (AAS). Brunauer, Emmett and Teller (BET) method was used to record the surface area of the activated prepared. The adsorption was best for the GSAC based on the BET surface area obtained. The study clearly showed that the prepapred ACs could find useful industrial applications. Furthermore, utilization of agricultural wastes as source materials for production of activated carbons could be beneficial both economically and environmentally because; it converts unwanted low-value agricultural waste to useful high-value adsorbents. Lastly, it can reduce the importation of activated carbon.

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Isolation of Multidrug - Resistant *Escherichia coli* from Urogenital Samples of Patients with Pelvic Inflammatory Disease in Niger State

***[1]Oyedum U. M., [1]Kuta F. A., [2]Saidu A. N. & [1]Babayi H.**

[1]Department of Microbiology, School of Life Sciences, Federal University of Technology, Minna

[2]Department of Biochemistry, School of Life Sciences, Federal University of Technology, Minna

Email of corresponding author: hemdi41@gmail.com

ABSTRACT

This study was conducted to determine the prevalence of multidrug resistant (MDR) *Escherichia coli* isolated from patients with PID attending nine hospitals in Niger State. A total of 1170 endocervical swabs (ECS) and urine samples were collected using sterile swap stick and sample containers. The samples were transported on ice pack to the Microbiology laboratory, Federal University of Technology, Minna for analysis.Screening for the presence of *Escherichia coli* was done using streak method of inoculation on Nutrient agar and Macconkey agar. Isolates of *Escherichia coli* were identified through Gram staining and other biochemical tests. Seventy two (22.5%) and 98 (24.5%) *Escherichia coli* isolates were obtained from both ECS and urine respectively. The antibiotic susceptibility profile of the isolates to ten (10) commonly prescribed antibiotics was determined using Kirby-Bauer disc diffusion method on Mueller-Hinton agar. The antibiogram showed that a total of 79 (34.6%) *Escherichia coli* isolates out of 228 multidrug resistant bacteria expressed multidrug resistant characteristics, and were resistant to more than three (3) classes of antibiotics. The multidrug resistant *Escherichia coli* exhibited 100% resistance to: Gentamicin in General Hospital Suleja; Cephalexin and Nalidixic acid in General Hospital Minna; Gentamicin and Streptomycin in General Hospital Kuta; Augmentin and Sulfamethoxazole in General Hospital Agaie; Cephalexin and Nalidixic acid in General Hospital Lapai; Augmentin in General Hospital Wushishi; Perfloxacin and Augmentin in General Hospital Nasko; followed by 92.9% resistance to Ofloxacin in General Hospital Bida and 80% resistance to Gentamicin and Cephalexin in General Hospital Kontagora. The results of this study confirmed the presence of multidrug resistant *Escherichia coli* in Niger State, hence there is a need for public health workers, to create awareness on the misuse of antibiotics, to prevent and curtail treatment failure due to antibiotic resistance.

Keywords: Pelvic inflammatory disease; Urogenital; Multidrug resistant *Escherichia coli*

INTRODUCTION

Pelvic inflammatory disease (PID) is basically a disease of various organs (such as the ovaries, fallopian tubes, uterus and endometrium) located in the upper genital tracts of a female. However, this disease is one of the top three prevalent gynaecological problems, basically associated with female reproductive damages such as; fallopian tubal blockage, endometriosis

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and oophoritis, which inturn leads to 10% infertility and 0.5% mortality among women in the reproductive ages of 25-34 years (Usman, 2016). The emergence of this life threatening disease is basically associated with organisms referred to as urogenital pathogens.

Overtime urogenital pathogens especially members of enterobacteriaceae such as: *Escherichia coli, Salmonella* sp, *Klebsiella* sp, *Pseudomonas* sp, *Staphylococcus* sp and *Proteus* sp (Meštrović, 2017) have been regarded as causative agents of PID in most developing countries such as Nigeria and as such, PID is basically regarded as a polymicrobial infection (Centre for Disease Control, 2015). However, based on quest to attain a stable healthy condition, most female patients misuse and abuse existing antibiotics, which inturn lead to the emergence of resistant urogenital pathogens, especially those resistant to multidrug classes of antibiotics.

Occurrence of multi drug resistant urogenital pathogens among females in hospitals and communities, have increased hospital delay, treatment failure, morbidity and mortality (Spencer *et al*., 2014). It is therefore imperative to determine multi drug resistant enterobacteriaceae such as *E.coli* which is fast becoming a urogenital pathogen associated with most PID patients in most developing countries.

METHODOLOGY

Description of the Study Area

The study was conducted in Niger State, Nigeria. Niger State is located in the middle belt zone of the country. It lies between latitude 8˚20'N and 11˚30'N and longitudes 3˚30'E and 7˚20'E. It shares common boundaries with other states namely: Zamfara States to the north-west; Kaduna State to the north-west; Kebbi State to the north-west, Kogi State to the north-cental; Federal Capital Territory (FCT) and Kwara State to the north-central and north-central respectively. The state covers a land area of about (76,363km^2) square km (29,484 square miles). About 85% of the populace in the state is involved in agriculture, particularly farming and they are majorly rural dwellers, while about 15% are involved in are urban dwellers, involved in white collar jobs, businesses, crafts and arts.

Plate 1: Map showing the various Local Government Areas in Niger State.
Source: Niger State Bureau of Statistics (2010)

The state has three zones, each zone with a distinct climate pattern and a defined agricultural system. Zone A is found in the southern part of the state and it comprises of Agaie, Bida, Edati, Katcha, Gbako, Lapai, Lavun and Mokwa Local Government Areas; while zone B comprises of Bosso, Chanchaga, Gurara, Kuta, Paikoro Rafi, Shiroro, Suleja and Tafa Local Government Areas and zone C comprises of Agwara, Borgu, Kontogora, Magama, Mariga, Mashegu, Rijau and Wushishi Local Government Areas. Nine (9) Local Government Areas (comprising of 3 Local Government Areas from each zone) were randomly selected for this study.

Sample Size

The number of samples collected, were calculated using the equation below (Idakwo, 2015).

$$\boldsymbol{n} = \frac{\mathrm{T}^2 \ \mathbf{x} \ \mathbf{P}(\mathbf{1}-\mathbf{P})}{\mathrm{m}^2}$$

where

n= required sample size

T= Confidence level at 95% (standard value of 1.96)

P= Prevalence rate of bacterial infection in Niger state (9.3%) (Source, Niger State Ministry of Health)

m= Margin of error at 5% (Standard value of 0.05)

n= $(1.96^2 \text{x } 0.093 \text{ x } 0.907)/0.05^2 = 129.6 \cong 130$

The sample size for each L.G.A was (n) =130 x 9= 1170 samples.

1170 samples were collected from nine (9) general hospitals located in selected local government areas of Niger state (Lapai, Bida, Agaie, Minna, Suleja, Kuta, Kontogora, Zungeru and Nasko).

Inclusion and Exclusion Criteria

Female patients within the age of 15-54years diagnosed of PID and are attendees of the selected hospitals were recruited for this study. Female patients above 54years and less than 15 years not diagnosed of PID and who are not attendees of the selected hospitals were excluded from this study.

Ethical Clearance

Ethical clearance for this study was sought from the Niger State Hospital Management board, Research and Ethics Committee.

Collection of Demographic and Clinical Data

A structured questionnaire was administered to obtain patient's demographic data (such as patient's location, age and awareness of the disease); patient's previous medical history; patient's socio-economic factors (such as patient's family status, occupation and education) and risk factors (such as douching frequency, source of water and type of toilet facility) as described by Kolo (2016) and Oseni *et al.* (2017).

Collection and Transportation of Samples

Sterile flexible swab stick was used for the collection of swab from the endocervical region of each patient enrolled for the study (Einwalter *et al.,* 2005; Pachori and Kulkarni, 2016; Oseni *et al.*, 2017).

The swab sticks were removed and submerged into normal saline and were taken to the Microbiology Laboratory of Federal University of Technology, Minna for further analysis (Enwa *et al.,* 2015).

Urine Samples: Five millilitres (5ml) of fresh urine was collected from each female patient diagnosed of PID into a universal bottle. The urine samples were transported to the

Microbiology Laboratory of Federal University of Technology, Minna under cold condition (Hunter *et al*., 2013). The urine samples were stored at 4^{o}C for 24 hours for further analysis (Hunter *et al*., 2013).

Direct Examination

Saline wet preparation was carried out in order to rule out the presence of *Trichomonas vaginalis* which is characteristically associated with a yellow-green discharge, itching, redness and swelling (Spencer *et al.*, 2014).

Culture of Bacteria

The endocervical swabs and urine samples were cultured and incubated on the following media such as Nutrient agar, MacConkey agar and Salmonella- Shigella agar at 37^{o}C for 24hours for the isolation of Gram negative and Gram positive bacteria. Pure culture of each isolate was obtained by continuous sub-culturing using the streak method. The pure isolates were stored on a nutrient agar slant for further identification and characterization (Kolo, 2016).

Gram Staining Technique

Smear of the isolate were prepared with a wire loop by emulsifying a colony of the isolate with a drop of distilled water on a clean glass slide free of grease and was used to make a thin smear. The smear was air – dried and was heat fixed. The smear was flooded with crystal violet stain for sixty seconds and was washed with clean running tap water. The slide was tipped off and flooded with lugol's iodine for sixty seconds and washed with clean water. The smear was decolourized rapidly using alcohol and washed immediately with clean water. The smear was flooded with neutral red stain (safranin) for two minutes, washed with clean water and the back was wiped with clean cotton wool and the smear was allowed to air dry. The dried slides were examined microscopically under oil immersion, using x40 objective lens and the results recorded (Cheesbrough, 2010; Kolo, 2016).

Biochemical Tests

The bacterial isolates was identified based on the following conventional biochemical tests such as; Coagulase, Oxidase, Catalase, Citrate, Urease, Indole and Triple sugar iron test (Cheesbrough, 2010).

Coagulase test

A drop of distilled water was placed on two separate slides and a colony of the test isolates was emulsified on the two slides to make a thick suspension. A loopful of plasma was added to one of the suspension. Observation for agglutination reaction was done within 10seconds of adding plasma cells (Cheesbrough, 2010).

Catalase test

Three milliliters of 3% hydrogen peroxide solution was added into a sterile test tube. A sterile wire loop was used to pick colonies of the test isolates and was immersed in the hydrogen peroxide solution. Observation for bubbles was done immediately and results recorded (Cheesbrough, 2010).

Triple sugar iron agar test

The test was performed to determine the ability of bacteria to ferment various carbohydrates such as glucose, lactose and sucrose. Inoculation with the test organism was done by stabbing through the centre of the medium to the bottom of the tube and the test organism was streaked on the surface of the agar slants. The agar slants were incubated at 37^{o}C for 24hours. Observations for colour change of the phenol red indicator to yellow both at the butt and slants (due to the fermentation of either glucose,

lactose or sucrose), gas production indicated by bubbles or cracks on the medium and hydrogen sulphide (H_2S) production indicated by black pigment coloration was done and recorded (Cheesbrough, 2010).

Citrate test

Simmon citrate agar slants were prepared and the surfaces streaked with isolates and incubated at 37^{o}C for 24hours. Observation for colour change was done and the results recorded (Cheesbrough, 2010).

Indole test

A wire loop of the test organisms were inoculated in the test tubes containing peptone broth at 37^{o}C for 24hours. After 24 hours, 0.5ml of Kovac reagents was added into the test tubes and the solution was thoroughly mixed. Observations for colour change was made and results recorded (Cheesbrough, 2010)

Oxidase test

A piece of filter paper was placed in a sterile Petri dish and two drops of freshly prepared oxidase reagent (referred to as tetra-p-diaminechloride) was applied onto the piece of filter paper. A colony of the test organisms was introduced onto the soaked filtered paper. Observation for blue- purple colour within few seconds was done and result recorded (Cheesbrough, 2010).

Antimicrobial Susceptibility Testing of Isolates

Preparation of turbidity standard for the inoculums

The McFarland standard was employed in the standardization of the test organisms. Morphologically similar colonies of each test organism were transferred aseptically from an agar plate culture into a tube containing 4 to 5 ml of nutrient broth. The broth was subjected to agitation and was incubated at 37°C until it achieved or exceeded the turbidity of the 0.5 McFarland standard. The turbidity of the actively growing culture in the broth was adjusted with sterile saline to obtain turbidity that was optically comparable to that of the 0.5 McFarland standard (Lalitha, 2004).

Inoculation of Plates

Susceptibility test of the isolates was carried out using Kirby- Bauer disc diffusion method on Mueller-Hinton agar (Clinical and Laboratory Standards Institute, 2014). A sterile cotton swab stick was dipped into the adjusted suspension. The swab stick was rotated several times by pressing it firmly on the inside wall of the tube above the fluid level to remove excess inoculums from the swab stick (Kolo, 2016). The surface of the sterile agar was inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking two or more times, rotating the plates approximately 60^o each time, to ensure uniform distribution of bacteria on the plates. The inoculated plates was left for 10 minutes to ensure prediffussion of the organisms and to allow excess surface moisture to be absorbed, before the agar plates were impregnated with discs. Each disc was pressed down to ensure complete contact with the agar surface. The plates were inverted and incubated at 37^{o}C for 24h (Clinical and Laboratory Standards Institute, 2014).

Reading Plates and Interpreting Results

After 24h hours of incubation, each plate was examined and the diameters of the zones of inhibition were measured, including the diameter of the disc. Zones of inhibition were measured to the nearest whole millimeter using a meter rule (Kolo, 2016).

Screening for Antibiotics resistant bacterial isolates

Antibiotics available in the study area were used for the study. The antibiotic include penicillin G, Augmentin, cefotaxime, ceftriaxone, tetracycline, trimethoprim/sulfamethoxazole, gentamycin, ofloxacin and chloramphenicol test discs (AB BIODISK, Sweden) were employed (Spencer *et al.,* 2014).

Screening for Multi drug resistance bacteria

Bacteria isolates resistant to three or more classes of antibiotics according to the clinical laboratory standard institute (CLSI, 2014) guidelines were termed multi-drug resistant (MDR) bacteria (Magiorakos *et al.*, 2012; Iliyasu *et al.,* 2015).

RESULTS AND DISCUSSION

Results

Out of 1170 endocervical swaps and urine samples screened, only 720(62%) samples revealed the presence of bacteria (Table 1).

Table 1: Prevalence of PID in nine general hospitals

Samples	NSS	NPS	Prevalence (%)
Endocervical swap	1170	320	27.3
Urine	1170	400	34.2
Total		720	62

Key: NNS= Number of Samples Screened; NPS= Number of Positive Samples

The *Escherichia coli* isolated and identified are indicated in Table 2. It was observed that *E.coli* occurred in both endocervical swaps (ECS) and urine samples (Table 2).

Table 2: Occurrence of *E.coli* in nine general hospitals

	ECS		URINE		TOTAL	
	Number of *E.coli* isolates	% Frequency	Number of *E.coli* isolates	% Frequency	Number of *E.coli* isolates	% Frequency
Escherichia coli	72	22.5	98	24.5	170	23.6
Total	320		400		720	

A total of 228 multidrug resistant bacteria were isolated from this study. *Escherichia coli* isolates from endocervical 31 (34.5%) and urine 48 (34. 8%) samples were isolated respectively. Table 3 shows the percentage frequency of multidrug resistant *Escherichia coli* from urine and endocervical samples respectively.

Table 3: Multi drug resistant *E.coli* in patients with PID from nine general hospital

Organisms	ECS		URINE		TOTAL	
	Number of MDR- *E.coli*	% Frequency	Number of MDR- *E.coli*	% Frequency	Number of MDR- *E.coli*	% Frequency
Escherichia coli	31	34.4	48	34.8	79	34.6
Total	90		138		228	

Key: ECS= Endocervical swap

Table 4: Susceptibility Pattern of multidrug resistant *Escherichia coli* in patients with PID from nine general hospitals

Hospitals	No of Isolates	Susceptibility pattern	OFX(%)	PEF(%)	CPX(%)	AU(%)	CN(%)	S(%)	CEP(%)	NA(%)	SXT(%)	PN(%)
G.H.S	8	S	1(12.5)	2(25)	0(0)	2(25)	0(0)	0(0)	1(12.5)	0(0)	2(25)	0(0)
		I	0(0)	1(12.5)	4(50)	1(12.5)	0(0)	2(12.5)	0(0)	1(12.5)	3(37.5)	0(0)
		R	7(87.5)	5(62.5)	4(50)	5(62.5)	8(100)	6(75)	7(87.5)	7(87.5)	3(37.5)	8(100)
G.H.M	5	S	1(20)	1(20)	2(40)	3(60)	2(40)	0(0)	0(0)	0(0)	2(40)	2(40)
		I	1(20)	1(20)	0(0)	0(0)	0(0)	1(20)	0(0)	0(0)	2(40)	0(0)
		R	3(60)	3(60)	3(60)	2(40)	3(60)	4(80)	5(100)	5(100)	1(20)	3(60)
G.H.K	6	S	1(16.7)	0(0)	2(33.3)	0(0)	0(0)	0(0)	2(33.3)	0(0)	2(33.3)	0(0)
		I	0(0)	2(33.3)	0(0)	2(33.3)	0(0)	0(0)	0(0)	1(16.7)	0(0)	1(16.7)
		R	5(83.3)	4(66.7)	4(66.7)	4(66.7)	6(100)	6(100)	4(66.7)	5(83.3)	4(66.7)	5(83.3)
G.H.B	14	S	0(0)	9(64.3)	9(64.3)	2(14.3)	5(35.7)	2(14.3)	1(7.1)	0(0)	6(42.9)	3(21.4)
		I	1(7.1)	2(14.3)	3(21.4)	3(21.4)	0(0)	4(28.6)	4(28.6)	2(14.3)	3(21.4)	2(14.3)
		R	13(92.9)	3(21.4)	2(14.3)	9(64.3)	9(64.3)	8(57.1)	9(64.3)	12(85.7)	5(35.7)	9(64.3)
G.H.A	11	S	3(27.3)	1(9.1)	0(0)	0(0)	0(0)	0(0)	1(9.1)	1(9.1)	0(0)	1(9.1)
		I	3(27.3)	2(18.2)	3(27.3)	0(0)	2(18.2)	1(9.1)	1(9.1)	1(9.1)	0(0)	1(9.1)
		R	5(45.4)	8(72.7)	8(72.7)	11(100)	9(81.8)	10(90.9)	9(81.8)	9(81.8)	11(100)	9(81.8)
G.H.L	12	S	2(16.7)	7(58.3)	1(8.3)	1(8.3)	3(25)	1(8.3)	0(0)	0(0)	2(16.7)	0(0)
		I	0(0)	2(16.7)	5(41.7)	1(8.3)	0(0)	2(16.7)	0(0)	0(0)	1(8.3)	2(16.7)
		R	10(83.3)	3(25)	6(50)	10(83.3)	9(75)	9(75)	12(100)	12(100)	9(75)	10(83.3)
G.H.KN	10	S	5(50)	0(0)	4(40)	2(20)	1(10)	0(0)	0(0)	2(20)	2(20)	4(40)
		I	1(10)	5(50)	1(10)	1(10)	1(10)	3(30)	2(20)	1(10)	1(10)	1(10)
		R	4(40)	5(50)	5(50)	7(70)	8(80)	7(70)	8(80)	7(70)	7(70)	5(50)
G.H.W	8	S	0(0)	0(0)	5(62.5)	0(0)	1(12.5)	0(0)	1(12.5)	0(0)	3(37.5)	2(25)
		I	1(12.5)	3(37.5)	2(25)	0(0)	1(12.5)	4(50)	3(37.5)	1(12.5)	1(12.5)	2(25)
		R	7(87.5)	5(62.5)	1(12.5)	8(100)	6(75)	4(50)	4(50)	7(87.5)	4(50)	4(50)
G.H.N	5	S	1(20)	0(0)	0(0)	0(0)	1(20)	0(0)	0(0)	0(0)	1(20)	2(40)
		I	1(20)	0(0)	1(20)	0(0)	0(0)	1(20)	3(60)	1(20)	0(0)	0(0)
		R	3(60)	5(100)	4(80)	5(100)	4(80)	4(80)	2(40)	4(80)	4(80)	3(60)

Key:OFX:Ofloxacin;PEF:Perfloxacin;CPX:Ciprofloxacin;NA:Nalidixicacid;CN:Gentamicin;ST:Streptomycin;PN:Ampicillin;Cep:Cephalexin;AU:Augmentin;SXT:Sulfamethoxazole; S: Susceptible; I:Intermidiate; R:Resistance; G.H.S: General Hospital Suleja; G.H.M: General Hospital Minna; G.H.K: General Hospital Kuta; G.H.B: General Hospital Bida ; G.H.A: General Hospital Agaie; G.H.L: General Hospital Lapai; G.H.KN: General Hospital Kontagora; G.H.W: General Hospital Wushishi; G.H.N: General Hospital Nasko

DISCUSSION

Pelvic inflammatory disease is basically the disease of female genital organs. It is usually said to occur when bacterial ascends from the vagina to the upper genital tract. This disease occurs as cervicitis, endometritis, oophoritis and salpingitis and in severe cases it can result to infertility (Oseni *et al.,* 2017; Ahmed 2017).

This study indicates that 720(62%) o f the samples collected from PID patients were positive for bacterial growth. This is based on the silent spread of bacterial organisms to the upper genital tract which results to high degree of damages such as miscarriage, preterm labor and ectopic pregnancy in the infected females (Naaz *et al.,* 2016). These therefore lead to infertility among the female population. This is in agreement with the findings of Pachori and Kulkarni (2016) and Naaz *et al.* (2016) who reported that higher rates of bacterial infections such as 60%, 57% and 30% in Africa, Asia and Indian respectively.

The high occurrence of *E.coli* 170(23.6%) revealed in this study could be based on the fact that *E.coli* predominantly colonize the gastrointestinal tract and as such is the main causative agent of urinary tract infections, and this frequently exposes this organism to the vagina due to its proximity to the periurethral openings and the perianal areas. This results is in agreement with the findings of Erdem *et al.* (2018), who revealed that majority of the organisms isolated from patients with urogenital infections are *E.coli*.

Furthermore, the high occurrence of multidrug resistant *E.coli* in this study could be attributed to the fact that *E.coli* is highly associated with the transfer of genetic materials such as plasmids, referred to as the resistance (R) - plasmids through various gene transfer processes (such as conjugation) among non-resistant *E.coli*. This result agrees with the findings of Isighoni (2016) and Nikado (2009).

The hundred percent (100%) resistance exhibited by multidrug resistant *E.coli* to eight (8) antibiotics namely; Gentamicin, Ampicllin, Cephalexin, Nalidixic acid, Streptomycin, Augmentin Sulfamethoxazole and Perfloxacin could be attributed to the fact that these multidrug resistant *E.coli* have been exposed to various antibiotics due to the misuse of these antibiotics by the populace in these study area. This finding agrees with the results of Anuli *et al.* (2016) and Anejo-Okopi *et al.* (2015).

Conclusion and Recommendations

This study basically revealed high resistance profile of multidrug resistant *E.coli* associated with pelvic inflammatory disease (PID) and as such have resulted to the rapid dissemination of resistant genes in the study area, which led to prolonged hospital stay, high mobidity and high mortality among reproductive age women with pelvic inflammatory disease (PID). Therefore, it is necessary that the government enforce certain measures such as; constant awareness on the misuse of antibiotics and laws against self medication and illegal purchase of drugs across the counter, to help curb the menace associated with pelvic inflammatory disease resulted from resistant organisms.

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Assessment of Turmeric Curing on *Heterotis niloticus* Smoked with Peanut Shell Briquettes as an Alternative to Firewood

***Adejola E. O., Ibrahim S. U. & Oyero J. O.**

[1]Department of Water Resources, Aquaculture and Fisheries Technology Federal University of Technology, Minna, Niger State

*Corresponding author: hadassahadejola@gmail.com; +234(0)9015178591

ABSTRACT

Smoking operations are mostly carried out using fuelwood that emit harmful gases to the environment causing adverse climate change and deforestation. The study focused on determining efficiency of peanut shell as biofuel to mitigate the release of carbon (iv) oxide into the atmosphere as an alternative source to fuelwood. Fish samples were smoked with peanut briquettes and fuelwood using the improved smoking kiln. Briquettes were made using a briquetting press. The proximate composition of smoked fish sample was determined using the AOAC method. Samples smoked with briquettes has the highest moisture mean value of 12.03 ± 0.02^{a} and the lowest 9.23 ± 0.02^{d} from sample smoked with firewood which is as a result of the energy source used. The result observed from ash content shows that the samples were significantly different with the samples for control having the highest mean value of 17.96 ± 0.01^{a} and treatment 2 having the lowest mean value of 12.50 ± 0.06^{d}. This is an indication that high ash content is a factor of fuel consumption since control has the highest, fire wood is not an economic source of energy source as compared to briquettes. The fat, carbohydrate and crude protein content of the samples were significantly different with treatment 2 having the highest mean value of crude protein being 49.21 ± 0.01^{a} and the lowest 44.30 ± 0.17^{d}, fat highest mean value was recorded at 9.12 ± 0.01^{a} and the lowest at 5.64 ± 0.01^{d}, carbohydrate has its highest mean value at 22.74 ± 0.02^{a} and lowest at 14.53 ± 0.01^{d}. Organoleptic parameters of the samples were determined and the results subjected to statistical analysis using One-way Analysis of variance (ANOVA). Except for appearance and texture, the samples cured with spice were significantly different at ($p<0.05$) in taste, flavor and overall acceptability which proves spice addition improves fish taste. Using briquettes helps to reduce energy scarcity and promote environmentally friendly practice in waste management.

Keywords: smoke, briquettes, fuelwood, turmeric, *Heterotis niloticus*

INTRODUCTION

Fishes are known to be the most rapidly perishable food item which are susceptible to rapid spoilage at ambient temperature and are often preserved to delay spoilage. Amuneke *et al*. (2020) from their studies established that due to inadequate storage facilities and poor handling practices catches of fishery product is lost to post harvest losses which gives rise to the

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need to process and preserve fish to sustain the quality of fish.

The African bony-tongue fish, *Heterotis (Clupisudis) niloticus* (Cuvier, 1829), is the only species of the family *Osteoglossidae* and is a widely distributed freshwater fish. Preservation technologies have made tremendous efforts to ensure that raw spices in particular are free of microbial and other contamination and that their shelf-life is extended. Turmeric being a common spice is ideal as a preservative for smoke cured fishery products as it is easily acceptable by the layman and its abundant availability.

Turmeric (*Curcuma longa*) is a plant possessing branched rhizomes which are peculiar to warm humid areas. It has been incorporated as one of the essential ingredient for cooking any type of food for a very long time. In some part of the world like Bangladesh it is occasionally used by local fishermen as preservative for small sized fishes (Mossarat *et al*., 2017). Turmeric improves the quality of food by giving the food a golden color and a spicy taste. Turmeric is an additive to many ingredients in dry seasonings, curry powder, sauces and sometimes added to improve the flavor of pepper. Furthermore, turmeric functions as a multipurpose spice as a result of its medicinal functions which includes stimulating digestion, detoxifying the liver, balancing cholesterol level, fighting allergies and increasing immunity in the body (Norma *et al*., 2017).

Curing has been practiced as a procedure for preserving fish longer than any other preservation method. Curing procedure may include salting and air drying. Fish smoking is an age long method of processing fish in Nigeria which involves the application of wood smoke to impart a smoky flavor and to partially dry a fish, or part of fish such as fillets, to produce a smoked fish product and also to extend the shelf life of the product under some conditions. In various parts of the world, preservation is still the main purpose of smoking. Besides increasing the shelf life of fish, smoking improves the taste and appearance of the product. Ibrahim *et al*. (2018) explained that smoking extends the shelf life of fish by inhibiting the growth of bacteria and slowing down the spoilage of fat known as rancidity.

Miguel *et al*. (2018) in their studies concluded that unprecedented climate change is the one of the greatest threat to humanity and the release of greenhouse gases such as carbon dioxide (CO_2) which is one of the most abundant gas in the atmosphere via the continuous use of fuelwood).

In Nigeria, fuelwood has been a source of energy for smoking fish for decades in form of fuelwood, charcoal and twigs which accounts for 51% of the total annual energy consumption (Ajobo, 2014). Deforestation is one of the environmental problems that is commonly associated with fuelwood and it occurs in areas where they are utilized unsustainably (Ludwig *et al*., 2002). However, increased use of fuelwood is not encouraged as it causes soil erosion and desertification. Biomass burning has a significant influence on climate and biogeochemical cycles through emission of trace gas and aerosols.

Forest products act as a “sink” to absorb greenhouse gas emissions and thereby store large quantities of carbon for extended period of time (Ajobo *et al*., 2014). As a result, the use of agricultural residues as alternative sources of energy becomes imperative because they are environmentally friendly and readily available.

Briquetting is the process of using mechanical pressure to reduce the volume of biomass

material thereby converting the biomass residues into more acceptable fuels by improving the physical properties of the biomass in terms of higher density, better handling and combustion characteristics than the loose materials (Ajobo, 2014).

The peanut (*Arachys hypogaea*) is a plant of the *Fabaceae* family (legumes). Also called ground-peas manila-nut, groundnuts, earthnut and peanuts which is part of the bean family. After removing the nut, the shell constitutes a nuisance to the environment so processing them into briquettes to be used as source of energy in smoking fishes will aid to reduce environmental pollution.

METHODOLOGY

Experimental Sites

The fish smoking experiment was carried out in the Teaching and Research Farm of the School of Agriculture and Agricultural Technology Gidan Kwano campus on longitude 09^0 31.02'N latitude 006^0 26.42'E at 200m high, Federal University of Technology, Minna, Niger State, Nigeria. The laboratory analysis was conducted at the Water resources, Aquaculture and Fisheries Laboratory, Federal University of Technology, Minna on Longitude 09^0 32' 15.248'N and Latitude 06 27' 13.0242'E situated in Bosso Local Government Area of Niger state.

The briquetting process was carried out at the Federal University of Technology, Minna, Central workshop of the School of Engineering and Engineering Technology Gidan kwano Campus on longitude 9^o 32' 69'' N and latitude 6^o 27' 60'' E at 243.3m high, situated in Bosso Local Government Area of Niger state, Nigeria.

Procurement of Samples

Fish Samples

Four (4) fresh *Heterotis niloticus* with total weight of 11.7 kg was purchased from Mobil market, Minna, Bosso Local Government Area metropolis Niger State and transported to the Fisheries Laboratory by sealing them in airtight bags and then placing them in a plastic cooler to preserve the fish.

Procurement of Turmeric

Turmeric was purchased from Kure Ultra-Modern market with co-ordinates 09^0 61.84′N, 06^0 53.16′E.

Procurement of energy source

Peanut shell

Peanut shell was collected from Kure Ultra-Modern market, Bosso Local Government Area, Niger state, Nigeria. A total of 100 kg was collected and kept under room temperature.

Firewood

African Mahogany (*Afzelia Africana*) wood was purchased from Kure Ultra-Modern market Minna, Bosso Local Government Area Niger state, Nigeria with co-ordinates 09^0 61.84'N, 06^0 53.16'E. The choice of this wood is because smoke emitted from this wood imparts a sweet aroma and flavor on the fish.

Preparation of Samples

Four (4) fresh *Heterotis niloticus* with a total weight of 11.7 kg were purchased from the Mobil fish market in Minna, Niger State, Nigeria. The fishes were degutted and thoroughly washed with clean tap water to remove blood and slime. The fishes were then cut into 24

pieces. Fish Samples were replicated three times as well as the control in preparation for smoking with peanut shell briquettes and firewood using the improved smoking kiln. The initial mean weight of the fishes was recorded in kg. Turmeric powder was weighed with an electronic scale and 40 g of turmeric was added to 20 g of salt and dissolved in 100 ml of water for two treatments. The remaining treatment was cured with 20 g of salt as well as the control respectively.

The first and second batch of the fish samples containing six (6) fishes each were cured with the spice extract and placed in a bowl for 45 minutes to improve the flavor of the fish after which it was allowed to drain for 30 minutes before smoking with peanut shell and firewood respectively. The remaining treatment and control group was cured with 20g of salt without turmeric.

Smoking Process

The fishes were arranged on top of the wire gauze placed in a smoking kiln for each of the treatment labelled as - Control (Fish smoked with firewood and cured with salt)), Treatment 1 (Fish smoked with firewood and cured with salt +turmeric), Treatment 2 (Fish smoked with peanut briquettes and cured with turmeric +salt) and Treatment 3 (Fish smoked with peanut briquettes and cured with salt). The briquettes and firewood are added into the combustion chamber of the improved smoking kiln at intervals. The fish were smoked for 18 hours and turned regularly to prevent charring and to ensure samples were smoked evenly till dried to a constant weight. The modified smoking kiln was used for the smoke-drying process. A perforated damper was incorporated above the furnace to prevent charring of fish samples. The smoking chamber was separated into three compartments using “chicken” wire mesh 10 cm above the damper. The briquette fire was set up in the combustion chamber and lighted. Care was taken to ensure that temperature of the smoke generated in the smoking chamber reaches the required temperature (50-70 ^{0}C) is obtained using a mercury-in-glass thermometer. After smoking, the smoked fish products were packed in low-density polyethylene (LDPE) zip look bags and stored at ambient room temperature of 25-30 ^{0}C. Smoked fish samples was stored for a day at ambient temperature and samples were subjected to organoleptic evaluation, and chemical analysis.

Briquetting Process

Briquettes was fabricated with the aid of a manually operated hydraulic briquetting machine. After the production of the briquettes it was sun dried for 14 days before commencement of the study. The procedure involved includes: pelleting (particle reduction) with the aid of a grinding machine, preparation of binder (paper)-This was done by soaking paper for 48 hours and using to form a mash that serves as binder with a ratio of 1:3 (0.5 kg of binder to 1.5 kg of Peanut shell and briquetting with the use of the briquetting compounder, cooling and sun drying of briquettes

Organoleptic quality assessment

This was carried out to evaluate the taste, odor, mouth feel (texture) and general appearance (color) of the smoked fish products. Taste panels of six members already familiar with scoring smoked fish were given the product scores at every two-week interval according to Faruk *et al*. (2018). Products was scored on a 9-point hedonic scale of 9 – Like Extremely, 8 – Like very much, 7 – Like moderately, 6 – Like slightly, 5- Neither like/ dislike, 4-Dislike slightly, 3- Dislike

moderately, 2-Dislike very much and 1 – Dislike extremely.

Chemical analysis

The proximate composition of the smoked sample products was determined using the standard methods of AOAC (1994).

Statistical analysis

The data collected was subjected to statistical analysis using one-way Analysis of variance (ANOVA) and Duncan Multiple Range Test for differences in the mean.

Experimental Design

A complete Randomized Design (CRD) was used. Treatment was replicated three times of 3 pieces of *Heterotis niloticus* fish with one control randomly distributed with 5% turmeric inclusion level.

RESULTS AND DISCUSSION

Table 1: Sensory scores of smoked *H. niloticus* using different processing methods

Parameters	Taste	Flavor	Appearance	Texture	Overall Acceptability
Control	07.33±0.21^{a}	06.50±0.43^{a}	05.83±0.40^{a}	06.17±0.30^{a}	06.67±0.33^{a}
Treatment 1	06.50±0.92^{a}	06.17±0.65^{ab}	06.17±0.65^{a}	05.50±0.34^{a}	05.00±0.73^{b}
Treatment 2	07.67±0.42^{a}	06.83±0.70^{a}	06.17±0.79^{a}	05.50±0.50^{a}	07.17±0.40^{a}
Treatment 3	04.83±0.40^{b}	04.67±0.42^{b}	06.83±0.54^{a}	05.17±0.31^{a}	04.60±0.24^{b}

Control (Fish smoked with firewood and cured with salt), Treatment 1 (Fish smoked with firewood and cured with salt +turmeric), Treatment 2 (Fish smoked with peanut briquettes and cured with turmeric +salt) and Treatment 3 (Fish smoked with peanut briquettes and cured with salt).

Table 2: Proximate composition of smoked *H. niloticus* using different processing methods

Parameters	%Moisture	%Fat	%Ash	%CP	%CF	%CHO
Control	09.23±0.02^{d}	07.85±0.03^{b}	17.96±0.01^{a}	49.21±0.01^{a}	01.22±0.01^{b}	14.53±0.01^{d}
Treatment 1	10.18±0.01^{c}	09.12±0.01^{a}	12.50±0.06^{d}	44.30±0.17^{d}	01.16±0.02^{c}	22.74±0.02^{a}
Treatment 2	12.03±0.02^{a}	06.37±0.01^{c}	15.49±0.01^{b}	45.94±0.01^{c}	01.28±0.01^{d}	18.89±0.01^{c}
Treatment 3	11.62±0.01^{b}	05.64±0.01^{d}	14.70±0.06^{c}	48.00±0.58^{b}	01.03±0.01^{a}	19.01±0.01^{b}

Control (Fish smoked with firewood and cured with salt), Treatment 1 (Fish smoked with firewood and cured with salt +turmeric), Treatment 2 (Fish smoked with peanut briquettes and cured with turmeric +salt) and Treatment 3 (Fish smoked with peanut briquettes and cured with salt CP-Crude protein, CF-Crude fiber, CHO- Nitrogen free extract, %- percentage.

From table 1, the results obtained from the six-man panel using the 9-point hedonic scale showed that treatment 2 yielded the best results and was the most preferred in terms of taste. This agrees with the studies of Norma *et al*. (2017) that the use of turmeric extract imparts a spicy taste on food. There was no significant difference ($p<0.05$) in the texture and appearance sensory attributes of fish smoked with peanut shell briquettes and firewood. However, there was a statistical significant difference in the taste, flavor and overall acceptability ($p<0.05$) with taste of treatment 2 having the highest mean value of 7.67 ± 0.42^{a} and the lowest being treatment 3 having mean value of 4.83 ± 0.40^{b}. The taste result is in accordance with Amuneke *et al*. (2020) that natural spice addition is effective in improving consumer's palatability in smoked *Heterotis niloticus* and is needed for pre-treatment of smoked fish prior to processing.

The flavor was statistically different at ($p<0.05$) with treatment 2 having the highest mean value 6.83 ± 0.70^{a} and the lowest being control with mean value of 6.50 ± 0.43^{a}. Preference in flavor could be attributed to the presence of vanillin found in peanut hull which agrees with the studies of Miguel-Angel *et al*. (2018).

From table 2, the result from the proximate analysis of the smoked fish indicated that the percentage moisture content of the treatment was statistically significantly different at ($p<0.05$) with treatment 2 having the highest mean value of 12.03 ± 0.02^{b} and control having the lowest mean value of 9.23 ± 0.02^{d}. The difference could be due to variation in the curing method used in the pre-treatment of the samples which agrees with Ayeloja *et al*. (2013) that variation in moisture absorption capacity of spices affects moisture content. There was a statistically significance difference at ($p< 0.05$) between the crude fiber of each treatment. Treatment 2 has the highest mean value of 1.28 ± 0.01^{d} and treatment 3 having the lowest mean value of 1.03 ± 0.01^{a} which agrees with Merlin *et al*. (1986) that fiber in peanut hull is usually exceeds 60% of dry matter.

The fat, carbohydrate and crude protein content of the samples were significantly different with treatment 2 having the highest mean value of crude protein being $49.21\pm0.01^{a}$and the lowest being 44.30 ± 0.17^{d}, fat highest mean value was recorded at 9.12 ± 0.01^{a} and the lowest at 5.64 ± 0.01^{d}, nitrogen free extract has its highest mean value at 22.74 ± 0.02^{a} and the lowest at 14.53 ± 0.01^{d}. This could be due to the smoke drying method. Similar findings were reported by Huda *et al*. (2010) that the proximate content of fish is always by processing method.

The result observed from the ash content shows that the samples were significantly different with the samples for control having the highest mean value of 17.96 ± 0.01^{a} and treatment 2 having the lowest mean value of 12.50 ± 0.06^{d}. This is an indication of that high ash content is a factor of fuel consumption since control has the highest, fire wood is not an economic source of energy source as compared to usage of briquettes which agrees with the report of Faruk *et al*. (2018) that biomass of higher ash content tends to consume more fuel than the biomass of lower ash content. Proper handling and management of biomass bridges the gap in energy requirement.

CONCLUSION

Using peanut shell briquettes as an alternative source of fuel helps to reduce energy scarcity and promote environmentally friendly practice in waste management. Samples cured with turmeric were imparted with a spicy flavor in

terms of taste and flavor which further reinforce that natural spice addition is effective in improving consumer's palatability in flesh of smoked *Heterotis niloticus.* Improved palatability of smoked dried *Heterotis niloticus* cured with turmeric will increase its market value. Proper handling and management of biomass bridges the gap in energy requirement.

Conflict of interest

There was no conflict of interest between corresponding authors.

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Influence of Day-Length Conditions on Immature Fitness Attributes of *Aedes aegypti* (Diptera: Culicidae)

*[1]Sule B. U., [1]Ukubuiwe A. C., [1]Olayemi I. K., [2]Salihu I. M., [1]Sodangi C. J. & [3]Ukubuiwe C. C.

[1]Department of Animal Biology, Federal University of Technology, Minna, Niger State
[2]Biological Science Department, IBB University, Lapai, Niger State
[3]Department of Microbiology, Federal University of Technology, Minna, Niger State

*Corresponding Author's email: sulebright1109@gmail.com, phone No: 08038251346

ABSTRACT

Photoperiod is the amount of light and darkness in a 24 hour clock is a physical factor affecting the physiology and behaviour of insects. This study investigated the influence of day-length on developmental attributes of *Aedes aegypti*. Eggs gotten from the wild were incubated in the laboratory. Day old larvae were exposed to five photoperiod regimens; 0, 6, 13 (control), 18 and 24 hour of light (hL). Rearing of immature stages and other entomological variables were monitored following standard protocols. Results revealed significant effects of photoperiod on all parameters measured. Total larval and total immature development ranged from 7.87±1.88 to 16.29±4.53 days and 9.67±1.94 to 18.08±4.48 days respectively, while average larval and average immature survivorship ranged from 94.08±4.25 to 99.46±0.87 and 94.71±3.88 to 98.38±1.44 %. This study showed photoperiod had significant effect on immature duration and survivorship of *Ae. aegypti.*

Keywords: *Aedes aegypti,* Day-length, Survivorship.

INTRODUCTION

Aedes aegypti originates from Africa but today are seen in all regions of the both developed and underdeveloped places worldwide (Powell and Tabachnick, 2013). In Sub-Sahara Africa where yellow fever epidemics and other aboviral diseases have had serious effects on human, they are the major cause of relative incidence of disease and mortality (Morrison *et al.,* 2008; Marcondes and Ximenes, 2015). The vector of these diseases constantly exposed to varying physical factor that significantly influence the physiology, behaviour and development (Delinger and Yocum, 2019).

Among these physical factor is photoperiod; defined as the amount of light available within a 24 hour clock (Gillot, 2005; Shi *et al.,* 2017). The number of hour of light and darkness L:D or hours of light (hL), has great influence on insects physiology (Saunders, 2012). It give rise to knowledge about yearly changes received and processed by mosquitoes, resulting to differences in developmental indices (Yee *et al.,* 2012; Lacour *et al.,* 2014; Armbruster, 2016). Day length variations serves as signal for changes in ecosystem and insects have what it takes to detect these changes before it arrives allowing

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them to make necessary responses (Denlinger *et al.,* 2017). More so, variations occur in insect's developmental indices due to the fact that photoperiodic response is species specific (Ukubuiwe *et al.,* 2018).

Knowledge about the right biological and physiological activities exhibited by species of insect to changes in photoperiod is vital in the development of a potent control protocols, particularly for medically important insect pest.

METHODOLOGY

Sourcing and Handling of Aedes egg

Ovitraps (plastic troughs of 400 mL capacity) half-filled with distilled water lined with white cloth inwardly were set in the wild and monitored daily for collection of eggs. Oviposited eggs were retrieved by removing the white cloth from the ovitrap as eggs are attached to the cloth. Retrieved eggs were transferred to the Insectary unit Animal Biology Department, Federal University of Technology, Minna, Niger State for incubation.

Simulation and Maintenance of Day-length Regimens

Simulation and maintenance of photoperiod regimens followed the method of (Ukubuiwe *et al*., 2018) while mosquitoes were reared as described by Olayemi and Ande (2009). Five photoperiod durations 0, 6, 13, 18 and 24 hours of light (hl) were simulated by varying the duration by which mosquitoes are exposed to light. The test mosquitoes were exposed to this light variation from larval stage through to adulthood.

Rearing of Experimental Mosquitoes

Eggs were introduced into six (6) replicate troughs (100 mL capacity) at twenty five (25) eggs/ trough and monitored until hatching. Hatched larvae were introduced into a separate rearing trough at 4 mL water per larva and fed with yeast every day and the water changed everyday until pupation as described by Ukubuiwe *et al.* (2016).

Influence of day-length conditions on Immature Fitness Attributes of *Ae. aegypti* Duration of development

This represented the time taken for an immature stage to transform to another immature stage. The mosquitoes were monitored twice (6 am and 6 pm) every day, the time and numbers of immature life stages that transform to the next stage (LI-IV), pupa stage and adult was taken note respectively (Ukubuiwe *et al.,* 2016).

Immature survivorship

This is the proportion of mosquitoes at the start of a life stage that effectively enters the next stage. It was determined for immature life stages expressed in percentages and computed using the formula described by Ukubuiwe et al. (2018).

$$S_i = (n_i/n_{i-1}) \times 100$$

Si = survival rates in instars stage i in percentage; ni is the number of larvae entering instars stage i and ni-1 the number of larvae that entered the preceding instar stage.

Data Analysis

Data generated from the independent study were processed into means and standard deviation using Microsoft Office Excel 2016. Variables from various regimens were compared for significant difference using one-way and two-way analysis of variance (ANOVA) as appropriate with means separated using Duncan Multiple

Range Test (DMRT). Differences in mean were considered to be significant at P<0.05

RESULTS AND DISCUSSION

Results

Influence of day-length on duration of immature development (days) of *Aedes aegypti*

Analyses showed significant (p<0.05) effect of photoperiod in developmental time exhibited by larvae at different photoperiod regimen from L1 to LIV. There was no significant (p>0.05) effect in total larval duration from 0, 6, 13 and 18 hours of light (hL). Immature reared at shorter day lengths 0, 6 and 13 hl displayed fast developmental rate as they took short time for their development, while 18 and 24 hl took longer time for their development (Table 4.1). The range of values for immature development are L1 0.71±0.18 to 1.31±0.17 days; L2 0.79±0.31 to 1.32±0.31 days; L3 1.41±0.53 to 4.39±2.27 days; L4 4.69±1.74 to 9.55±3.98 days and pupa 1.54±0.38 to 1.90±0.10 days, respectively (Table 1).

These variations in larval and pupa duration of development led to a significant (p<0.05) difference in total larval and immature duration for the species. Total larval development ranges from 7.87±1.88 to 16.29±4.53 days andtotal immature development ranges from9.67±1.94 to 18.08±4.48 days, respectively (Table 1)

Influence of day-length on immature survivorship (%) of *Aedes aegypti*

Analyses revealed significant (p<0.05) effect of day-length conditions on survival rate of immature life stages of the species except at second larva instar (LII). Average larval survivorship of those exposed to 0, 6, 13 and 18 hL show no difference but at 24 hL, there was significant difference as survival rate was low. At the pupa stage, there was no significant (p>0.05) effect of day-length on survivorship. The range of values for the survivorship of larva instars and pupa stage are L1 97.96±3.31to 100.00±0.00 %; LII 97.17±3.11 to 100.00±0.00 %; LIII 88.61±17.65to 100.00±0.00%; LIV87.73±8.51 to 98.41±2.47 % and pupa 94.05±4.25to 97.22±4.30 %, respectively (Table 2).

Average larval and average immature survivorship also varied significantly (p<0.05) with range of values 94.08±4.25to 99.46±0.87 % and 94.71±3.88 to 98.38±1.44 %, respectively (Table 2)**.**

Table 1 Effect of Photoperiod on Duration (Days) of Immature Development of *Aedes aegypti*

Photoperiod (hL)	First larva instar (L1)	Second larva instar (L2)	Third larva instar (L3)	Fourth larva instar (L4)	Total Larval Duration	Pupal Stage Duration	Total Immature Duration
0	0.71±0.18^{a*}	0.79±0.31^{a}	1.41±0.53^{a}	4.90±1.60^{a}	7.87±1.88^{a}	1.81±0.11^{b}	9.67±1.94^{a}
6	1.11±0.25^{b}	0.83±0.17^{a}	1.84±0.65^{a}	4.69±1.74^{a}	8.47±2.31^{a}	1.54±0.38^{a}	10.01±2.01^{ab}
13	0.72±0.19^{a}	0.89±0.18^{ab}	1.61±0.71^{a}	5.53±1.58^{a}	8.74±2.14^{a}	1.78±0.11^{b}	10.52±2.11^{ab}
18	1.31±0.17^{b}	1.32±0.31^{c}	2.07±0.69^{a}	6.55±1.67^{a}	11.24±1.50^{a}	1.90±0.10^{b}	13.14±1.83^{b}
24	1.18±0.33^{b}	1.18±0.30^{bc}	4.39±2.27^{b}	9.55±3.98^{b}	16.29±4.53^{b}	1.79±0.63^{b}	18.08±4.48^{c}

*Values in a column having same superscript are not significantly different at P≤0.05

Values are expressed as Mean±SD

Table 2:Effect of Photoperiod on Immature Survivorship (%) of *Aedes aegypti*

Photoperiod (hL)	First larva instar (L1)	Second larva instar (L2)	Third larva instar (L3)	Fourth larva instar (L4)	Total Larval Duration	Pupal Stage Duration	Total Immature Duration
0	100.00±0.00^{b*}	100.00±0.00^{a}	100.00±0.00^{b}	97.83±3.49^{b}	99.46±0.87^{b}	94.05±4.25^{a}	98.38±1.44^{b}
6	97.96±3.31^{a}	97.79±3.53^{a}	98.48±2.36^{ab}	98.41±2.47^{b}	98.16±1.41^{b}	96.81±3.66^{a}	97.89±0.66^{b}
13	100.00±0.00^{b}	97.17±3.11^{a}	100.00±0.00^{b}	97.55±4.29^{b}	98.68±0.85^{b}	94.20±5.24^{a}	97.78±0.73^{b}
18	100.00±0.00^{b}	98.55±3.55^{a}	96.70±5.17^{ab}	97.53±9.74^{b}	98.19±3.41^{b}	95.91±6.57^{a}	97.74±1.85^{b}
24	100.00±0.00^{b}	100.00±0.00^{a}	88.61±17.65^{a}	87.73±8.51^{a}	94.08±4.25^{a}	97.22±4.30^{a}	94.71±3.88^{a}

*Values in a column having same superscript are not significantly different at P≤0.05

Values are expressed as Mean±SD

DISCUSSION

Effects of day-length conditions on development of *Aedes aegypti* mosquitoes

From this study, immature development of mosquitoes reared under day-length ≤ 13hLspent shorter day for their development while day-length ≥18 hL spent longer time for their development. This is similar to what was observed by Kollberg *et al.*, (2013) in pine sawfly (*Neodiprion sertifer*) and Ukubuiwe *et al*. (2018) in *culex quinquefasciatus.*The faster growth rate at (13 hL) in this study is same as observed by Lopatina *et al*. (2011) in carabid beetle (*Amara communis*).

On a contrary, Bradshaw and Holzapfel (1975) reported rapid growth rates at long light duration for *Toxorhynchites rutilus* and retarded development during short duration. Chocorosqui and Panizzi (2003) also reported longer developmental time in shorter day-length for *Dichelops melacanthus*. These contradictions may be due to the fact that different species respond differently to photoperiod.

The fast growth rate by mosquito reared under shorter photoperiod might be physiological or behavioural responses to beginning of rainy season, a season having short day length mostly (Leimar, 1996). Wet season favours mosquito development simply because relative humidity is high and breeding sites available, hence mosquito species' population increases. The danger of larvae been flooded during the wet season might have triggered the fast developmental rate and short light duration might have favoured feeding by immature stage, hence increase accumulation of teneral reserve for pupation (Kollberg *et al*, 2013). Furthermore, longer developmental time experienced for longer light duration might either be stress and diapause-related physiological stimulus (Lopatina *et al*., 2011).

Effects of day-length conditions on survivorship of *Aedes aegypti*

From this study, day-length ≤ 13 hL recorded higher survival rate while ≥18 hL survived less. This finding is similar to the work of Ukubuiwe *et al* (2018) in their study accessing the effect of photoperiod on survivorship of *Culex quinquifasciatus.* High survivorship observed at shorter day-length indicates that they are favourable for mosquito survivorship. Mathias *et al*. (2006) reported differently to photoperiod responses of *Wyeomyia smithii*, *Polyommatus icarus* and *Anopheles quadrimaculatus*. According to Kollberg*et al*. (2013), immature survivorship of European pine sawfly (*Neodiprion sertifer*) is not dependent of photoperiod. The differences observed in the present study with others could be due to the fact that photoperiod is species specific.

CONCLUSION

This study showed that photoperiod had significant effect on immature developmental indices measured. Further, rate of development is fast at short day-length also survivorship is high at short day-length suggesting that short day-length favoured development and survivorship. This information gotten from this study is vital in developing a robust control strategy.

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Production, Nutritional and Sensory Properties of Cashew Apple Jam

*Kolo S. I., Shehu A. A., Jubril B., Ayuba A. & Hassana M. S.

Department of Food Science and Technology, Ibrahim Badamasi Babangida University, Lapai, Niger State

*Corresponding Author: E-mail: slmtkolo6@gmail.com, Phone number: 09090002385

ABSTRACT

Cashew Apple was used in the production of Jam to study the nutritional and sensory properties of the product and to determine its suitability and acceptability. The product was analysed for some quality parameters (Total Titrable Acid (TTA), Total Soluble Solid (TSS or Brix), Vitamin C and pH), nutritional composition and sensory acceptability. The result showed a high vitamin C content (85.62%) and Total Soluble Solid (56%) in the quality parameters assessed. The proximate revealed high level of moisture content (44.32%) and an unusual low level of carbohydrate (27.20%) in cashew apple products. The recorded sensory scores which ranged from 7.20-8.60 using the 9-point hedonic scale indicated that the fruit jam samples were highly acceptable by the consumers. This study showed that an acceptable Jam can be produced from Cashew Apple with nutritional compositions and quality parameters favorably. It will be recommended that studies should be carried out to improve on the quality standards of the Cashew Apple Jam to conform to standards sets for Jam products.

Keywords: Cashew apple, Jam, Brix, Spreadability.

INTRODUCTION

Cashew (*Anacardium occidentale*) is a tropical fruit that was first grown in South America before its spread to other parts of the world. The fleshy part of the cashew fruit is known as the cashew apple. It is highly rich in tannins, reducing sugars (fructose and glucose), minerals such as calcium, iron and phosphorus, vitamin C, and some amino acids (Sanoppa, 2021). Cashew apple is an underutilized fruit. During the harvest operations the apples are generally discarded after collection of raw cashew nuts (Preethi *et al*., 2021). As reported by Oyeyinka *et al*. (2011) industrial application of cashew apple is limited in Nigeria. However, with the realization of their nutritional potential, their use in the preparation of value-added products is increasing and in high demand (Oyeyinka *et al*., 2011). But, there are only a few countable products *viz*. cashew apple jam, sauce, ketchup and pickle that could be prepared from whole apples (Preethi *et al*., 2021).

Various researches have been carried out to utilize cashew apple in the food industry. An enriched product was produced using Cashew apple powder to supplement biscuits made from cassava flour (Ogunjobi and Ogunwolu, 2010). Also, the mineral and proximate analysis of Cashew apple juice was conducted on different cashew species. The results obtained showed

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that cashew apple juice has the potential to contribute to daily diet needs (Lowor and Agyente-Badu, 2009). And more recently, the organoleptic and mineral properties of cashew apple juice preserved with some chemicals were analyzed. The results confirmed that combination of clarification and chemical preservation is suitable for preservation of cashew apple juice up to 24 hours under refrigeration. The method was also found efficient in decreasing the astringency and in retaining nutrient quality of the juice (Dania *et al*., 2021).

Jam is a popular food item due to its preserving qualities and organoleptic profiles. It looks like a jelly but less firm. Its production requires ingredients (fruit pulp, acid, pectin and sugar) at appropriate quantities to achieve a desired finished product. Raw material quality and formulation are the key to the quality of finished goods (Rana *et al*., 2021). According to Codex Alimentarius Commission standards (CAC)(2008) , Jam should contain Total Soluble Solids (TSS) greater than 65% (CAC, 2008, Rana *et al*., 2021). Jam is expected to contain approximately 67-68% total soluble solids (TSS) along with 45% fruit pulp at least (Baker *et al*., 2005). Cashew tree is highly populated in Lapai and most of the apple from the fruit goes to waste. Most of the dwellers are only interested in selling of the nut to make money. Hence, there is a need to reduce the number of fruits wasted yearly by farmers and sellers by converting some of these fruits into Jams which can be preserved for a longer time. This study therefore seeks to produce and evaluate an acceptable jam from cashew apple to encourage its utilization in the food industry.

METHODOLOGY

Fresh cashew apple, sugar and lime were purchased from Lapai market in Lapai Local Government Area of Niger State with co-ordinates 9.0449^{o}N, 6.5741^{O}E.

Jam preparation:

Cashew fruits were sorted by separating the nuts from the apple. The cashew apple was cut into small pieces, rinsed and drained. It was put in a boiling water with salt to remove the astringent taste. It was drained after boiling for about 5 minutes. The cashew apples were then placed in a pot with addition of sugar, lime and water to cover the mixture. The mixture was boiled at 80^{0}C for about 30 minutes until set. The resultant mixture (jam) was hot filled into a sterilized jar, sealed and rapidly cooled (Emelike and Akusu, 2019).

Quality Analysis:

Total Titrable Acid (TTA), Total Soluble Solid (TSS or Brix), Vitamin C and pH were determined using standard methods (AOAC, 1990).

Nutritional Analysis:

Nutritional composition was determined using AOAC methods (Carbohydrate, Moisture, crude protein, ash and crude fat) (AOAC, 2000)

Sensory Evaluation:

Sensory evaluation was carried out using a 9-point hedonic scale and 10 panelist (both trained and untrained). Samples were assigned to each of the panelist. The panelists were asked to evaluate each sample on given sensory score sheet for the attributes such as color, aroma, texture, appearance, after taste, sweetness, spreadabilty and overall acceptability after 3 hours.

The 9-point hedonic scale was represented as:
9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor

dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much, 1 = dislike extremely (IFT, 1981).

Statistical Analysis

All experimental data obtained were subjected to analysis of variance (ANOVA) procedure of SPSS version 15.0 at 95% significant level (P=0.05) and means were separated using Duncan's multiple range tests.

RESULT AND DISCUSSION

Quality Analysis

The quality analysis of the Cashew Apple Jam obtained is shown in Table 1. The values obtained shows a high vitamin C content (85.62%) and Total Soluble Solid (56%) followed by low contents in acidity (0.115%). The TSS (56%) was observed to be lower than the CAC standards set for Jam products (67-68%). This result coincides with the nutritional attributes and composition of both the fruit and other ingredients used in the Jam production (Dania *et al*., 2021, Oyeyinka *et al*., 2011).

Table 1. Quality Analysis of Cashew Apple Jam

Sample	TTA (%)	TSS (%)	Vitamin C (mg/100g)	pH
Cashew Apple Jam	0.115±0.01[a]	56±0.00[a]	85.62±0.01[a]	3.57±0.01[a]

Nutritional Analysis

The nutritional analysis as presented in Table 2. The Cashew Apple Jam shows a significant difference in the level of moisture content. The high moisture content observed in this study is comparable to that reported by Emelike and Akusu (Emelike and Akusu, 2019). Other parameters such as the crude protein, ash content and crude fat also fall in same range as observed in other Cashew Apple products except for the carbohydrate (27.20%) which was observed to be lower than average (60%). (Dania *et al*., 2021, Emelike and Akusu, 2019, Ogunjobi and Ogunwolu, 2010, Oyeyinka *et al*., 2011).

Table 2. Nutritional composition of Cashew Apple Jam

Sample	%Moisture content	%Crude protein	% Ash Content	% Crude Fat	%Soluble Carbohydrate
Cashew Apple Jam	44.32±0.00[a]	2.95±0.01[a]	3.86±0.01[a]	1.72±0.00[a]	27.20±0.01[a]

Sensory Analysis

The sensory analysis was carried out on two samples. Sample A is a fruit Jam randomly selected and purchased from the market as control, while Sample B is the Cashew Apple Jam. The recorded sensory scores which range from 7.20 -8.60 using the 9-point hedonic scale is an indication that the fruit jam samples were highly acceptable by the consumers. There were significant statistical difference (P<0.05) observed in some the sensory parameters as seen in Table 3; the aroma, texture, after taste, sweetness and spreadability. But the overall acceptability of both samples were beyond 5.50 on a 9-point hedonic scales. This reveals that both samples were acceptable by the panelists. This trend is evident with the occurrence in several studies of Jam production (Emelike and Akusu, 2019, Oyeyinka *et al*., 2011).

Table 3. Sensory analysis of sample A and B

Sample	Color	Aroma	Texture	Appearance	Aftertaste	Sweetness	Spreadability	Overall Acceptability
A	8.40±0.70^{a}	7.90±0.88^{a}	8.40±0.79^{a}	8.20±0.92^{a}	7.60±0.70^{a}	8.20±0.92^{a}	8.80±0.42^{a}	8.50±0.53^{a}
B	8.60±0.52^{b}	7.20±0.79^{b}	6.90±0.88^{b}	7.70±0.48^{a}	7.80±0.92^{b}	8.50±0.53^{b}	7.20±0.42^{b}	7.40±0.52^{a}

Means followed by different superscript with a column are significantly different (p < 0.05)

Key: sample A= Commercial Jam in the market; Samples B = Cashew Apple Jam

CONCLUSION

This research showed an acceptable Jam can be produced from Cashew Apple with nutritional compositions and quality parameters still within acceptable range. This will reduce wastage and increase economic value of these fruits in Lapai LGA and Nigeria at large. Furthermore, studies can be carried out to establish quality standards of the Cashew Apple Jam to conform to standards sets for Jam products.

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Conflict of Interest

The authors declare no conflict of interest.

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Dietary Exposure to Pesticide Residues from Fish and Livestock Products in Kogi East, Nigeria

***Apeh D. O., Suleiman M. S., Atanu F. O., Olajide J. E., Mohammed L. S., Momoh F. O. & Umar H. O.**

Department of Biochemistry, Faculty of Natural Sciences, Kogi State University Anyigba, Nigeria

*Corresponding Authors e-mail/Phone number: danapeh@gmail.com/ +234(0)7033395643

ABSTRACT

Fishes and livestock products play very important economic and socio-cultural roles to the wellbeing of households, such as food supply, source of income, soil fertility, livelihood and sustainable agricultural production. This study assessed the concentrations of organochlorine and organophosphate pesticide residues in three fish species (*Clarias gariepinus, Heterobranchus* and *Tilapia zilli*) obtained from Idah, Kogi East and in products from cattle obtained from abattoir at Anyigba central market, Kogi East both in Kogi state. Samples of fish, cow liver, beef and cow milk were prepared for analysis, extraction of the pesticide residues was done using Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method and analysis was carried out with Gas chromatography tandem Mass spectrometry (GC-MS/MS). Results of the analysis showed that organophosphate pesticide residues were not detected in all the samples but organochlorine pesticides residues including; dieldrin, endosulfan sulphate, γ-chlordane, α- chlordane and p,p'-Dichlorodiphenyldichloroethylene (p, p'- DDE) were detected in some of the samples. Dieldrin was the most persistent of all the residues as it was found in *Clarias gariepinus, Tilapia zilli*, beef and cow liver. Other detected pesticides except dieldrin were found at levels below WHO maximum residue level. Lower concentration of α-chlordane, γ-chlordane, p, p'DDE, and endosulfan sulfate in the livestock samples may not cause acute effect when consumed but the presence of dieldrin above maximum residue level (MRL) is not acceptable health wise. The estimated dietary intake (EDI in µg/kg. bw/day) of dieldrin from fish ranged from $9.28x10^{-5}$ to $1.06x10^{-3}$, while in beef and cow liver it was estimated at $9.0x10^{-5}$, and $4.0x10^{-5}$ respectively. Endosulfan sulphate in beef had an EDI of $3.0x10^{-5}$, and in the liver, estimated dietary exposure to γ-chlordane and α- chlordane were $9.0x10^{-7}$ and $2.0x10^{-7}$ respectively. The EDI of p,p'- DDE in milk was found to be $7.0x10^{-3}$. This study also suggests that farmers in Kogi East and its environs may be using less organophosphate pesticides in their farming activities compared to organochlorine pesticides. Therefore it is recommended to occasionally monitor levels of pesticides in livestock and other food sources.

Keywords: Organochloride pesticides, dieldrin, fish, livestock products, Kogi East

INTRODUCTION

The green revolution being a viable approach in achieving world's food security in the face of population explosion has been a major trigger of increased food production. But the other side is

Apeh D. O., Suleiman M. S., Atanu F. O., Olajide J. E., Mohammed L. S., Momoh F. O. & Umar H. O. (2021). Dietary Exposure to Pesticide Residues from Fish and Livestock Products in Kogi East, Nigeria. 2nd Faculty of Natural Sciences Annual Conference. IBB University Lapai held between 30th August to 2nd September 2021. Pp 225-232

that it has contributed to several environmental challenges such as the accumulation of agrochemicals and veterinary drug residues in plant and animal tissues (Carvalho, 2017). A pesticide refers to any chemical substance or mixture of drugs intended for preventing, destroying, repelling or mitigating the effect of pest on plants and animals. Pesticides are substances with high toxic effects and persistence within the environment and are primarily designed to kill insects, fungi and weeds (Oliver *et al*., 2018). Pesticide residues are quantifiable amount of pesticides that linger on/in agricultural produce after their intended use, or from contamination due to other secondary activities taking place within the environment (EFSA, 2019).

Pesticide residues may be found in many agro-ecosystems, but they pose health risk to human when exposed to them either from primary or derived agricultural products. Toxic effects of pesticide residues in human could be acute or chronic. Acute toxicity could lead to symptoms such as headaches and nausea, while chronic toxicity could trigger or increase the progression of cancers, birth defects, infertility, and endocrine disruption (Akan *et al.,* 2013). In particular, organochlorine and organophosphate pesticides are highly stable under different environmental conditions and are persistent in nature, exhibiting adverse effects on wildlife and humans (Monirith *et al*., 2000). Children, in particular, are more endangered by short-term and chronic exposure to pesticides (Jallow *et al*., 2017).

These pesticide residues may be washed into the river by run-off water when it rains. They can potentially bioaccumulate in fish's fatty tissues which may lead to biomagnification in human on the top of the food chain. Fish absorb these compounds directly from water or by ingesting contaminated food, while livestock accumulate pesticide residues either by its direct application to animals as insecticide (like impregnated ear tag, spray, self-treatment back rubber, dust bags, inhalation) or through ingestion of contaminated water or feed (Tongo and Ezemonye, 2015). The lipophilic nature of many pesticides encourages their accumulation within the fatty parts of meat and meat products like milk and fatty tissues and organs (Oliver *et al*., 2018).

This research seek to investigate the incidence and exposure of consumers of livestock products in Kogi East and the consumers fish products harvested from the riverine area of Kogi East, Kogi State, Nigeria.

METHODOLOGY

Study Area

This study was carried out in the Eastern region of Kogi State (Anyigba and Idah). Anyigba is a town in Okura district, Dekina Local Government Area of Kogi State, Nigeria. The town is located between latitude 7°27'-7°31' North of the equator and between longitude 7°09'-7°12' East of the Greenwich Meridian (Ifatimehin *et al.,* 2014). The altitude of Anyigba is about four hundred and twenty meters (420 m) above sea level in the derived Guinea Savanna Vegetation zone of Nigeria. Idah is located on the Eastern bank of the river in the middle belt region of Nigeria. It is the headquarters of the Igala kingdom and also a local government with an area of 36 km^2, coordinates 7 05'N 6 45'E. It is an important fishing port and market trading town in Nigeria (Ifatimehin *et al*., 2014).

Sample collection and preparation

Cow liver, beef and cow milk samples were collected from the only abattoir in Anyigba Central market, Kogi East, while the fish samples were obtained from Idah market. A total of twelve samples of each sample matrices were collected. The samples were homogenized into powder using a blender, and 5 g of each was weighed out.

Extraction and quantification of pesticide residues

The method employed for extraction of pesticide residues was QuEChERS method (AOAC, 2007). This method used a single extraction in Acetonitrile with the addition of large amounts of salt and buffers to aid the extraction of both polar and non-polar pesticides. Pesticides were subsequently determined using gas chromatographic method. The extract (2 µl) was injected into a Gas chromatography tandem Mass spectrometry (TQ8040) equipped with the following instrument parameters and operating conditions: Column: SH-RXi-55il MS-type fused silica capillary column (30m, 0.25mm id & film thickness 0.25µm), Carrier: Helium gas, flow control mode: Linear velocity, Sampling time: 1 min, Ion source temperature: 200^{o}C, Interface temperature: 250^{o}C, CID gas: Argon gas. The pesticide residues assayed for were six organochlorides (γ-chlordane, α- chlordane, Dieldrin, p,p'-Dichlorodiphenyldichloroethylene (P, P'- DDE), Endosulfan sulfate, Methoxychlor) and eleven organophosphates (Dichlorvos, Carbofuran, Dimethoate, Diazinone, Pirimicarb, Dichlofenthion, Pirimiphos methyl, Fenthion, Bromophos-ethyl, Ethion, Carbophenothion).

Data collection and Determination of Dietary Intake

Questionnaires were used as a tool for the collection of data. It was administered majorly to about 150 households in Kogi East to determine the consumption of livestock and fish products within the region. The estimated daily intake (EDI) of the pesticide residues present in the samples were calculated using the formula suggested by Saladino *et al.* (2017).

$$EDI\ (mg/kg.bw/day) = \frac{[mean\ conc.of\ toxin\ \left(\frac{mg}{kg}\right) \text{x}\ daily\ intake\ (kg/day)]}{average\ body\ weight\ (kg)}$$

.....equation 1

*Note that 61kg is the average body weight of an adult in Nigeria.

Data Analysis

Data obtained from the administered questionnaires, and data obtained from the GC-MS/MS analyses were analyzed using Ms Excel 2013, and Instat software. Thereafter, tables were used to present the results.

RESULTS AND DISCUSSION

Determination of food consumption

Table 1 shows the average daily intake of fish and livestock products per adult individual in Kogi East. All the animal products considered in this study are reported to be consumed once daily with the exception of *Clarias gariepinus*, *Hetebranchus* and liver with a consumption rate below one (1) on the average. Meat however, had the highest mean daily consumption value of 0.770 kg/day compared to other animal products consumed by residents of Kogi East.

Table 1: Food consumption pattern of fish/livestock product in Anyigba, Nigeria.

Parameter	*Clarias gariepinus*	*Tilapia zilli*	*Hetebranchus*	Beef	Liver	Milk
Number of times consumed daily (average)	0.31	1.06	0.5	1	0.3	1
Maximum number of times consumed daily	2	3	1	3	2	3
Average daily serving size/individual	1.46	2.14	1.76	2.08	1.91	2.46
Weight of the sample (kg)	0.086	0.027	0.057	0.033	0.037	0.025
Daily consumption (kg)	0.126	0.057	0.100	0.770	0.158	0.012

Table 2 shows the incidence and concentration of organochlorine pesticide residues in fishes obtained from Idah and livestock samples from Anyigba central market, Kogi East. Dieldrin was detected in 50% of *Clarias gariepinus* at a mean concentration of 0.751 ± 0.051 ppm and 50% of *Tilapia zilli* 0.213 ± 0.015 ppm respectively. Dieldrin was also detected in both liver and beef, and it had 50% incidence in both animal products, with mean concentrations of 0.096 ± 0.030 ppm and 0.245 ± 0.034 ppm respectively. Endosulfan sulphate was reported in 50% of beef having a mean concentration of 0.078 ± 0.005 ppm. γ-chlordane and α- chlordane were both found in liver (50%) alone at very low concentrations of 0.002 ± 0.002 ppm and 0.001 ± 0.000 ppm respectively. P, P'- DDE was also only detected in cow milk samples, with an incidence rate of 100% and mean concentration of 0.002 ± 0.000 ppm. The results of this study shows that most detected organochlorine pesticides were present at concentrations lower than the Maximum Residue Limit recommended by FAO/WHO except dieldrin levels which were higher than 0.05 ppm in both the fish and livestock products. These pesticides might have been washed into the river by rain or disposed into the rivers as waste, they may also be part of the feed and water of cattle, or originate from pesticides used to control ectoparasites on the cattle.

Table 2: Concentration and Incidence of pesticide residues in Animal products

Parameters	***Clarias gariepinus*** **ppm (%)**	***Tilapia zilli*** **ppm (%)**	***Hetebranchus*** **ppm (%)**	**Beef** **ppm** (%)	**Liver** **ppm** (%)	**Milk** **ppm** (%)
Dieldrin	0.751 ± 0.051 (50)	0.213 ± 0.015 (50)	nd	0.245 ± 0.034 (50)	0.096 ± 0.030 (50)	0
Endosulfan sulphate	nd	nd	nd	0.078 ± 0.005 (50)	0	0
γ-chlordane	nd	nd	nd	nd	0.002 ± 0.002 (50)	0
α- chlordane	nd	nd	nd	nd	0.001 ± 0.000 (50)	0
P, P'- DDE	nd	nd	nd	nd	nd	0.002 ± 0.000 (100)
Dichlorvos	nd	nd	nd	nd	nd	nd
Carbofuran	nd	nd	nd	nd	nd	nd
Dimethoate	nd	nd	nd	nd	nd	nd
Diazinone	nd	nd	nd	nd	nd	nd
Pirimicarb	nd	nd	nd	nd	nd	nd
Dichlofenthion	nd	nd	nd	nd	nd	nd
Pirimiphos methyl	nd	nd	nd	nd	nd	nd
Fenthion	nd	nd	nd	nd	nd	nd
Bromophos-ethyl	nd	nd	nd	nd	nd	nd
Ethion	nd	nd	nd	nd	nd	nd
Carbophenothion	nd	nd	nd	nd	nd	nd

Key: nd - not detected

Determination of Estimated Dietary Intake

This study shows that dietary exposure to pesticide residues from *Tilapia zilli* consumption (with EDI of $1.06x10^{-3}$ µg/kg) present highest tendency to predispose Kogi East residents to health effects of pesticide residues compared to that of *Clarias gariepinus* (EDI=$9.28x10^{-5}$) and livestock samples; liver (EDI=$9.0x10^{-7}$), beef (EDI=$3.0x10^{-5}$) and milk (ED1=$7.0x10^{-3}$).

Table 3: Estimated dietary intake values of the detected pesticides

Animal products	Pesticide residues	EDI (µg/kg. bw/day)
Clarias gariepinus	Dieldrin	$9.28x10^{-5}$
Tilapia zilli	Dieldrin	$1.06x10^{-3}$
Beef	Dieldrin	$9.0x10^{-5}$
	Endosulfan sulphate	$3.0x10^{-5}$
Liver	γ-chlordane	$9.0x10^{-7}$
	Dieldrin	$4.0x10^{-5}$
	α- chlordane	$2.0x10^{-7}$
Milk	P, P'- DDE	$7.0x10^{-3}$

For all the animal products, very low tendencies of deleterious health effect of pesticide residues through consumption of all the products was observed. Although the levels are low, *Tilapia zilli* consumption showed higher risk compared to other animal products.

Dieldrin possesses more than one mechanism of toxicity, with the liver and the central nervous system identified as its target organs (Berntssen *et al*., 2012). The chronic toxicity study of Walker *et al.* (1973) showed that dieldrin presented liver tumours in mice but not in rats and does not appear to be genotoxic (Fitzhugh *et al.* 1964). The International Agency for Research on Cancer (IARC, 2021) classified dieldrin in Group 3: not classifiable as to their carcinogencity to humans. The high rate of transfer of dieldrin from feed to milk, eggs and adipose tissue are among the highest reported for chlorinated pesticides, making it a highly accumulative compounds. As seen in our study, previous studies have shown high presence of dieldrin in fish products, fish oil was reported to contain very high levels of dieldrin (Berntssen *et al*., 2012). Feed products of plant origin occasionally showed levels of dieldrin above the limit of detection, which is mostly in the range of 1 to 10 µg/kg (EC, 2005), this then becomes a very potential source of dieldrin in fish and fish products.

Dieldrin has earned itself ban in the European Union nations, and many other countries, this resulted in reduction in human exposure over the years, and also it's concentration in food is less than 10 % what it was over thirty years ago (Tsiantas *et al*., 2021). Berntssen *et al*. (2012) also reported a daily intake of dieldrin from food ~~is~~ in the range of 1 to 10 ng/kg b.w., which is considerably lower than the permissible tolerable weekly intake (PTWI) of 0.1 µg/kg b.w. established by the joint FAO/WHO meetings on pesticide residues (JMPR). The level of estimated dietary intake of dieldrin as reported in our studies is much lower than the PTWI.

CONCLUSION

The study revealed that no organophosphate pesticide was reported in either fish or livestock product, while about five of the analyzed organochlorine pesticides were detected. Dieldrin, an organochlorine was detected at concentrations many times more than the MRL value established by WHO in both fish and livestock samples, but also less than the PTWI established by JMPR. The concentration of γ-chlordane and other organochlorine pesticides

present in the livestock samples were below the MRL value established by WHO. This indicates that the level of contamination by γ-chlordane and other organochlorine pesticides present may not cause acute effect when consumed. Presence of dieldrin above MRL is not acceptable health wise. Therefore, it is necessary to monitor the livestock and fishes as well as feeds destined for animal consumption regularly.

Conflict of Interest Statement

There is no conflict of interest to declare

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Comparative Study of the Use of Natural Coagulants (*Moringa oleifera* and Watermelon) and Artificial Coagulant (Alum) for Water Treatment

***Muhammed J. J. & Jimoh A.**

Department of Chemical Engineering, Federal University Technology Minna, Nigeria.

*Corresponding Author's Email: jiyamohd7@gmail.com; Phone Number: +2348130785009

ABSTRACT

This paper presents a comparative study of the effectiveness of natural coagulant (*Moringa oleifera* combined with watermelon) and artificial coagulant (alum) as primary coagulant for raw water treatment from lower Usuma dam, Abuja. The research was aimed at investigating the potential of *Moringa oleifera* seed in conjunction with water melon seed as a possible replacement for alum in treating water. Stock solutions of the coagulants were prepared, and jar test experiments were performed using varying mixing ratios (by weight) of the coagulants to determine the effect of dosage, speed, and composition on coagulation. Optimum dosage of 0.7 g/L, optimum speed of 50 rpm, and optimum composition of 80% *Moringa oleifera* in conjunction with 20% watermelon were obtained. In comparison with alum, combining the advantages of both *Moringa oleifera* and watermelon yielded overall best results with all parameters within World Health Organization (WHO) drinking water standards. The result showed that alum can be successfully replaced with these combined natural coagulants; thereby creating non-toxic quality portable water, biodegradable sludge, and lesser the cost of water treatment.

Keywords: water melon, *Moringa oleifera,* seed, coagulant, comparative study, turbidity, colour, water treatment.

INTRODUCTION

Water is an important resource for life. Entire living organisms on earth need water for life. However, current misuse of water coupled with growing population size, industrialization, and change in climate, has increased the shrink in cleaning the water reserves; these and increasing cost of other factors have resulted in increase in cost of water treatment (Muhammad *et al.*, 2015; Kumar *et al.*, 2017). Developing countries (like Nigeria) and third world countries are facing potable water supply problems because of inadequate financial resources. Safe drinking water is essential to the health and welfare of a community, and water from all sources must have some form of purification before consumption (Muhammad *et al.*, 2015). The ideal water should have some characteristics such as clear, colourless, tasteless, odourless, pathogen-free, harmful chemical-free and non-corrosive. In this context, water treatment is the removal of suspended and colloidal particles, organic matter, micro-organisms and other substances that are deleterious to health. To achieve this standard, there is one common

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technique applied in water treatment process, which is coagulation-flocculation (Hendrawati *et al.,* 2016).

The common method of water treatment involves the use of aluminium sulphate (alum) as water coagulants. These synthetic coagulants are actually expensive and as chemicals, when used for water purification may have negative effects on health of human, animal and plants if not properly administered during the water treatment process (Kukwa *et al.*, 2017). Recent studies have indicated a number of serious drawbacks linked to the use of aluminium salts such as; Alzheimer's disease associated with high aluminium residuals in treated water, excessive sludge production during water treatment and considerable changes in water chemistry due to reactions with the OH– and alkalinity of water (Muhammad *et al.*, 2015). A number of studies have pointed out that the introduction of natural coagulants as a substitute for metal salts may ease the problems associated with chemical coagulants (Muhammad *et al.*, 2015). Using natural coagulants instead of aluminium salts might give advantages, such as lower costs of water production, less sludge production and ready availability of reagents (Muhammad *et al.*, 2015).

Researchers have proved that the active coagulant agents in plant extract are proteins. Some studies on natural coagulants have been carried out and various natural coagulants have been produced or extracted from plants such as *Moringa oleifera, Prosopis juliflora, Jutropha curcas, Zea mays, Opuntia dillenii, Citrullus lanathus, and Cactus latifaria* (Kukwa *et al.*, 2017). Among plant materials that have been tested over the years, the seeds of *Moringa oleifera* has been shown to be the most effective natural plant coagulant for water purification (Kukwa *et al.*, 2017). *Moringa oleifera* coagulant has been found to have high coagulation activity only for high turbidity water. The activity is low for low turbid water (Manyuchi and Chikomo, 2016). Therefore, it is important to improve the characteristics of this plant. This necessitates the need to explore the potentials of combining water melon and Moringa extracts as substitute for alum.

This research aimed at investigating the potential of *Moringa oleifera* seed and watermelon seed in comparison with that of alum as coagulant in water purification. The objectives of this study is to investigate the coagulation characteristics of crude protein extracts of these coagulants and to determine optimum coagulation dosage, optimum mixing speed, and optimum composition.

METHODOLOGY

The materials used in this research are *Moringa oleifera* seed, watermelon seed, and alum which were obtained from Kure market Minna, Niger State, Nigeria. The raw water from lower Usuma dam in Abuja Nigeria, magnetic stirrer (Remi), digital pH meter (HACH Senson 3), weighing balance (MP 300), thermometer, drying oven (SWT 320 1999), pipette, flocculator (Stuart SW6), beakers, turbidimeter (Eutech TC201), conductivity meter (Eutech CON 700), filter paper, spectrophotometer (HACH DR/2000), measuring cylinder, mortar and pestle, stop watch (REED SW600), and sieve.

Water melon seed cake (coagulant) and *Moringa oleifera* seed cake (coagulant) preparation

A methodology clearly described by Muhammad *et al*. (2015) was adopted for preparation of the natural coagulants. The seeds were removed

from the fruit, thoroughly washed and sun-dried for six days after which they were deshelled and ground with a mortar and pestle, and oil was then extracted from the seeds with the aid of n-hexane using the magnetic stirrer. The residue was washed with distilled water severally until no trace of n-hexane present. The washed residue was weighed and oven dried in an oven at 70^{o}C until constant weight was obtained. Proximate analysis of the cake was carried out and then the active coagulation agents were extracted (crude extract).

Jar test experiment (analysis)

The effect of coagulant dosage and mixing speed on coagulation was carried out using jar test as described by Muhammad et al (2014). The jar test apparatus was used to carry out coagulation and flocculation on the water samples. Coagulant mixtures of 0.1 g (water melon with *Moringa oleifera*) in different compositions of 100% water melon; 80% water melon (W) with 20% *Moringa oleifera* (M); 60% water melon with 40% *Moringa oleifera*; 40% water melon with 60% *Moringa oleifera*; 20% water melon with 80% *Moringa oleifera*; and 100% *Moringa oleifera* were used for the treatment. By adding the raw water sample to the jars containing these compositions to make up the 250 ml mark, the jars were placed in the jar test kit and the stirrers lowered into each. The stirring speed was set at 150 rpm for rapid mixing for 2 minutes and 80 rpm, 8 minutes for slow mixing. The samples were then allowed to settle for 30 minutes and the flocs filtered using a filter paper. The test parameters (temperature, turbidity, pH, colour, TDS and conductivity of the turbid water) were measured on the filtrate using the thermometer, the turbidimeter, the digital pH meter, and the conductivity meter respectively.

RESULTS

The physicochemical properties of the raw water sample used in this study are presented in Table 1.

Table 1: Analysis of Lower Usuma Dam Raw Water, Abuja

Parameter	Unit	Initial Result	WHO Standard (Muhammad *et al.,* 2015)
pH		7.30	6.5-8.5
Temperature	^{o}C	28.2	25-30
TDS	mg/L	48.7	933 Max.
Conductivity	µS/cm	60.9	1400 Max.
Colour	PtCo	333	15 Max
Turbidity	NTU	21.8	5 Max

Table 2 shows the results of the effect of Watermelon in conjunction with *Moringa oleifera* as coagulant. The compositions were varied at an interval of 20%.

Table 2: Effect of Watermelon in conjunction with *Moringa oleifera* as Coagulant

Composition Wt.%	pH	Temperature °C	TDS mg/L	Conductivity µS/cm	Colour PtCo	Turbidity NTU
100%W	6.94	27.3	74.8	92.7	111	6.82
80%W+20%M	6.95	27.2	62.9	78.9	76	5.07
60%W+40%M	6.96	27.3	97.2	121.6	28	2.60
40%W+60%M	6.92	27.3	86.7	108.5	21	2.07
20%W+80%M	6.86	27.3	99.3	124.3	4	1.19
100%M	6.95	27.3	121.3	151.7	40	2.93

Figure 1 shows the pattern followed by the effect of varying Composition of Natural Coagulant on Turbidity

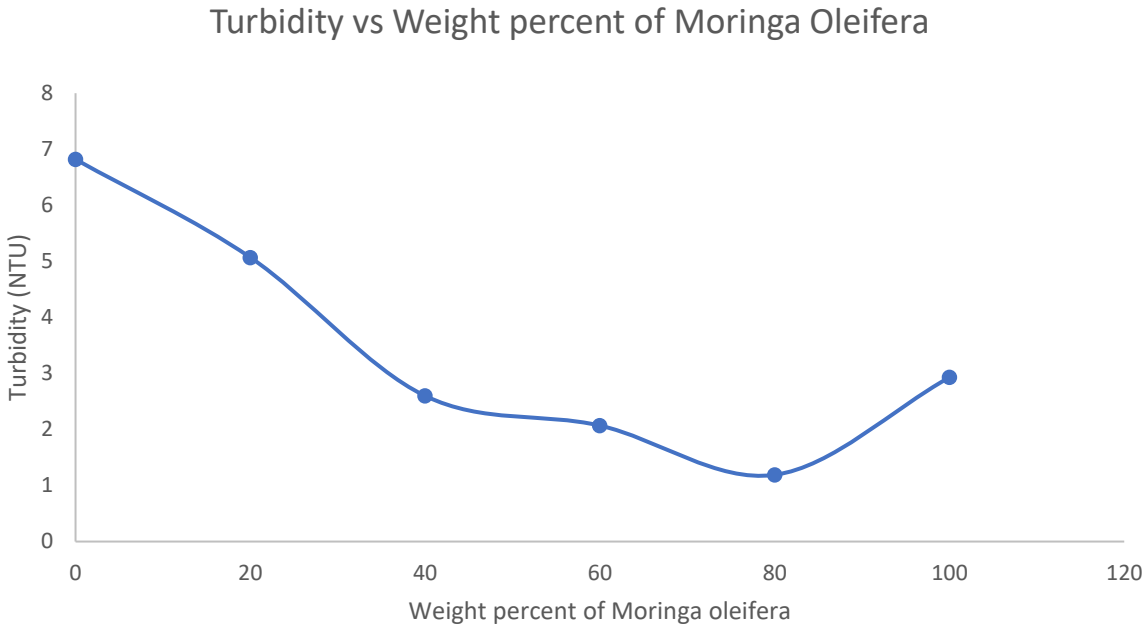

Figure 1: Effect of varying Composition of Natural Coagulant on Turbidity

Table 3 shows the results of the effect of coagulant dosage on coagulation. The dosages were varied from 0.6 g/L – 1.0 g/L for each sample treated.

Table 3: Effect of dosage of the combined natural coagulant on coagulation

Dosage g/L	pH	Temperature °C	TDS mg/L	Conductivity µS/cm	Colour PtCo	Turbidity NTU
0.6	7.03	26.8	95.8	119.8	18	1.17
0.7	7.04	27.1	94.7	118.4	15	1.12
0.8	7.05	26.9	108.9	136.3	19	1.27
0.9	7.06	26.9	103.8	129.7	15	1.12
1.0	7.07	27.2	120.0	150.0	23	1.54

Figure 2 shows the pattern followed by the effect of dosage on water turbidity using the combined natural coagulant.

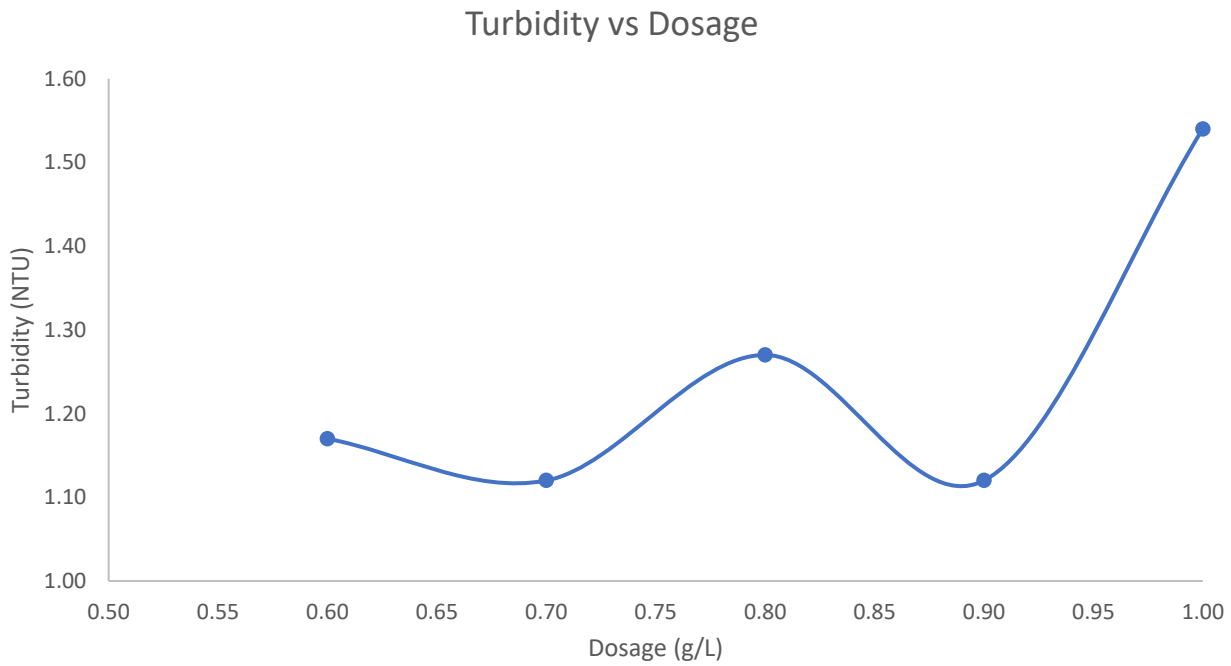

Figure 2: Effect of Dosage on Turbidity

Table 4 shows the results obtained when the effect of stirring speed on coagulation was studied by varying the stirring speed at a constant coagulant dosage using the combined natural coagulants.

Table 4: Effect of stirring speed on coagulation using the combined natural coagulants

Speed rpm	pH	Temperature °C	TDS mg/L	Conductivity µS/cm	Colour PtCo	Turbidity NTU
250	7.08	27.3	97.2	121.2	17	1.12
200	7.05	27.4	92.1	115.3	17	1.04
150	7.04	27.3	95.2	118.9	17	1.04
100	7.06	27.2	92.4	115.6	13	0.80
50	7.11	27.3	93.4	116.8	11	0.68

Figure 3 shows the pattern followed by the effect of stirring speed on turbidity using the combined natural coagulant.

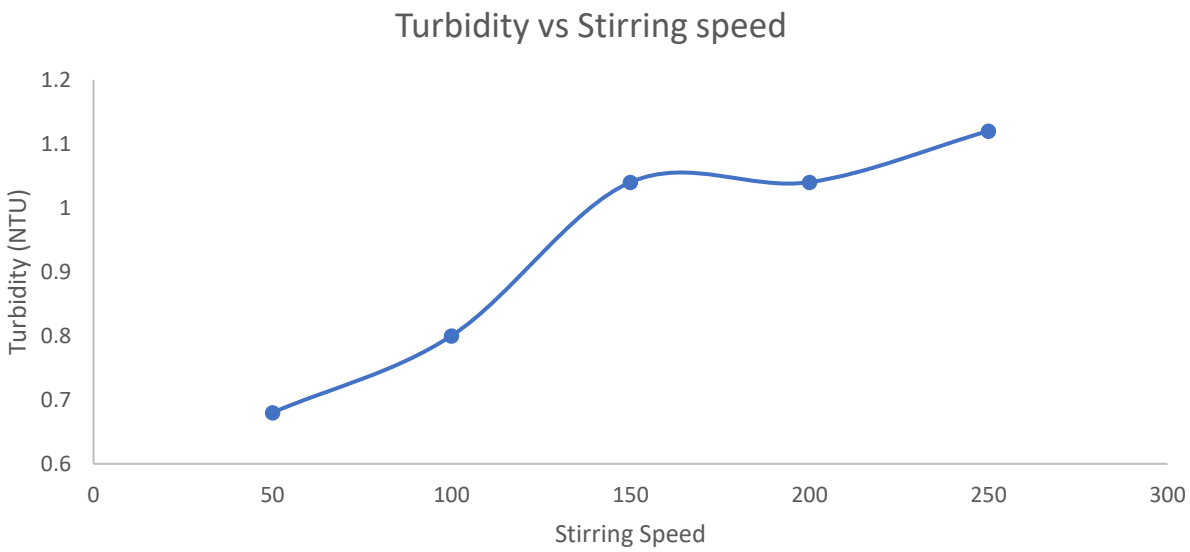

Figure 3: Effect of Stirring Speed on Turbidity

Table 5 shows the results obtained after coagulation when Alum was used as coagulant aids in conjunction with Watermelon and *Moringa oleifera* as the primary coagulant. Where: A: Aluminium sulphate; M: *Moringa oleifera* and W: Watermelon

Table 5: Effect of Watermelon and *Moringa oleifera* as coagulant aids in conjunction with Alum

Composition	pH	Temperature	TDS	Conductivity	Colour	Turbidity
Wt.%		°C	mg/L	µS/cm	PtCo	NTU
30%W+40%M+30A	6.08	27.2	110.7	138.5	30	1.90
60%W+30%M+10%A	6.48	27.1	78.3	98.2	25	1.70
90%W+10%A	6.46	27.1	54.4	68.1	32	1.90
30%W+60%M+10%A	6.29	27.1	102.4	128.0	31	2.00
90%M+10%A	6.04	27.1	132.8	166.3	24	1.60
100%A	6.04	27.0	96.6	121.2	10	1.10
10%M+90%A	6.09	26.8	89.6	112.0	23	1.70
10%W+30%M+60%A	6.01	26.9	88.8	110.9	32	2.10

DISCUSSION

Analysis of Lower Usuma Dam Raw Water

From Table 1, the turbidity value of the raw water sample was within the range of 0-50 NTU which is classified as low turbidity water (Ugwu, *et al*., 2017). It can be seen that the turbidity and the colour are above the WHO's recommended value for good quality drinking water. Hence the need for treatment.

Effect of Watermelon in conjunction with *Moringa oleifera* as Coagulant

From Table 2, the addition of a combined coagulant of watermelon in conjunction with *Moringa oleifera* to the raw water sample has a negligible effect on the temperature and pH. However, there was a drastic reduction in TDS and conductivity values of the water sample. This observation is in consonant with previous studies on coagulation and flocculation ability of some seeds (Ugwu, *et al*., 2017). The optimum result was obtained when the composition is 20% watermelon + 80% *Moringa oleifera.* This gives the highest reduction in turbidity and colour. The result obtained at this optimum are well within the WHO recommended standards.

Effect of Dosage of the Combined Natural Coagulants on Coagulation

From Table 3; at varying dosage, negligible changes were observed in pH and temperature for the water sample treated with a combined coagulant of 20% watermelon and 80% *Moringa oleifera*. However, there was a notable increase in conductivity and TDS; and also decrease in the turbidity and colour of the water sample after treatment. The observation on pH, colour and conductivity made in this study were in accordance with previous studies on coagulation and flocculation ability of some seeds (Ugwu, *et al*., 2017). The greatest decrease in turbidity and colour were seen at the dose of 0.7 g/L and 0.9 g/L which reduced the turbidity from 21.8 NTU

to 1.12 NTU respectively. This value is within the WHO recommended level of 5NTU, however according to Muhammad *et al.* (2015), the optimal dosage for a specific water is defined as the dosage which gives the lowest turbidity in the treated water; therefore, the optimum dosage is 0.7g/L. At this dosage the efficiency of the coagulant in removing colour was also highest, reducing the colour from 333 PtCo to 15 PtCo. This value is also within the WHO recommended standard of less than 15 PtCo. According to the findings of Muhammad *et al.* (2015), it was indicated that with increase in coagulant dosage, the conductivity increases, this result is in agreement with his.

Effect of Stirring Speed on Coagulation using the Combined Natural Coagulant

Table 4, present the results of effect of stirring speed on coagulation. the effect of mixing speed on coagulation was observed to only have a moderate effect on the coagulation process. This is in accordance with the findings of Muhammad *et al.* (2015). There was no much difference in the pH and temperature change before and after coagulation. There was notable decrease in the turbidity and colour of the water sample after treatment. Also, at 250 rpm the turbidity and colour removal are least and best at stirring time of 50 rpm with lowest turbidity value of 0.68 NTU and colour value of 11 PtCo. It can therefore suggest that the lower mixing speed may improve the removal of turbidity due to reduced shearing of the flocs during initial formation which is in agreement with the findings of Muhammad *et al.* (2015).

Effect of Watermelon and *Moringa oleifera* as Coagulant aids in conjunction with Alum

The various coagulant combinations in Table 5 was used to treat the raw water sample. The results indicated notable decrease in pH. With an initial raw water pH of 7.30, the obtained pH values after coagulation are below the range of WHO standards of 6.5 to 8.5. The presence of alum causes a decline in the pH value (Ugwu, *et al*., 2017). This is in consonance with the results of Adejumo *et al*. (2013). In practical terms, this indicates that when using the combined coagulants of Watermelon and *Moringa oleifera* with alum as coagulant aid; no combination of this coagulants would give optimum result since the pH will be below the WHO recommended standards.

CONCLUSION

Coagulants from *Moringa oleifera* and watermelon were successfully extracted and tested at different ratios and doses in comparison with the alum solution. An optimum dosage of 0.7 g/L, optimum speed at 50 rpm, and optimum composition of 80% *Moringa oleifera* in conjunction with 20% watermelon were obtained. This therefore establishes that *Moringa oleifera* seed powder as a natural coagulant can be more efficient when used with 20% watermelon seed powder as a coagulant aid. Combining the advantages of both *Moringa oleifera* and watermelon produced overall best results with all parameters within WHO drinking water standards.

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Construction and Evaluation of Two Bladed Savonius Vertical Axis Wind Turbine

[1]Musa M., [2]Ibrahim A. G., [1]Argungu G. M., [3]Chika C. & [*2]Ibrahim H. I.

[1]Department of Physics, Usmanu Danfodiyo University Sokoto, Nigeria

[2]Department of Physics, Federal University of Technology Minna, Nigeria

[3]Department of Pure and Applied Chemistry, Usmanu Danfodiyo University Sokoto, Nigeria

[*]Correspondence: Email address: habson4ya@gmail.com: Tel: +234(0)8036806528

ABSTRACT

Nigeria (Sokoto, Kebbi Zamfara and Niger State in particular) is blessed with abundant wind, if carefully and properly harnessed with the aid of a wind machine. This can be used to generate electricity for the rural areas where power is less consumed by the inhabitants. The rate at which people living within rural and urban regions use fossil fuels thereby polluting the environment is not to be over emphasized. The release of CFC's gases which in turn leads to global warming needs to be addressed. Wind is a source of energy which has full potential in satisfying the energy demand of a given nation, yet, Nigeria is not exploiting the energy in the wind to the fullest, and instead, she suffers from either lack of electricity in some regions or high epileptic power supply in other regions. North western states have an average monthly wind speed of about 5.5m/s, which at times reaches 9.2m/s during the windy period of the year. Wind turbines are devices used to capture the energy of the wind and convert to mechanical energy that can be used to turn an electrical generator. Basically, there are two types of wind turbines: the vertical axis (which are also of two types: Savonius and Darrieus) and horizontal types, with the vertical axis wind turbine having so many advantages over the horizontal type. Yet, being less deployed for generation of electricity. The simple construction of Savonius rotor without any aerodynamic design and its ability to self-start at a lower wind speed makes it advantageous over the Darrieus type. A Savonius vertical axis wind turbine with a cut-in wind speed of 2.5ms^{-1} and cut-out wind speed of 12ms^{-1} was built to convert kinetic energy of the wind to mechanical energy of the turbine which turns a generator to produce electricity. The turbine was able to produce a maximum of 93rpm at a wind speed of 9.3ms^{-1} and a minimum of 43rpm at a wind speed of 2.5ms^{-1}. The turbine also proved to self-start at a wind speed of 1.5 - 2.0m/s.

Keywords: Savonius, Turbine, Rotation per minute, Wind, speed, Cut-in speed, Cut-off speed.

INTRODUCTION

Nigeria is finding it very difficult to meet its electricity needs from hydropower and fossil fuel generation alone due to increasing population and industrialization. Wind is a source of energy

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which has full potential in satisfying the energy demand of a given nation. Yet Nigeria is not exploiting the energy in the wind to the fullest. The power supply from the national grid is not enough, ineffective and most time not reliable. Hence, there is need to provide alternative source of power. It is very important that the country follow the trend of sustainability of energy adopted by developed countries in order to be self-sufficient in power generation, transmission and distribution (Adewumi, Oluwatoyinbo & Augustus, 2015). Wind is caused by the thermal movement of air particles and it is an environmentally friendly source of energy that has got huge potential of satisfying the energy needs of people living around areas with potential resources and can also serves as a means for mitigating the climate change from greenhouse gases, emitted by the burning of fossil fuels. It has been estimated that roughly 10 million mega Watt of energy are available in the earth's wind (GWEC, 2006). So, the potential of wind energy is reflected in the increase in capacity growth of wind energy systems across the globe. As of 2020, the total installed capacity of wind power system in the world had reached nearly around 743 GW (Statista, 2020). It was also recorded in 2017 that, there was a slight decrease from 2016 to 2017 bringing the total world wind power capacity to 539 GW (GWEC, 2017). Comparing world energy capacity of 2020 with that of 2010 of 194,390 MW, it is about 26% increase since 2010. On a yearly scale, wind energy production increases to about 17%, contributing immensely to worldwide electric power usage (GWEC, 2017), and proving its importance to the electrification of Europe by 11.6%, with about six European countries and Uruguay producing more than 10% of their energy from wind (Elie, Amne & Michel, 2019), and in 2019, the Asian countries took over by producing more energy from wind than any other country.

Currently, Nigeria is among the countries of the world harnessing the energy of the wind but on a very low scale, with recent development of 37 wind turbines installed in Lamber Rimi village of Katsina state and the electricity that would be generated from these wind turbines will be contributing to the National grid supply. There are at least more than 83 other countries around the globe generating their national electricity supply from wind power.

On a nationwide scale, utilization of wind energy for electricity generation can only be achieved with cost effectiveness in limited locations such as Gusau, Jos, Kano and Sokoto. It is found that although, Nigeria is blessed with reasonable wind energy resources for power generation, the country is still engrossed in blackouts and lack of electricity, leading to underdevelopment and crippling economic growth, both in rural and urban areas of the country. However, latest results show that the Southern and Northern states of the federation are capable of experiencing mean wind speeds between 3.0m/s to 3.5 m/s and 4.0m/s to 7.5 m/s (Source). On the Beaufort scale, the country is rated between 1 to 3 in the Southern state and between 3 to 4 in the Northern state (IJATER, 2014), meaning that, there is huge prospect within the country for power generation through wind if associated challenges hindering wind energy technology (WET) advancement are overcome (Ajayi, 2010). Nigeria is blessed with renewable energy sources in addition to abundant fossil fuel sources. Despite the seeming large number of pilot projects, their aggregate contribution is yet to significantly address the massive poverty of modern energy services, so, there is need for large scale deployment of renewable energy, by

getting the legal and regulatory support from government, since in Nigeria's mainland wind speed reaches at about 2m/s to 4m/s at 10m height (Sambo, 2012). This is an indication that greater wind resources are available at the country's hinterlands and coastal areas.

On a very basic level, a wind turbine is a large rotor wane called blades placed or mounted at the top of a high tower. The blades spin as wind hits them, producing electricity with the help of a generator attached to it. The amount of wind captured by the blade depends on the length of the turbine blades. The reason for maximizing the length of the blades is that, the larger the swept area of the turbine (this is the circle that the turbine produces while spinning), the more wind it will collect and therefore, this increases the energy it can generate. Due to enough wind and speed at higher altitudes, wind turbines are best installed on a high ground due to blockage of wind by trees and buildings around the installation areas.

Wind can be tapped by wind turbines that convert its kinetic energy into mechanical power, which will in turn be converted into electricity by a generator connected to the wind machine. There are basically two types of wind turbines namely: the Horizontal Axis Wind Turbine (HAWT) and Vertical Axis Wind Turbine (VAWT). These turbines have one or two advantages over the other, with the VAWT mostly installed in locations with lower wind speed while the HAWT is best installed in locations with higher wind speed giving it its higher rotational speed over the VAWT. Since the HAWT has higher rotational speed when compared to the VAWT, this work is based on Savonius VAWT rotor. Though, there are different types of VAWT, the Savonius, Helical Savonius, Eggbeater Darrieus, H-Darrieus, combined configurations of Savonius and Darrieus rotors etc.

Significant evidence exists that the environmental impact of chlorofluorocarbon (CFCs) gases to climate change is rapidly gaining momentum and its alleviation has paramount importance. The emissions of carbon dioxide (CO_2) by the burning of fossil fuel is a primary cause of climate change and reducing these pollutants in our atmosphere is crucial to human survival and sustainability. Also, wind power has expanded rapidly to make a significant contribution to global electricity generation.

So, constructing a wind turbine locally for the replacement of non-renewable energy sources and a long-lasting solution should be encouraged, intensified and supported by stakeholders and government. In order to augment for the electrical power condition in the country, the government has been forced to tap into wind energy by the buying wind turbines. Many researchers have done several valuations on the capability and accessibility of wind resources in Nigeria at several heights. An average wind speed of 5.4m/s at a height of 66m was conducted with 10 years speed data (Ogbonaiya, Okoro & Gvender, 2007). The characteristics of wind speed and energy potential in Minna, Niger state was examined using daily wind speed and direction data of 2 years at a height of 10m, the research showed that, the average wind speed was about 10.35m/s with an extractable power of 38.03kW. The average wind speed is actually above the minimum speed 6m/s needed for wind turbine to generate electricity (Moses, Oyedum & Oyewumi, 2019). Wind turbine has been investigated and even produced in Nigeria for electricity production in smaller scale as far back as 1990. The first wind farm in Nigeria was

completed in 2021 but not yet into production, located in Katsina, having close to 40 Horizontal Vertical Axis Wind Turbines capable of producing 275kW each and will be fed into the transmission grid for general use. The turbines were designed to feed Katsina grid with a total of 10MW of electricity. Since independence from UK, Nigeria has built 12 power plants and produced as much electricity as North Dakota for 246 times more people, with blackouts 320 times per year (Hinshaw, 2014). Therefore, Nigeria will need about 207,000MW per day for its 211 million people.

In Nigeria, the interest in windmills was shown in the late fifties and early sixties, in which the few windmills designed and developed could not yield expected results. An important reason could be that wind velocity in Nigeria, apart from coastal regions, which is estimated to be 2.3-3.4m/s at a height 10m and 3.0-3.9m/s for high land areas and semi-arid regions (Muhammad Argungu et al, 2013) is relatively low and varies appreciably with seasons. Since wind is intermittent, turbines tend to halt their operation for a while and lower wind can also have a retarding effect on the performance of the turbines. Thus, the challenge lies with the design of a wind turbine which can be used in a small-scale manner in remote and rural areas where electricity is very scarce.

Vertical axis wind turbines are gradually gaining momentum but their performance is still low when compared to horizontal axis wind turbines. However, vertical axis wind turbines have so many advantages over the horizontal axis, some of which are:

1. The materials needed for the construction of the turbine can easily be sourced within the environment, so it is cost effective
2. They can accept wind from any direction without the need of a yawning device to orientate it in the direction of wind flow, so their performance is independent of the direction of wind
3. They can be installed both in rural and urban areas. In addition, the turbine can be installed on the roof of a building and the ground. This gives them the advantage of not needing an expensive high support tower
4. They can easily be constructed and maintained since they do not need any complex aerodynamic design
5. They are best suitable for extreme weather conditions areas (Akwa, Vielmo & Petry, 2012).

THEORY OF WIND ENERGY CONVERSION

The kinetic energy of the wind was converted to the kinetic energy of the turbine. The maximum power P_{max} of the turbine is calculated from equation 1.

$P_{max} = ½ C_p \rho A V^3$ (1)

where:

A = swept area of the blades of the turbine (m^2) = length x diameter for VAWTs system

C_p = power coefficient of turbine (16/27) for Vawt Turbine

P_{max} = maximum power of the turbine (W)

ρ = density of air ($1.25 kgm^{-3}$)

V = speed of the wind (m/s)

Power in the Wind

Since wind is air in motion, it possesses kinetic energy. This energy per unit volume is expressed as;

$K.E = ½ mv^2$ (2)

where;

m = mass flow of the air (kg/s) = $\rho * A * v$ (3)

v = velocity of the wind (m/s)

ρ = density of the air (1.25kgm^{-3})
A = area swept by the blades

Important parameters for wind turbines are wind speed, turbine swept area and air density of the site of installation (Mari & Kirubakaran, 2014). The power output of the turbine is a function of the cube of the wind speed, so doubling the wind speed gives a higher wind energy potential. This is why vertical axis wind turbine requires a cut in wind speed of about 2.5 to 4m/s to start it up, and a cut out wind speed of about 12-25 m/s. While relatively few areas have significant prevailing winds in this range, many have enough to be harnessed effectively and to give better power output. Larger turbines with large swept area tend to have higher capacity factors.

The power available (energy per unit time) is expressed as:

$P = ½\rho AV^3$ (4)

where:
P = power present in the wind [W]
For a VAWT, the swept area is described by:

$A = 2rh$ (5)

where:
r = radius of the turbine [m];
h = length of the blades [m];
Naturally, the power extractible by the rotor is less than the expression (4). To calculate the power extractible by the rotor from the wind, consider Figure 1

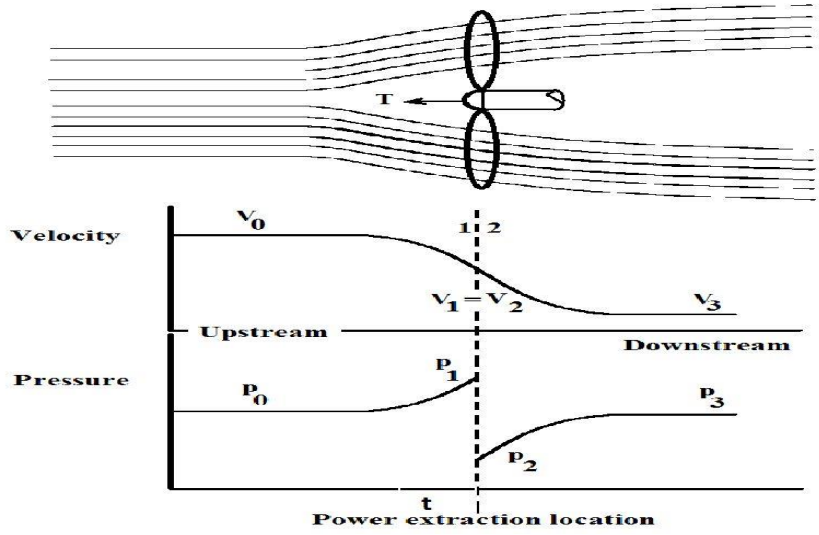

Figure 1: Downstream and Upstream Wind Velocity in a Location

Assume V_1 and V_2 to be upstream and downstream velocity respectively

$K.E = ½ m(V_1 – V_2)^2$ (6)

The difference between upstream and downstream provides upthrust equal to

$Thrust = m(V_1 – V_2)$ (7)

The power (Energy per unit time) is

$m(V_1 – V_2)v = ½ m(V_1 – V_2)^2$ (8)

Where $v = \frac{V1 + V2}{2}$ (9)

The power in the wind becomes

$P = m(\frac{V1 + V2}{2}) (V_1 – V_2)$ (10)

But m = ρAv

$= \frac{\rho A}{4} (V_1 + V_2)^2 (V_1 – V_2)$ (11)

$= (\frac{\rho A}{4})V_1^3 (1 + \frac{V2}{V1})^2 (1 - (\frac{V2}{V1})^2)$ (12)

The axial induction factor (α) defined as the fractional decrease in wind velocity between the free stream and the energy extraction device is expressed as

$\alpha = \frac{V2}{V1}$ (13)

$P = (\frac{\rho A}{4})V_1^3 [(1 + \alpha) (1 - \alpha^2)]$ (14)

$P = (\frac{\rho A}{4})V_1^3 (1 - \alpha^2 + \alpha - \alpha^3)$ (15)

Maximum power extractible implies

$\frac{dP}{d\alpha} = 0$ (16)

$0 = (\frac{\rho A}{4})V_1^3 (-2\alpha + 1 + \alpha - 3\alpha^2)$ (17)

$0 = 3\alpha^2 + 2\alpha - 1$

$\alpha = 1/3$ or -1

$\alpha = -1$ is not desirable because it implies

$V_1 = -V_2$ (no power)

So $\alpha = 1/3$ is the desired value

$P_{max} = ½ \rho A V_1^3 \frac{16}{27}$

$P_{max} = ½ \rho A V_1^3 (0.593)$ (18)

Comparing (18) and (3) and assuming $v = V_1$

$\frac{Pmax}{P} = 0.593$

This implies that, $P_{max} = 0.593P$

Harnessing power from wind is subject to Betz's law, which says that no turbine can capture more than 59.5% of the kinetic energy in the wind (or water). That is to say that, only 59.3% of the available power can be extracted from the wind.

In assessing the wind power potential of a site, the statistical data of its availability is necessary. So, the speed of air is low near the ground due to frictional effects according to the Hellman's exponential law given by

$V_z = V_s (\frac{Z}{10})^b$ (19)

where

b = Hellman's exponent

V_s = measured wind speed at a given height

V_z = wind speed expected at a height Z

Equation 19 is used to extrapolate the raw wind speed recorded from anemometer to a desire height. The Hellman's exponent is about 0.1429 for ground (sea level).

METHODOLOGY

Materials used for the construction

The Rotor

The rotor is made up of a thick iron rod which is 3.2m long, 4.0cm in diameter and was connected to two properly greased ball bearings which were placed at the opposite ends of the rod. The rotor helps to transfer the mechanical energy of the turbine to kinetic energy that helps to turn the generator

The Shaft

The shaft is made up of 2.9m long and 5.0cm in diameter iron pipe, strong enough to hold the weight of the Savonius blade. The rotor was placed inside the shaft and holes were drilled through the shaft in order to knot the shaft and the rotor together to enable the rotor to rotate as the shaft is rotating.

Ball Bearings

The ball bearings were properly greased to reduce friction and noise, the rotor was connected to them to ensure easy and higher rotational efficiency of the turbine. The size of ball bearings was carefully and calculative chosen to ensure them fittings

Shroud

The shroud was made from wood; a board with a diameter of 1.2m almost the same diameter of the Savonius rotor which was knotted to the both ends of the Savonius blade to enable

enclosure of the blades in order to harvest more wind and for safety measures and other necessary applications.

The Base

The base was constructed and built out of 2-inch angle iron; it was built to ensure that it can stand the weight of the turbine and speed of the wind from any direction, strong enough to prevent excessive vibration and noise from the turbine during high wind regimes. The base or stand is 1.17m high and 2m wide at the bottom which is constructed only with a cast or angle iron, held in place with a stay wire strong enough to withstand any resistance at any wind speed in Nigeria.

The Savonius Blade

The Savonius blade was built out of a metal (drum) of diameter 1.0m and height 0.9m. The drum was cut into two equal half and welded to a shaft of 2.9m long. Another metal was welded round the diameter and length of the Savonius blade to ensure stability of the blade during rotation. The two half drums cut to make the Savonius blade were joined together by placing the half drums side by side, back and front to one another and then welded to the shaft and finally, the blades were painted to prevent corrosion or rusting of any kind.

RESULTS AND DISCUSSION

Wind Analysis

The wind analysis was an important part of this project because it allowed for perspective time at the same location where the turbine was installed at a height of 8.17m above the ground level. Through analyses done using thermo anemometer, tachometer and multimeter, all the data was combined to see what potential lies around the area. Using Microsoft excel, a large amount of data was looked at in an organized manner. The program provided wind roses for the directional data that was collected.

Figure 2 helps to show the average wind potential directly surrounding the area where the turbine was installed at 15min intervals at three different locations for one week each.

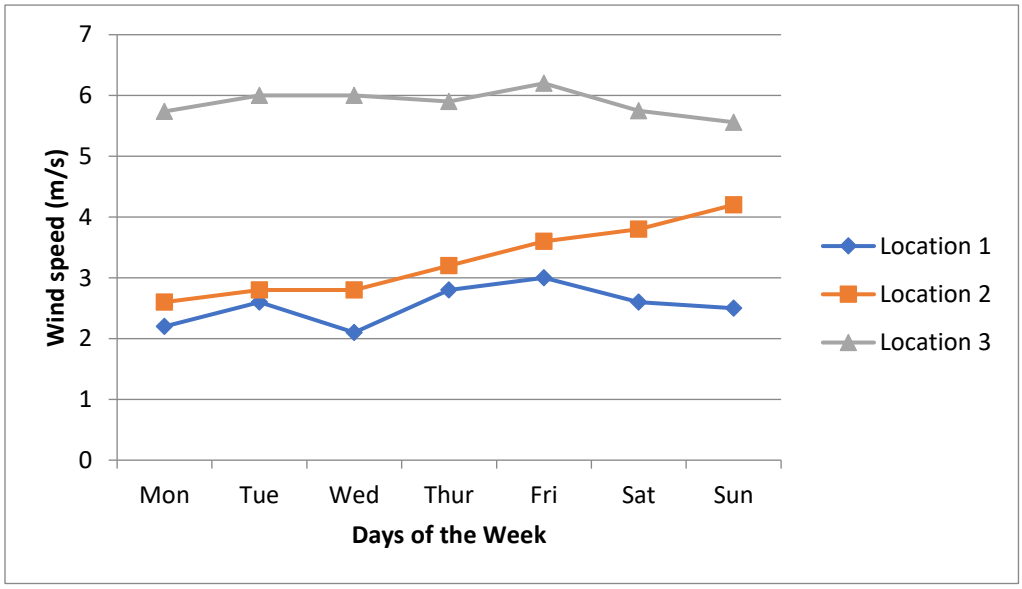

Fig. 2 Wind variation for three different locations

It was observed from Figure 2 that the wind potential of location 1, which was 1.8m above the ground level, was the least because wind speed increases with an increase in altitude. So, this location does not provide the required wind speed for the turbine to function efficiently.

In location 2, the wind speed reached a maximum of 4.2ms^{-1} on Sunday at a height of 1.8m above the ground level, this is because the area was open and there were no trees to disturb the flow of the wind. Although, the speed of the wind on this location is large enough to start up the turbine and also enable the turbine to rotate, the number of revolutions per minute will be too small, for the generator specification used during this research.

However, based on the result collected from three different locations, the third location which was at a height of 5.8m above the ground level was chosen because the speed of the wind reached a maximum average of about 6.2ms^{-1} on Friday which is best for the turbine specification and the turbine was installed there.

More so, since wind increases and decreases at any time, there is no certainty that the wind speed will remain constant for even a minute.

Savonius Rotor Analysis

The Savonius rotor was tested and the variation of Rev. per minute vs. Wind Speed of the Savonius Rotor and Turbine Power vs. Wind Speed of the Savonius Rotor were plotted as shown in Figures 3 and 4.

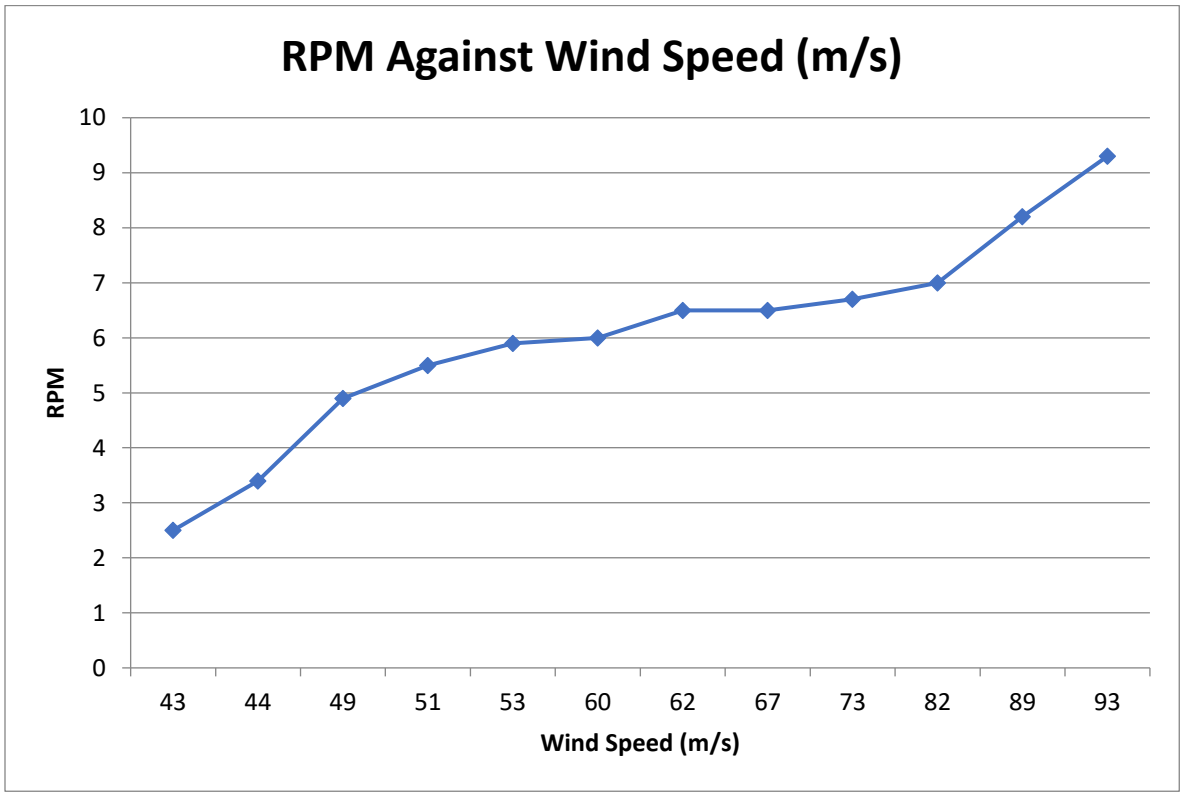

Fig. 3. Variation of Rev. per minute and Wind Speed of the Savonius Rotor

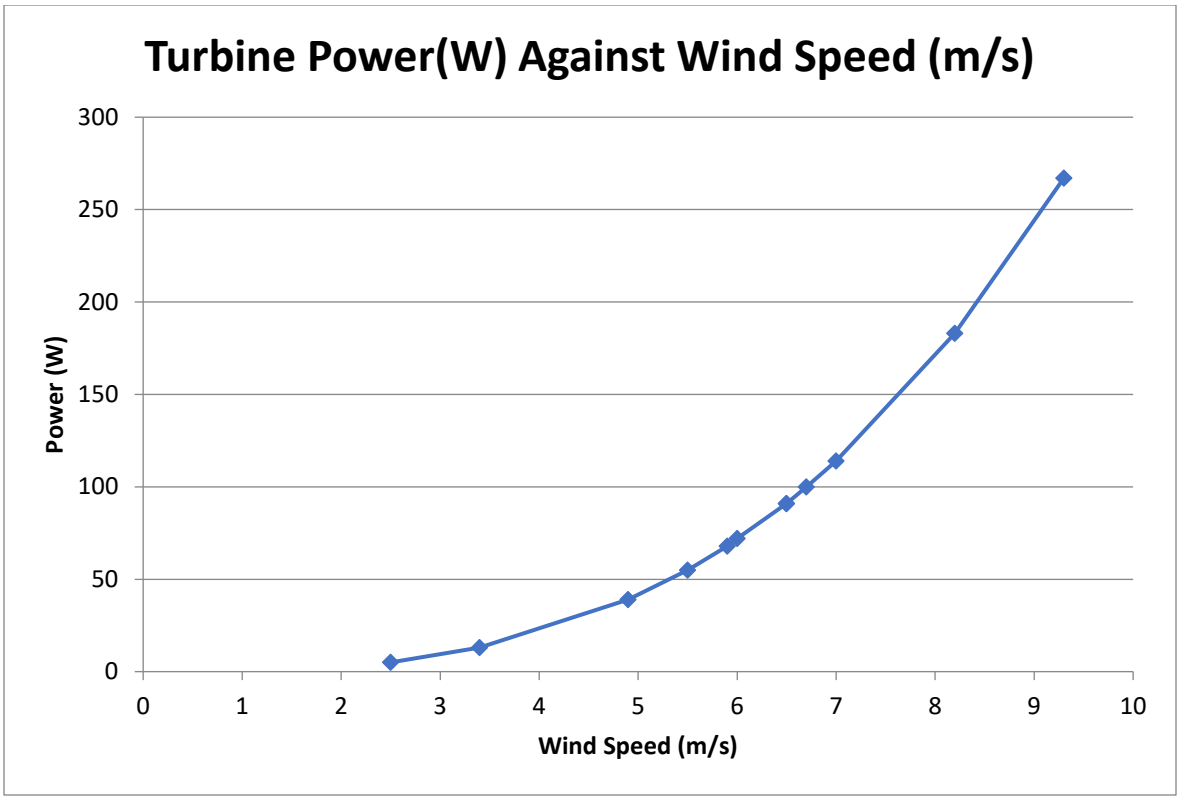

Fig. 4. Variation of the Turbine Power and Wind Speed of the Savonius Rotor

From Fig. 3 and 4, it shows that, the highest rotational speed per minute of the Savonius rotor was 93rpm at a wind speed of 9.3ms^{-1} and the lowest was 43rpm at a wind speed of 2.5ms^{-1} a cut-in wind speed of 2.5ms^{-1} and cut-out wind speed is 12ms^{-1}. At this wind speed (9.3ms^{-1}), the turbine functioned optimally as expected.

CONCLUSION

The Savonius Turbine was designed to withstand a wind speed of about 12m/s but with the help of a stay wire, which gives it a high stability. The turbine was tested and the results proved that it functioned very well. It was observed that, due to the weight of the Savonius rotor, a high wind must be required to start up the turbine but instead of reducing its weight, the swept area of the turbine was increased so as to get a higher lift. The turbine functioned well at low and high wind speeds, producing 43rpm and 93rpm at a wind speed of 2.5ms^{-1} and 9.3ms^{-1} respectively. There were times the Savonius rotor proved to self-start at a wind speed of 1.5-2.0m/s. so, further research will be carried out to test the output power of the turbine using a generator.

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Assessment of Partially Purified Urease from Spouted *Citrullus vulgaris* Seeds

*[1&2]Joseph P. S., [2]Musa A. D., [3]Evans C. E., [2]Uthman A. & [3]Ezikanyi G. K.

[1]Department of Chemistry, Air Force Institute of Technology, Kaduna, Kaduna State
[2]Department of Biochemistry, Ibrahim Badamasi Babangida University, Lapai, Niger State
[3]Department Biochemistry, Federal University of Technology Minna, Niger State.

*Corresponding author's contact: jpshabs@afit.edu.ng; Phone No: +2348175082009

ABSTRACT

Urease is a well-known enzyme which catalyzes urea into ammonia and carbon dioxide. Large quantities of urease in some species of the Cucurbitaceae family has been identified. It is an important enzyme for plants as it converts atmospheric nitrogen into absorbable form of nitrogen. It is also found in some bacteria and fungi and sometimes acts as defense mechanism. This research aimed at Assessing partially purified urease from sprouted *citrullus vulgaris* seeds. The seed sample was made to undergo enzyme extraction using precipitation and dialysis methods. The optimum urease activity and protein of the sprouted *citrullus vulgaris* was observed at day 7. The optimum pH and temperature were found to be 7.0 and 30°C respectively while activity of urease was highest at substrate concentration of 0.3M. The extracted urease can be used as alternative to either regulate the urea level in diabetic patients or could replace commercially available urease for carbonate precipitation.

keywords: Urease, *Citrullus vulgaris*, Optimum pH, Optimum temperature

INTRODUCTION

Nitrogen is the most important nutrient for plant growth and development (Hafnawy *et al*., 2014). It has been proposed that plant urease functions in the assimilation of urea normally formed in plants due to the hydrolysis of arginine to ornithine which is catalyzed by the enzyme arginase (El-Shora, 2001). Ureases (urea amidohydrolase, EC 3.5.1.5) is nickel (Ni^{+2}) dependent metalloenzymes for its activity, produced by plants, fungi and bacteria, but not by animals which are found to be ubiquitous and the most proficient enzyme known to date (Witte *et al*., 2002). Ureases catalyze the hydrolysis of urea into ammonia and carbamate which further decomposes into another ammonia molecule nd carbon dioxide. This reaction rate is being accelerated by Urease with a factor of at least 1014 when compared to the urea decomposition by elimination reaction (Maroney and Ciurli, 2014); In addition to the nitrogen assimilatory function of urease, plant ureases appear to have defensive roles against herbivore and fungal attack (El-Shora, 2001). Environmental factors such as temperature, pH, and substrate concentration can affect the enzyme activity (Pervin *et al.,* 2001). In medical laboratories, it is used for diagnostic purpose, in the determination of urea in biological fluids

Joseph P. S., Musa A. D., Evans C. E., Uthman A. & Ezikanyi G. K. (2021). Assessment of Partially Purified Urease from Spouted *Citrullus vulgaris* Seeds. 2nd Faculty of Natural Sciences Annual Conference. IBB University Lapai held between 30th August to 2nd September 2021. Pp 252-258

such as urine and blood (Chellapadian and Krishnan, 1998). Some of urease applications include treatment of industrial waste, the industry of alcoholic beverages, use in hemodialysis, and its potential use in space missions as life supporter (Kayastha and Srinivasan, 2001). Urease is also found useful since urea has commercial importance in the manufacture of hair conditioner, glues, fertilizer, plastic, animal feed and as a browning agent in factory-produced pretzels. Urease have been reported for screening various environmental pollutant, mainly heavy metals and pesticides. It has been extensively used as a model enzyme to elucidate the applicability of inhibitory assay for mercury II (Tsai and Doong, 2005). Urease utilizing bacteria has also been used to produce bio-cement for cement strengthening (Varalakshmi and Anchanadevi, 2014).

According to previous reports, large quantities of urease in some utespecies of the Cucurbitaceae family have been observed (Fahmy, Mohamed and Karmel, 1994). Therefore, in this gpresent study we determined an alternative source for urease using sprouted *Citrullus vulgaris* seeds.

Materials

Citrullus vulgaris (Water melon) were purchased from local market. Nessler's reagent, Ammonium sulfate, Dialysis turbing cellulose (sigma-alorich), Sephadex G-100 and other chemicals used were of analytical grade.

Methods

Preparation of *Citrullus vulgaris seeds sample*

Sprouting process: *Citrullus vulgaris* seeds were placed on a woolen material and covered while water was regularly applied. Under this humid and warm condition, the seeds became swollen and began to sprout. The sprouting process was monitored for 10 days.

Sample Preparation: 50 g of *Citrullus vulgaris* seeds was grinded into a fine powder and mixed with equal amount of water to form a paste-like product. After 24 hours the soaked melon samples was swirled for 2 minutes and sieved with four layers of pre-washed and dried muslin cloth. The filtrate was centrifuged at15000 rev/min for 15 minutes at 4^{o}C. The clear supernatant was collected as the crude enzyme and stored in the refrigerator for further analysis.

Ammonium Sulfate Precipitation: The crude enzyme was purified by precipitation using ammonium sulfate. The ammonium sulfate was added to solution of the crude enzyme in a concentration less than the point of precipitation of proteins. After centrifugation, the unwanted precipitated proteins were discarded, and more salt was added to the supernatant at a concentration sufficient to salt out the desired protein. After a second centrifugation, the protein was recovered as a precipitate.

Dialysis: This was carried out according to the method adopted by Bali *et al.,* (2020). Semipermeable membrane is used to separate small molecules and protein base on their sizes. The urease enzyme was dialyzed 12 hours against the extraction buffer. Changing the buffer three times after each 4 hours interval.

Protein Determination: The total protein was determined using the method described by Whitaker and Granum (1980).

Calculation: To calculate the protein concentration calcar's empirical formula was used;

$X = 1.45\ E_{280} - 0.74\ E_{260}$

Where X is the protein concentration in solution g/litre and E is the absorbance. E_{280} and E_{260} are the absorbance at 280 and 260nm respectively.

Urease activity

To measure the urease activity, the ammonia liberated on incubating the enzyme with urea for 30 minutes was determined using Nessler's reagent as described by Kulshretha and Husain, (2006).

Optimum pH: This was determined according to the method described by Kulshretha and Husain, (2006). pH of 0.05M Tris-acetate buffer ranged from 5.5 and 8.0 were used to assay for the urease activity incubated at constant temperature 60^{o}C and 0.2M urea.

Optimum temperature: This was determined according to the method described by Kulshretha and Husain, (2006). The free urease enzyme was suspended in Tris-acetate buffer (0.05 M) at the optimum pH above, and incubated at different temperatures (20 to 80°C) for 30 min.

Statistical analysis

Statistical package for social sciences (SPSS) was used to analyzed the data

RESULT AND DISCUSSION

The results obtained from this study reveal that sprouting significantly increase the protein level in *Citrullus vulgaris* with optimum protein level of 0.059 mg/g at day 7, after which the protein level began to decline as shown in figure 1. This result corresponds with the study by Dikshit and Ghadle (2013) who observed that Sprouts are rich in proteins, digestible energy, vitamins, minerals, amino acids, and phytochemicals, as these are necessary for plant germination. A study reveals that there was increase in the percentage of protein in germinated grains which shows that there is occurrence of protein synthesis during imbibitions leading to hormonal changes which plays an essential role in order to achieve a complete germination (Nanogaki., Bassl & Bewley, 2010).

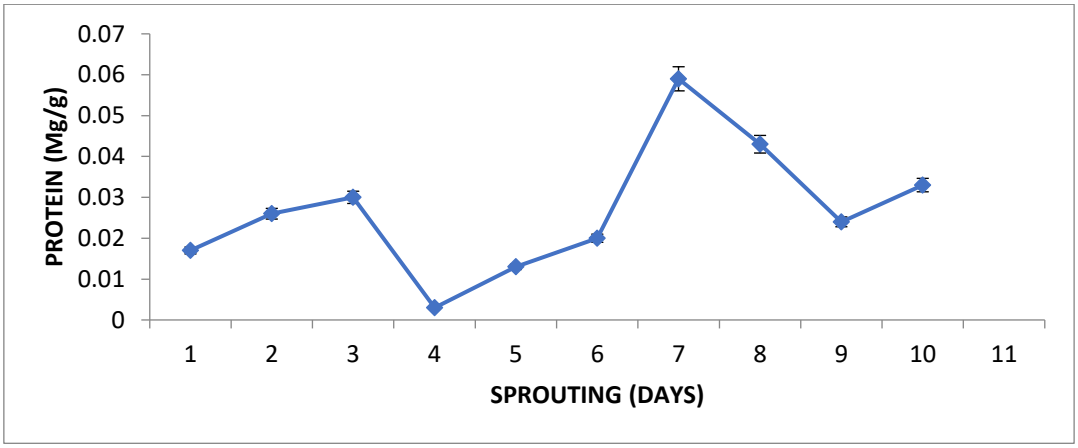

Figure 1: Effect of sprouting on the protein level of *Citrulus Vulgaris*

Different sprouting days (1-10 days) were carried out to optimize urease enzyme activity. The result reveals that urease activity was increased due to the sprouting process. *Citrulus vulgaris* was shown to have its optimum urease activity of 0.000168 U/sec at day 7 and then decline rapidly as shown in figure 2. This is consistent with Sanhita and Samita, (2019) that

reported increase in urease activity with germination time. Urease activity increased during plant germination, and this is attributed to role of the seedling accessory protein involved in nickel incorporation at the active site (Polacco and Holland, 1993). Urease maturation is very necessary because of its involvement in seedlings´ nitrogen metabolism (Polacco and Holland, 1993). Increase in urease specific activity is also associated with plant urea metabolism, most especially when urea is supplied as fertilizer (Mobley and Hausinger, 1989). Ordinarily, the nitrogen present in urea is unavailable to the plant unless hydrolyzed by urease. Therefore, plant urease activity is needed to be significantly increased to metabolize the large urea flow which could lead to leaf necrosis if available in excess (Stebbins *et al*, 1991).

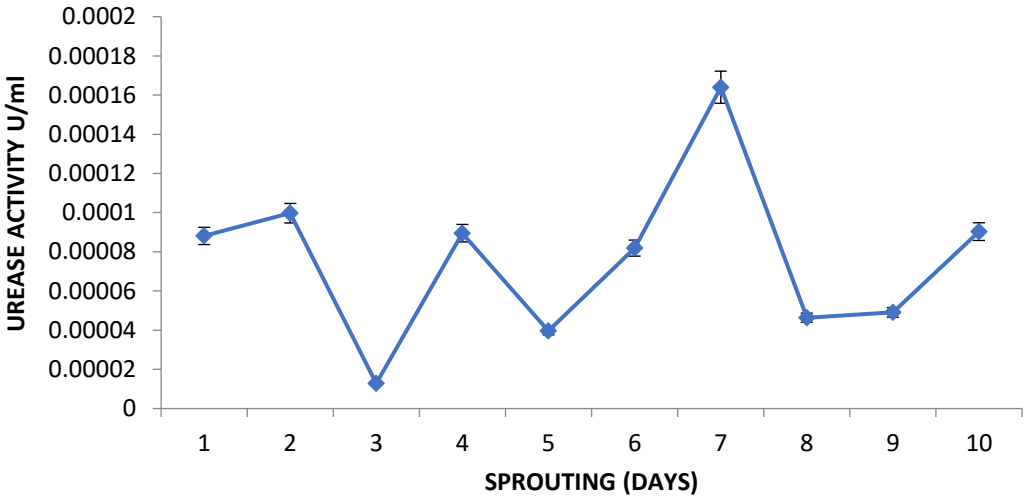

Figure 2: Effect of sprouting on urease activity

The pH value obtained from the purified urease studied has its optimum urease activity at 7.0 as shown in figure 3. Adewoyin and Egwim (2011) noted that urease possessed optimum pH between 6.5 and 7.0. The stability of urease activity at their respective pH may be explained by the structural changes occurring in enzyme proteins caused by pH variation.

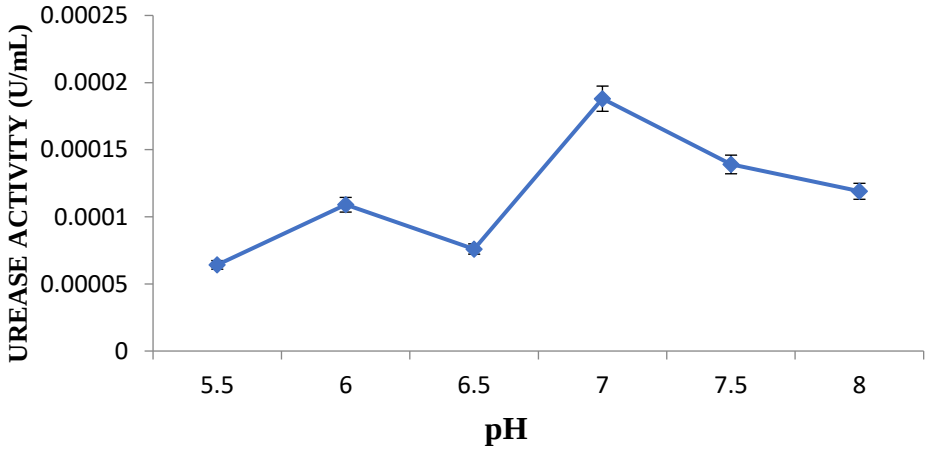

Figure 3: Effect of pH on partially purified urease activity

The optimal temperature for the partially purified urease enzyme is 30°C as shown in figure 4. As urease enzyme is proteinous in nature, it loses its three-dimensional conformation as the temperature increases and the enzyme is disintegrated. Although Krajewska (2009) observed that urease is a thermo stable enzyme, some studies on optimum temperature of urease in chenopodium album leaves (El-Shora, 2001) and Pisum Sativvum L. seed (El-Hafnawy and Sakran, 2014) were found to be 40^{0}C.

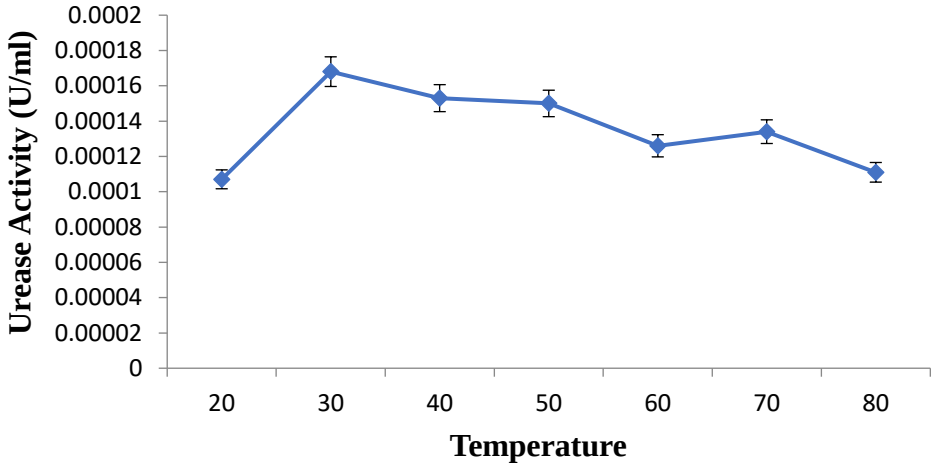

Figure 4: Effect of Temperature on partially purified urease

The optimum substrate concentration for the urease enzyme has been found to be 0.3 M. Urease enzyme activity initially increased with increasing substrate concentration but decreased with rising substrate concentration as shown in figure 5. At high concentration, urease activity became independent of substrate concentration.

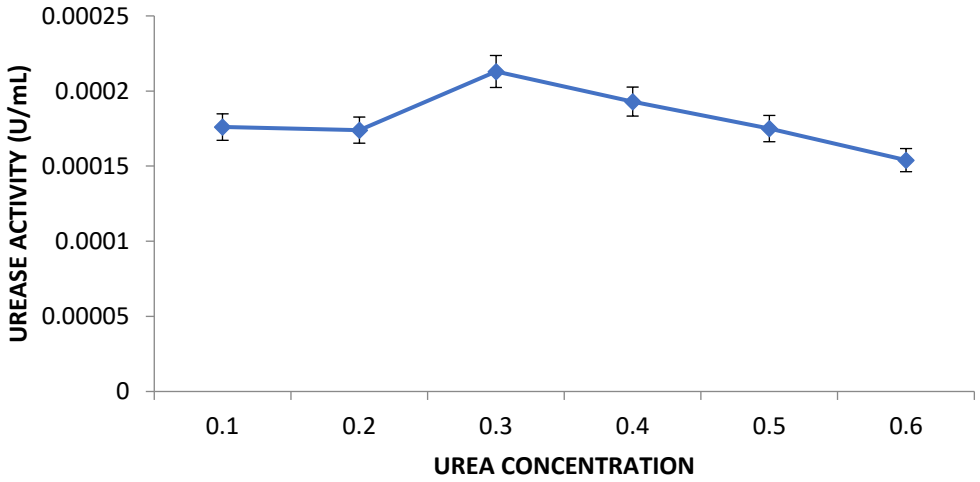

Figure 5: Effect of substrate concentration on partially purified urease

CONCLUSION

Urease from *citrillus vulgaris* was sprouted for 10 days to optimize the protein and urease activity which were found at day 7. The enzyme was partially purified by ammonium sulfate precipitation and dialysis. The partially purified enzyme was maximally active at 30 ℃ and at pH 7.0. The enzyme had optimum substrate substrates concentration at 0.3 M. The findings of the present study suggest that urease isolated and partially purified from *Citrullus vulgaris* will meet the numerous biotechnological applications such as clinical estimation of urea.

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Nutritive and Pharmacological Properties of *Annona senegalensis* Leaf Extract

[*a]Ayeni G., [a]Ejoba R., [b]Yahaya A., & [b]Larayetan R. A.

[a]Department of Biochemistry, Faculty of Natural Sciences, Kogi State University Anyigba, Nigeria, PMB 1008.
[b]Department of Pure and Industrial Chemistry, Faculty of Natural Sciences, Kogi State University Anyigba, Nigeria, PMB 1008.

*Corresponding author: gideon4.ayeni@gmail.com; +234-7068317885

ABSTRACT

Nutritive and pharmacological properties of the leaf's extract of *Annona senegalensis* using different models, was investigated. Quantitative analysis of proximate composites of the leaf revealed adequate moisture contents, protein, carbohydrate, crude fibre, ash contents. *In vitro* free radical scavenging ability of *A. senegalensis'* leaf extract displayed promising inhibitory activity (73.49 ± 12.03) against *2,2-diphenyl-1-picrylhydrazyl* (DPPH) at a high extract concentration. The protective effects of the crude extract in the carbon tetrachloride (CCl_4)-intoxicated rats model via intraperitoneal route of administration was evaluated. The results showed reduction in serum activities of aspartate, alanine amino transaminases, alkaline phosphatase, cholesterol, creatinine, uric acid, while increased serum total protein and albumin contents were observed in all the intervention (either standard drug, silymarin or crude extracts) groups. Co-treatment with crude extract was found to enhance serum liver of vitamin C, reduced glutathione (GSH), catalase and depleted malondialdehyde (MDA) contents, significantly ($p < 0.05$) compared to the untreated (induction) control animal group. The acute toxicity test (LD_{50}) of the crude extract in oral route of administration to experimental rats, recorded no adverse effect at 3000 mg/kg body weight. These findings have however provided adequate support of the role most locally herbs play in food supplementation for the treatment option of various free radical associated diseases.

Keywords: *Annona senegalensis*, Antioxidant, Induction, Supplementation, Carbon tetrachloride

INTRODUCTION

Medicinal plants are unique with diverse biological-related chemicals which have been exploited in the curative medicine and food industries, bridging the anticipated gabs between nutritional deficit and limited therapeutic agents (Ogle *et al.*, 2003). The pharmacologically active components of these plants have been exploited over the years in the traditional medical settings for the treatment of various ailments, including most disorders stemming from nutritional imbalances, with or without adequate credence of the empirical validation to that effect (Hassan *et al.*, 2010; Ayeni *et al.*, 2019). Literature revealed that most rural populations depend largely on plants to meet their health and nutritional needs. Several

Ayeni G., Ejoba R., Yahaya A., & Larayetan R. A. (2021). Nutritive and Pharmacological Properties of *Annona senegalensis* Leaf Extract. 2nd Faculty of Natural Sciences Annual Conference. IBB University Lapai held between 30th August to 2nd September 2021. Pp 259-275

of these medicinal plants have been attested to by their therapeutic impacts, with significant references made to their acclaimed success till-date (Farombi, 2000; Hassan *et al.*, 2010, 2015; Abo-elhamd *et al.*, 2016). They are antidotes to numerous health challenges emanating from oxidative initiators (Amadi *et al.*, 2019; Kulabhusan *et al.*, 2020) and have been shown to improve the nutritive quality of food in the neutriceutical setting (Day, 2013; Nemzer, Al-Taher and Abshiru, 2020). Hence, the need for a systematic search and application of the medicinal herbs in complimentary medicine, pharmaceutical industries and nutriceuticals, owing to the increasingly trend in the utilization of plant's extracts in the contemporary medicine of this age (Ribeiro *et al.*, 2015; Osorio, Flórez-López and Grande-Tovar, 2021).

There exist a good number of toxins, known to create acute liver injury in intervention studies (Muriel, 2008). Carbon tetrachloride (CCl_4) is one of the chemicals capable of inflicting acute injury to the liver with concomitant attack on the biological protein and membrane bilayer of the hepatocytes (Raj *et al.*, 2010). On the basis of the biotransformation channels to a toxic moiety, CCl_4 was selected as a model for evaluating acute liver damage in this work. Carbon tetrachloride (CCl_4) is a toxic organic solvent, often used in animal studies to induce acute and severe liver damage (Brautbar and Williams, 2002; Manibusan, Odin and Eastmond, 2007; Zamzami *et al.*, 2019). The cytochrome protein complex family is responsible for the metabolism of CCl_4 through Cyt.P450 dependent mono oxygenase and CYP2E1 both of which are localized in the mitochondria and endoplasmic reticulum portion of the hepatocyte (Ahmad and Tabassum, 2012; Delgado-Montemayor *et al.*, 2015). The intermediate products of CCl_4; trichloromethyl (CCl_3*) and trichloromethyl (peroxyl) anion (CCl_3O_2*) radicals, are potent hepatotoxins, as previously reported (Srivastava, Chen and Holtzman, 1990; Sanmugapriya and Venkataraman, 2006; Hassan *et al.*, 2015). The intermediary products of CCl_4 have been shown to induce protein aggregation and oxidize polyunsaturated membrane lipids (Ritesh *et al.*, 2015).

Annona senegalensis is a family member of *Annonaceae*. The specie is commonly known as Wild Custard Apple and widely distributed in Africa continent (Okoli *et al.*, 2010; Abe and Ohtani, 2013; Mustapha, 2013). In Nigeria, the specie is mostly found in the North Central and South Eastern part of the country. The plant is being revered in the traditional setting due to its medicinal value. *A. senegalensis* has been locally used as stimulant, pain reliever, vegetable in most food and supplement against commonly forms of energy deficiency. The pharmacological activities reported for *A. senegalensis* include, antioxidant, antimicrobial, antidiarrheal, anti-inflammatory, anticonvulsant, anti-malarial (Bongo *et al.*, 2017; Quílez *et al.*, 2018; Bhardwaj *et al.*, 2020; Kusmardiyani *et al.*, 2020). To the best of our knowledge, information revealing the nutritive and pharmacological properties of the crude extract of *A. senegalensis'* leaf are yet to be established in a comparative wise or validated by empirical means. This study however, aimed to investigate the nutritive value, at the *in vitro* level and the pharmacological activities, using rats in a model involving carbon tetrachloride (CCl_4)-induced hepatic challenged.

METHODOLOGY

Chemicals and Equipments

Unless otherwise stated, all chemicals and reagents used in this work were of analytical

grade, purchased through the designated outlets of their respective company. Equipments were those owned by Kogi State University Anyigba, Nigeria including those on collaboration with the research group, excerpts.

Plant Collection and Authentication

Fresh leaves of *A. senegalensis* were collected in March, 2021, Dekina Local Government Area of Kogi State, Nigeria. Authentication was done by Mr. Ayegba Sule, in the Department of Plant Science and Biotechnology of the same institution. A voucher specimen reference number-BC/002 was issued and herbarium specimen issued and herbarium specimen deposited. The collected leaves were shade-dried for a period of 21 days.

Preparation of the Extracts

The dried plant parts (1kg) were crushed to a reasonable size using modern laboratory grinding pistle. Extraction with methanol (1:5 w/v) were performed for 72 h. The extract mixture was separated using Whatman No. 1 filter paper. The organic solvent was recovered at 40 ºC ± 2 ºC, with the aid of a Heidolph rotary evaporatory machine. Concentrated extracts were dried under the fume hood overnight. The crude extracts were stored for further studies.

Free radical scavenging assay
***2,2-diphenyl-1-picryl hydrazyl* Assay**

Scavenging activity of the crude leaf extract of *A. senegalensis* were assessed on *2,2-diphenyl-1-picrylhydrazyl* (DPPH) radical solution. The outlined protocol of Simelane *et al.*, (2010), with slight modifications, were followed. 2 ml of the methanol extracts at concentrations range (25 µg/ml to 125 µg/ml) was added to an equal volume of DPPH (2 ml radical solution). The combined solution was completely mixed and allow to stand for about 30 min to 60 min maximum, for proper colour maturation. Calibrations of absorbance values for the combined solution was read at a wavelength of 517 nm using ascorbic acid (AA) as a reference-standard synthetic antioxidant.

Animal Research Protocol

An Ethical Research Clearance binding the use of animals for experimental purpose were obtained from College of Health Sciences Research Ethics Committee (CHSREC), Kogi State University Anyigba with reference number CHSREC/2021/0001. Animals were placed on acclimatisation under standard condition approved for the use of animals.

Experimental Animals

Thirty male *wistar albino* rats with weight range 190 to 200 g were procured from the Departmental designated company for the supply of experimental animals for Biomedical Research purpose as approved by Ethical Clearance Unit. Animals were kept in standard cages with beddings and were adequately changed throughout the period of the studies. They were acclimatised for one week in a standard laboratory conditions and maintained on standard animal feeds and clean tap water *ad libitum.*

Acute Toxicity (LD_{50}) Evaluation

The median lethal dose (LD_{50}**)** of the crude leaf extract was tested on six experimental animals. Rats were orally administered with (using oral gavage) 500, 1000, 1500, 2000, 2500, 3000 mg/kg body weight. The animals were observed for 72 h and follow up for possible signs of delayed toxicity and death in accordance with Erhirhie, Ihekwereme and Ilodigwe (2018).

Induction Process

Liver protective activities of the extract of *A. senegalensis* was carried out on *wistar strain* rats model. The rats were orally administered by gavage with the crude extract. Induction of hepatotoxicity was conducted in line with the outline protocols of Farombi (2000) and Rao *et al.* (2006).

Animal Grouping and Intervention Studies

The animals were divided into 6 groups of 5 rats each as follows:

Group I (Normal untreated), received daily dose of olive oil (1 ml/kg body weight orally, *per os*) for fourteen days. **Group II (**Induction untreated) were injected by means of intraperitoneal administration at 1 ml/kg body weight of 30% CCl_4 (in olive oil), 3 times a week for 14 days. **Group III** received silymarin at 70 mg/kg b.w and 30% CCl_4 (in olive oil) for 14 days. Group **IV** received a daily dose of crude leaf extracts of *A. senegalensis* at 20 mg/kg b.w and 30% CCl_4 (in olive oil) for 14 days. Group **V** received a daily dose of crude leaf extracts of *A. senegalensis* at 40 mg/kg body weight 30% CCl_4 (in olive oil) for 14 days. Group **VI** received a daily dose of crude leaf extracts of *A. senegalensis* 60 mg/kg body weight and of 30% CCl_4 (in olive oil) for 14 days. Experimental animals were sacrificed using the approved anaesthetic agent on the last day of the experiment. Blood and organs of interest were harvested for further biochemical assays.

Preparation of Serum Liver Homogenate

Excision of fresh liver tissue of about 0.5 g, followed by the homogenisation in a 5 ml solution containing 50 mM sodium phosphate buffer at pH 7.5. The homogenised liver was centrifuged at 15000 g for 15 min on a centrifuge operated at temperature of 4 °C to obtain the liver serum. The supernatant was kept in a refrigerator at -20 °C for other biochemical estimation.

Assay on Lipid Peroxidation

The procedure outlined by Oboh, Akinyemi and Ademiluyi (2012) , was employed. The solution is a mixture of 15 µL of sodium dodecyl sulphate (SDS) solution (8.1%), 56 µL of acetic acid (20%), 150 µL of thiobarbituric acid (TBA) solution (0.25%) and 63 µL of deionised water were added into the homogenate mixture (15 µL) on a 96 wells plates, heated for 60 min to a level of boiling point. The temperature of the mixture was adjusted to 25 °C. Spectroscopic measurement of the mixture was taken at a wavelength of 532 nm. Lipid peroxide products were read from the standard curve as malondialdehyde (MDA) equivalent

Vitamin C Determination

An outline method of Baker and Frank (1968), with minor modifications, was adopted. In each case, 4 ml of trichloroacetic acid was separately added to a 1 ml of protein content of the serum. The resultant mixture was centrifuged at 2000 g for 10 minutes. Following the incubation procedure, observed at 40 ^{o}C for 30 minutes, the mixture was allowed to cool to a temperature of 25 ^{o}C. 2ml of 2,4 dinitro phenyl hydrazine was added to the existing solution to precipitate hydrazone. Furthermore, 2 ml of sulphoric acid was added to the reaction system. A subsequent formation of orange-red colour, was measured spectrophotometrically at 520 nm.

Catalase Determination

The catalase determination employed the procedure stated Hadwan and Abed (2016), with minor modification. The reacting mixture consist of 10 µL of the serum liver homogenate, 58 µL of

hydrogen peroxide (65 µM) with sodium phosphate buffer (6 Mm) at pH 7.4. The resultant solution were completely mixed and left for incubation for 3 min at 37 °C. The reaction was terminated by the addition of 232 µL ammonium molybdate (32.4 mM) which was indicated through the formation of colour complex (yellow) of molybdate-hydrogen peroxide conjugate. Absorbance of the complex mixture as against the blank was read at a wavelength of 347 nm. Catalase activity was extrapolated.

Reduced Glutathione Determination

The procedure outlined by Ellman (1959), following slight modification was employed in this assay to evaluate glutathione (GSH) concentration in the serum liver homogenate. The serum liver samples were precipitated in to the reacting system containing 10% trichloroacetic acid (TCA) (98 µL each of sample and 10% TCA). The sample mixtures were centrifuged at 15000 x g for 10 min at 25 °C . 26 µL of the supernatant or standard blank with 13 µL of 0.5 mM 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB) and finally 65 µL of 0.2 M sodium phosphate buffer (pH, 7.8) were added respectively into the 96 well plates containing the reacting system. The solution were gently mixed and incubated for 15 min at 25 °C. Absorbances of the blank and tissue samples were determined at a wavelength of 415 nm. Values for the reduced glutathione (GSH) concentrations were determined from the GSH standard curve.

Estimation of Serum Parameters for Hepatic and Renal Indices

Serum total cholesterol, aspartate aminotransferases (AST), alanine aminotransferases (ALT), alkaline phosphatase (ALP), urea, uric acid and creatinine were analyzed using an Automated Chemistry Analyzer (Labmax Plenno, Labtest Co. Ltd., Lagoa Santa, Brazil) with commercial assay kits according to manufacturer's protocol.

Proximate Composition Determination

Determination of moisture, ash, crude fibre, fat, carbohydrate and protein contents were carried out by the outlined laboratory procedure of AOAC (Baur and Ensminger, 1977)**.**

Statistical Analysis

Data were represented as mean ± standard error of the mean of five replicates (n=5). The percentage inhibition was calculated from the graph of percentage inhibition. Percentage inhibition of each parameter was estimated using the following formula stated below:

$$\% \text{ inhibition} = \left[1 - \frac{(\text{Sample})}{(\text{Control})}\right] \times 100$$

The *in vivo* results were analysed statistically by one-way analysis of variance (ANOVA), using Graph Pad Prism, version 5.0, Bonferroni multiple comparison test. A *p*-value less than 0.05 was considered to be significant.

RESULTS

The crude leaf extract displayed promising scavenging effects at graded doses by inhibiting DPPH radical solution. The inhibitory activities were optimum at a concentration of 75 to 125 µg/ml of the extract solution (**Table 1**).

Table 1: Free Radical Scavenging Activity of the Crude Leaf Extract of *A. senegalensis.*

Concentration (µg/ml)	Ascorbic Acid	Extracts				
	100	25	50	75	100	125
% Inhibition	68.15±10.23	47.27±19.14	48.27±19.14	56.83±14.41	62.94±13.49	73.49±12.03

Values are mean± standard error of the mean of five replicates (n=5). Results are expressed as a percentage of their inhibitory concentration against DPPH radical solution.

The results of the acute toxicity test showed no adverse effect on the experimental animals. The median lethal dose (LD_{50}) of the crude extract was greater than 3000 mg/kg body weight. Induction of CCl_4 induce acute liver damage, leading to direct effects in the levels of liver biomarkers and associated kidney parameters, in animal studies. Application of the crude extract at 20 to 60 kg/bw dosages on daily basis for 14 days leads to a reduction ($p < 0.05$) in the serum levels of ALT, AST, ALP, total bilirubin, uric acid, creatinine, total cholesterol (elevating biomarkers in liver injury). Results showed a concomitant increase in the level of protein moieties (total protein and albumin) contents (being component of a reduced biomarkers in a liver injury), with a significant ($p < 0.05$) level of difference compared to induction control group (**figures 1 & 2**).

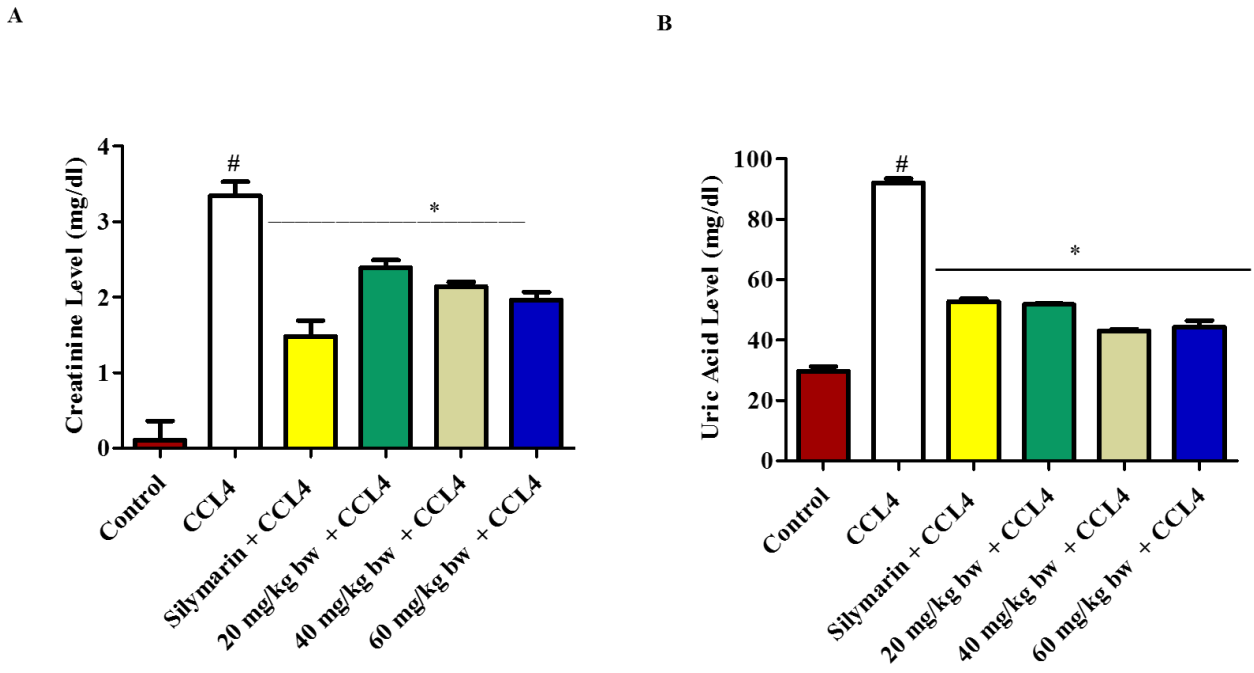

Figure 1: Effects of *A. senegalensis* on the serum renal indices in different animal groups. Data are presented as mean ± standard error of mean of five replicates (n=5); **#** = significantly ($p < 0.05$) different from normal reference control, *****= significantly ($p < 0.05$) different from CCl_4 group (unduction control).

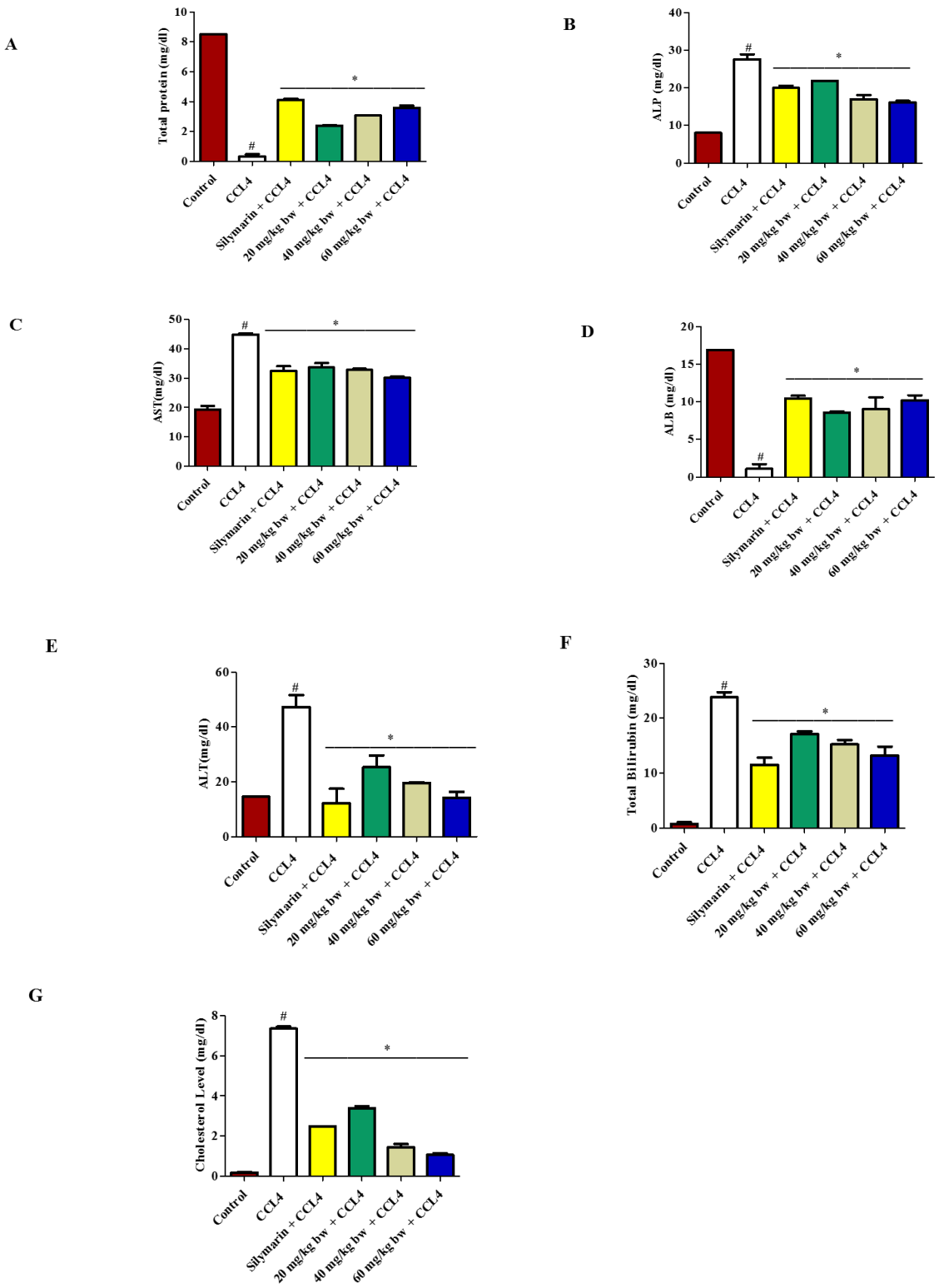

Figure 2. Effects of *A. senegalensis* on both liver function enzymes and non-enzymatic parameters on the experimental animal groups (graphs **A-G**). Data are presented as mean ± standard error of mean of five replicates (n=5); **#** = significantly ($p < 0.05$) different from normal reference control, *****= significantly ($p < 0.05$) different from CCl_4 group (unduction control).

In the same vein, pharmacological activities of the crude extract on the vitamin C, catalase, reduced glutathione and lipid peroxidation product (malondialdehyde, denoted as MDA) of the serum liver homogenate contents was further investigated on animal model, in the current study **(figure 3)**. Enhanced levels of the concentration of *in vivo* vitamin C, catalase, reduced glutathione moieties and decreased in the previous elevated concentration in the malondialdehyde contents of the liver homogenate were observed, with significant levels (p < 0.05) different from the induction control animal (CCL_4-treated) group. The crude extract displayed protective trend similar in effects with the standard drug of choice (silymarin) for the assessment. The promising inhibitory capacity which is an index of increase in a significant scavenging ability (Ayeni *et al.*, 2019) at a graded concentration (25 to 125 µg/ml), taking together with the *in vivo* protection of the liver cells against CCL_4-mediated toxic onslaught, provide adequate credence for the therapeutical value of the extract.

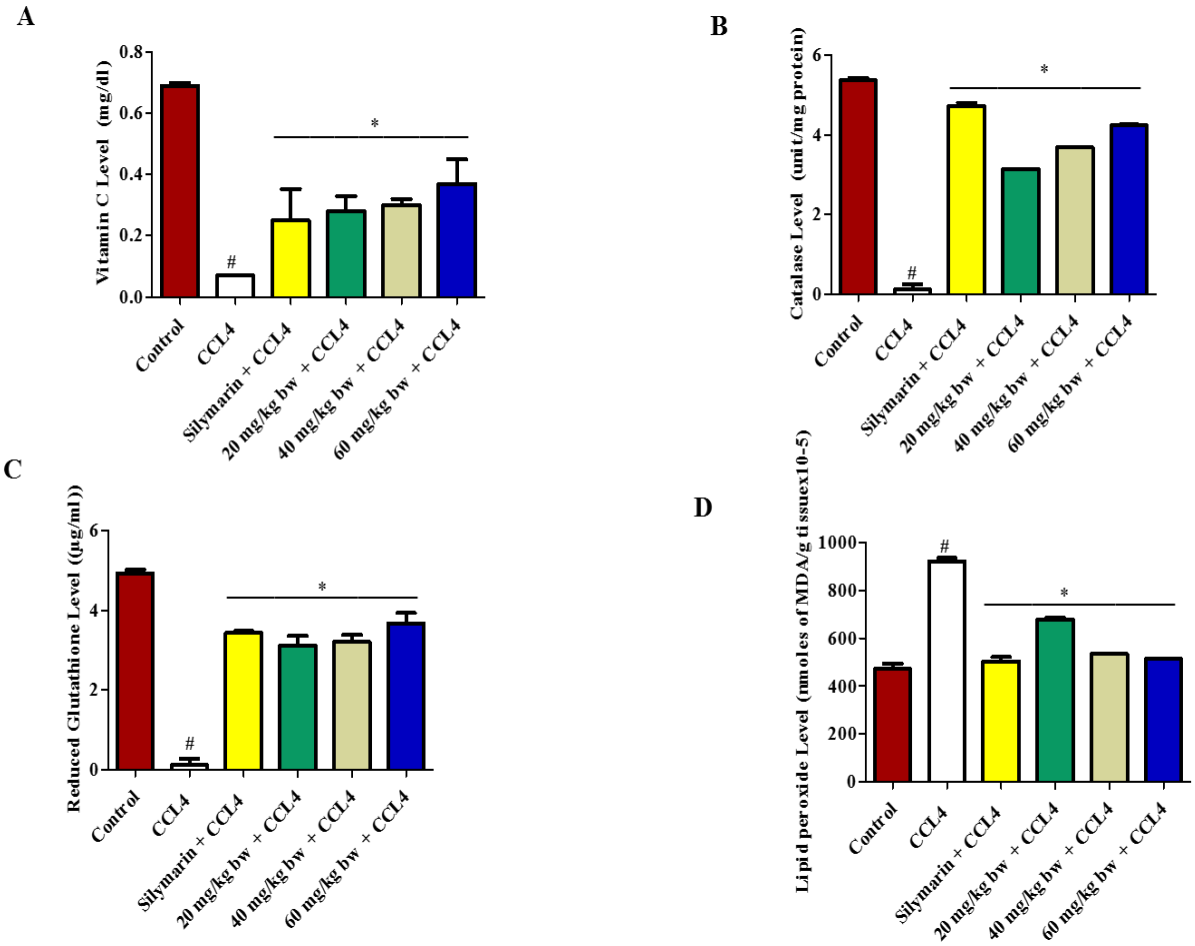

Figure 3. Effects of *A. senegalensis* on the serum liver antioxidant and non-antioxidant parameters on the experimental animal groups (graphs **A-D**). Data are presented as mean ± standard error of mean of five replicates (n=5); **#** = significantly (p < 0.05) different from normal reference control, *= significantly (p < 0.05) different from CCl_4 group (unduction control).

Investigation of the chemical composites of crude leaf extract of *A. senegalensis* revealed various organic compounds. The various natural components detected were quantitatively analysed as presented in **table 2.**

Table 2: Proximate Composite of the Crude Leaf Extracts of *A. senegalensis*

S/N	Proximate Analysis	Values (%)
1	Moisture Content	5.10 ± 0.06
2	Ash	1.65 ± 0.09
3	Crude Fibre	1.95 ± 0.03
4	Fat	0.80 ± 0.06
5	Protein	1.80 ± 0.03
6	Carbohydrates	88.71 ± 0.08

Values are mean ± standard error of the mean of five replicates (n=5).

DISCUSSION

The crude leaf extract of *A. senegalensis's* plant displayed strong inhibition against DPPH radical propagation at a concentration range of 75 and 125 µg/ml, employed *in vitro*. The inhibitory activities of the extract against DPPH radical solution are thought to be mediated by certain phytochemicals such as phenolics and flavonoids, known for the scavenging activities as reported by Kagan *et al.* (2002); Rathee, Hassarajani, Chattopadhyay, (2007); Sanni *et al.* (2018); Ayeni *et al.* (2019). Free radicals have been identified with usual alteration of important biomolecule, and a specific target to DNA base sequence (Golla and Bhimathati, 2014; Ayeni *et al.*, 2019). It is most likely that the plant act basically as an *in vitro* electron donating agent aimed at averting the proliferation of the radicals emanating from the synthetic free radical (DPPH) employed.

Acute liver injury rooting from hepatotoxins are known to cause a range of pathologies with increased deleterious effects (Heise *et al.*, 2015; Zamzami *et al.*, 2019). The mode of hepatotoxicity of CCl_4 is mediated through its reactive intermediates serve as the precursors of oxidative damage of the liver tissues, protein conjugate, membranes layer and ultimately liver failure (Yang *et al.*, 2010; Lee *et al.*, 2014). Increased level of activities and concentration of these oxidative parameters; ALT, AST, ALP, total bilirubin, uric acid, creatinine, total cholesterol and decreased in the protein moiety (albumin and total protein) contents in the serum instigated by exogenous toxins are effective indicators of liver injury (Bag and Mumtaz, 2013). Cholesterol in conjunction with other biological lipids are relevant in the maintenance of physiological integrity and well-known to play key role at the advent of pathogenesis of atherosclerosis (Levine, Keaney Jr and Vita, 1995). In the current study, elevated serum concentration of total cholesterol, being one of the indicators of oxidative stress, observed in an increasing generation of free radicals *in vivo* (Shukla, Bigoniya and Soni, 2015). The oral administration of the extract of *A. senegalensis* at varying doses (20 to 60 mg/kg bw) were observed to mitigate the previously elevated concentrations of ALT, AST, ALP, uric acid, creatinine, total bilirubin, total cholesterol in all the intervention rats groups. Thus, ascertain a protective ability of the potency of the medicinal plant with a nutritional value on a liver previously challenged with a toxic inducer comparatively with silymarin (standard hepatoprotective drug).

In vivo antioxidants are known to play important roles in protecting intracellular compartment against toxic inducers and other opportunistic invaders (Yi *et al.*, 2014). *In vivo* defence system

are set of enzymatic and non-enzymatic antioxidants such as catalase, superoxide dismutase, glutathione reductase and peroxidase and other antioxidant vitamins. They are constitutive in their biological configuration, whose functions are in line of scavenging free radical invaders of bodily architecture, detoxification of harmful substance to a predestined harmless moiety, distribution, recycling and incorporation of vital exogenous substance into the biological stream (Balendiran, Dabur and Fraser, 2004; Okokon, Simeon and Umoh, 2017). In this study, estimation of catalase, reduced glutathione and vitamin C contents of the serum liver homogenate in the crude leaf extract of *A. senegalensis's* co-treatment animal groups with CCL_4, revealed an impressive increase in the concentration of the liver antioxidant parameters, significantly ($p < 0.05$), compared with the CCL_4 animal infusion group. The results of the antioxidant contents in the serum liver homogenate of the co-treatment animal groups showed no significant different when compared to the standard liver protective drug (silymarin), under the condition of our investigation. Increased levels of these liver antioxidants molecules are important markers of homeostasis in a healthy liver state (Dudonne *et al.*, 2009). However, reduction in the activity and levels of CAT, GSH and Vitamin C in the liver indicate liver state of compromised functions, occasioned by the induction of hepatotoxicity (Venukumar and Latha, 2002).

Moreso, the product of lipid peroxidation (malondialdehyde, MDA) otherwise known as lipid peroxide has been regarded as an important parameter in the evaluation of liver status (Oboh, Akinyemi and Ademiluyi, 2012). Evaluation of the lipid peroxidation products in this study, showed significant ($p <0.05$) depleted MDA level in the serum liver homogenate contents of animals concurrently administered crude leaf extract and CCL_4, compared to the non-intervention (CCL_4-intoxicated animals') group. A depleted amount of MDA in the co-treatment animals (animal groups with extract intervention bias) serum liver homogenate, suggest an increased synthesis and replenishment of the damaged liver tissues and endogenous antioxidants (Yi *et al.*, 2014). The observed trends in these findings corroborate with previous researches updates (Hassan *et al.*, 2011; Hassan *et al.*, 2015; Ayeni, Ejoba and Olajide, 2016; Sanni *et al.*, 2018). It is probable, that *A. senegalensis's* crude leaf extract exerts phyto-antioxidant-dependent mechanism, through donation of electrons(s), chelating, reduction of metallic ion (Simelane *et al.*, 2010; Maltas and Yildiz, 2012) and induction initiative of the *de novo* synthesis template for endogenous antioxidative molecules (Hassan *et al.*, 2015; Sanni *et al.*, 2018).

The correlation between individual nutritional status and the state of health has been previously established (MMPrado *et al.*, 2013; Sartorelli *et al.*, 2019). In that regards, various components of the plant, in the form of herbal preparation or decoction, have been shown to improve dietary value and play vital role in the healing regimen in a complementary medicine (Nandal and Bhardwaj, 2012). Carbohydrate, protein, and fat contents, represent vital nutrients, serve to drive various metabolic activities as major plant's primary metabolites (Canarini *et al.*, 2019). The high carbohydrate value reported (88.71 ± 0.08) in this study, in comparison with protein (1.80 ± 0.03) and lipid (0.80 ± 0.06) composites, suggest to a large extent, high energy demand for basic-metabolic requirements by the specific organ of interest (leaf) and a seasonal variation factor. However, degradation sequel to the amino acid

requirements for nitrogenous need of the plants and other plant's segment could explain the basis for the reduction in protein moiety (Huffaker, 1990; Serventi *et al.*, 2010). Low yield of the lipid component under the condition of our investigation for the extract could probably due to the over dependency on starch (carbohydrate) for energy requirements, cellular preference for energy source and seasonal variation (Hartmann and Trumbore, 2016). Moisture, ash and fibre moieties have been shown to play an important role in nutrition (Chukwu, 2020). Moisture constituents is seen as been proportionally related to the relative water availability in the entire body mass of the plant (Tian *et al.*, 2018). Moderate amount of this component estimated in this study, probably, serves a means of internal defence against invading microbes (Melotto *et al..*, 2006). Ash content in the extract in any nutritional study, elucidate is the amount of valuable minerals deposite and bulk of organic residue in the plant extracts (Egharevba and Kunle, 2010). The presence of crude fibre has been shown to be associated with numerous health benefits ranging from ability to lower cholesterol level, reduce constipation and ease complex carbohydrate digestion (Tungland and Meyer, 2002).

CONCLUSION

The crude extract exhibited enhanced pharmacological activities through the scavenging effect of DPPH radical. The extract showed a normalising effect on the serum enzymatic and non-enzymatic parameters and induce synthesis of *in vivo* antioxidant system through proposed antioxidative mechanism of action. These activities demonstrate the protective effects of *A. senegalensis* against oxidative-induced liver damaged, attributed to its unidentified phytochemical constituents. Thus reporting data therapeutically justify *A. senegalensis* consumption either as dietary source or for curative purpose. Hence, structural elucidation of the active ingredients eliciting the bioactivities is therefore recommended for further investigation.

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CONFLICT OF INTEREST

Authors declare that no conflict of interest in this study.

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Mycological Evaluation of Barbing Saloon Tools in Lapai Town

*Mohammed J. N., Babangida M. K. & Muhammad I. L.

Department of Microbiology, Ibrahim Badamasi Babangida University, Lapai

*Email: ndejiko@gmail.com, ndejiko@ibbu.edu.ng Phone no. +2347067855089

ABSTRACT

Hairdressing and barbing saloons are personal service establishments that can constitute health risk to the general public due to the dangers of infection and, in some cases, injuries and damage to the skin. The aim of this study was to look at the fungal contamination of barbering shop tools in Lapai, Niger State, Nigeria. After seeking the shop owners' consent, swab sticks were used to collect samples from barbering tools including combs, brushes, and clippers. The samples were diluted serially before been plated on SDA. Pure distinct fungal colonies were subcultured on fresh SDA plates and maintained on Slant bottles for characterization and identification. Total fungal counts obtained from salon utensils across Lapai town ranged from $4.0x10^5$ to $1.0x10^5$ CFU/swab area. Seven fungal species isolated and identified included *Aspergillus niger, Candida spp. Mucor spp, Aspergillus flavus, Trichophyton spp, Torulopsis glabrata and Penecillium spp.* The highest percentage occurrence of fungi on salon utensils were *Aspergillus niger* (38.0%), and the lowest was *Penecillium spp* (4.8%). The presence of these potential pathogens is an indication that hairdressing and barbering salons could be contributing to the spread of infection within Lapai town.

Keywords: Barbing, Saloon, Fungi, Infection, *Aspergillus*.

INTRODUCTION

Barbing is one of the non-sexual public behaviors that can exposes people to blood and body fluid related infections through sharing of the tools used in the saloons. The nature of the service render by the barbers, as well as the tools and equipment they used can contribute to the health risks associated with their services (Al Yousef *et al*., 2021). A saloon is a shop or an apartment where hairstylists and barbers offer personal services to their customers using a range of tools or instruments (Jan Mohammadi *et al.,* 2015). Infectious agents mostly microorganisms can be accidently transmitted through blood during haircuts, especially in barbers' shops.

The fungi are the chief cause of salon infections, the common species include *Cladosporium, Candida, Mucor, Trichophyton, Penicillium, and Aspergillus*. Fungi are unicellular microorganisms that can cause range of diseases in humans. Some of them are normal human flora with high potential to become infectious under certain conditions (Naz *et al.,* 2012). The hair salon tools are mostly damp; an ideal habitat for the fungal proliferation that can easily come in contact with the hair of the clients during use (Müller *et al*., 2021).

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Fungi infections can spread from an infected client's scalp to their hair thereby spreading from hair to tools and to another customer in the barbering salon especially when the disinfection and sterilization procedures are not adhered to between uses. The hazards associated with the tools could affect both the operators and the customers considering the ease of transmission of microorganisms. Infections such as HIV, hepatitis B and C, warts and skin infections including *Tinea capitis* (scalp fungal infection), have been transmitted via saloon tools such as razors, scissors and clippers (Wagne, 1990).

The risk of transmission that follows parenteral exposure to HIV-infected blood was estimated to be around 0.3 percent but believed to be up to 100 times larger for HBV (30 percent), and between 3 and 10% for HCV. The communal cultural behaviour among most people shaving at a shop or roadside barber is an underappreciated and lack of understanding of pathway of blood-related transmission of infections (WHO, 2000). Two significant principles are integral part of any infection prevention and control measure: general precautions and risk assessment. The general precautions are established risk-lessening measures that salon owners should always apply especially when they come in contact with blood or other bloodly fluids. The concept of these precautions refers to the appropriate use of safety Personal protective equipment throughout the handling of sharps and the deployment of barriers. Salon owners are expected to treat all clients as infectious and take proper infection control precautions (CDC, 2003). The aim of the present study is to evaluate the barbers' tools in barbing shops within Lapai town.

METHODOLOGY

Samples Collection

The labelled sterile swab sticks were initially dampened in normal saline and used to swab the tools of interest at appropriate points. The tools used for this study included combs, brushes, clippers, towels and chars. The swab sticks were instantly corked appropriately and immediately returned to the container and sent to the laboratory for analyses.

Enumeration and Isolation of Fungal Isolates

The swabbed samples were diluted in 10 mL sterile normal saline and vigorously shaken for 10 minutes to make a stock solution. 1ml of the sample stock solution was pipetted aseptically into a test tube containing 9ml of normal saline to create a 10^{-1} dilution, from which 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} dilutions were made. Aliquots (0.1mL) of dilutions of all samples were plated on Potato dextrose agar and spread with a sterilized hockey stick. The plates were covered and wrapped with film tapes before incubation at 25°C for 48-72 hours. The number of colonies produced from each plate, which ranged from 30 to 300, were counted and recorded.

Sub-culturing and identification of Fungal Isolates

The fungal colonies with distinct colour and morphology were subcultured on fresh PDA plate for pure isolates and incubated for growth and spore formation. The isolated colonies were also preserved on agar slant for further use. Each of the distinct fungi growth was characterized morphologically (growth shape, color, perimeter, and size pace of development, and presence of pigmentation in the medium) and microscopically and compared with mycological atlas.

RESULTS

Fungal count on the saloon tools

The highest fungal count of $3.7x10^5$ CFU/Swab area was observed on a clipper while the lowest fungal load of $2.7x10^5$ CFU/Swab area was recorded on comb and char (Table 1). Each of the brush and the towel had 3.0×10^5 CFU/Swab area However, the total fungal counts acquired from various salons tools throughout Lapai town indicates that all the tools contained fungi contamination.

Table 1. Mean fungal counts obtained from barbing tools (std means standard deviation of the triplicate data)

Sample	Colonies count [CFU/Swab area]	Std
Clipper	3.7×10^5	0.57
Brush	3.0×10^5	0.82
Comb	2.7×10^5	1.20
Towel	3.0×10^5	0.21
Char	2.7×10^5	0.57

Morphological characteristics of the isolated fungi

Table 2 shows the morphological characteristics of the isolated fungi. In all seven fungi species were identified in the study, which include: *Aspergillus niger* (black, velvety to flaky surface, septate and long smooth colorless or brown), Candida spp. (white cream to yellow, septate, round and overly), Mucor (white, spherical, thick non septate and elliptical contain large number of poragia), *Aspergillus flavus* (yellowish green, velvety to flaky surface, septate and long rough conidia), Trichophyton spp. (white/light, pear or cone shape, septate and longish smooth walled), *Torulopsis glabrata* (cream, round colony, non-septate and non-conidia), Penecillium spp. (green / brugreen, powdery, septate and reses vertically from hyphae).

Table 2: Morphological characteristics and identification of the pure fungal Isolates

S/N	ISOLATE	COLOUR	SHAPE	HYPHAE	CONIDIA
1	*Aspergillus niger*	Black	Velvet to flaky surface	septate	Long smooth colorless or brown
2	*Candida spp.*	White Cream to yellow	Round and overly	septate	Round ovelly
3	*Mucor spp.*	White	spherical	Thick and non-septate	Elliptical contain large number in the poragia
4	*Aspergillus flavus*	Yellowish green	Velvet to flaky surface	Septate	Long conidia rough
5	*Trichophyton spp.*	White / light	Pear or cone shape	Septate	Longish smooth walled
6	*Torulopsis glabrata*	Cream	Round colonies	Non septate	None
7	*Penecillium spp*	Green	Powdery	Septate	Reses vertically from hyphae

Percentage occurrence of fungi

The highest percentage occurrence of fungi was *Aspergillus niger* (38.0%), and the lowest was *Penecillium spp* (4.8%) as shown in Table 3. Each of *Torulopsis glabrata* and Candida sp had 9.52%, *Aspergillus flavus* 16.7%, Mucor 14.2% and *Trichophyton spp* 7.14%

Table 3: Frequency of occurrence of isolated fungal species

Fungus Colony	Number of Colony	Percentage (%)
Aspergillus niger	16	38.0
Candida sp	4	9.52
Aspergillus flavus	7	16.7
Mucor	6	14.2
Trichophyton spp	3	7.14
Torulopsis glabrata	4	9.52
Penicillium	2	4.80
Total	42	100

DISCUSSION

The highest fungal count recorded on the clipper is due to the fact that it is the tool that is always in contact with the skin and scalp where fungal growths are rampart. Two (4.76%) of the 15-equipment analyzed had one fungus isolate (mono-fungi contamination), one (2.38%) had two fungi isolates (dual-fungi contamination), and twelve (28.57%) had more than two fungi isolates.

The findings of this study agree with Sekula *et al*. (2002), who undertook an environmental evaluation of four personal service salons. Swap samples from randomly selected instruments at each salon were examined for the presence of bacteria and fungus, and the results indicated that three of the four salons' instruments were contaminated with bacteria and fungi.

This shows that the existing salon disinfection methods were ineffective in minimizing health risks among customers as a result of the presence of these potentially harmful fungi. Sterilizing processes vary by service providers and are rarely implemented especially if the client looks illiterate of the possibility of infectious agent transmission via such tools (Stanley et al., 2019) The use of ethanol, flame, and ultrasonic cleaners are some local sterilization methods that could help prevent microbial transmission but are rarely or efficiently implemented (Abubakar *et al*., 2017). The presence of fungi in these salons' equipment and supplies implies that the operators' sterilization methods are poor, if they at all sanitizes them between customers

One of the fungi isolated from all of the saloon tools *(Aspergillus niger)* is one of the most common fungal pathogens in humans. *Trichophyton spp*. was found in most tools, indicating that *ringworm* or *dermatophytosis* can also be disseminated through these salons. These findings demonstrate that these salons' cleanliness procedures fall well short of what is required. This might indicate that the salon owners or staff are unconscious of the hazards they encounter on the job. This situation demands that public health organizations wake up to their duties in teaching and organizing sensitizations, training workshops, and seminars for barbering salon owners and staff in Lapai town (Edward et al., 2015). People who are aware of the dangers or hazards connected with their profession are more inclined to change their practices and, as a result, can help reduces disease transmission.

CONCLUSION

About Eight (8) fungal species including A. niger, *Torulopsis glabrata* penicillum spp, mucor spp and Trichophyton spp were isolated. The tools used in barbers' shops within Lapai town have a relatively high fungal load, which could cause an epidemic of the diseases associated with these organisms. Some of these fungal isolates for example A. niger can be pathogenic to human. The tools used in barbers" shops can act as reservoirs and carriers of microbes that can be passed from person to person. To minimize the spread of infections, all salons in Lapai town should be sensitized to maintain a high level of disinfection and sterilization.

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Production and Partial Characterization of Proteases Produced by *Bacillus lichenformis* Grown on Pineapple (*Ananas comosus*) and Watermelon (*Citrillus lanatus*) Peels as Carbon Sources

[*1]Tsado A. N., [2]Egwim E. C., [3]Oyeleke S. B. & [2]Shittu O. K.

[1]Department of Biological Science, Niger State Polytechnic, Zungeru, Niger State
[2]Department of Biochemistry, School of Life Science, Federal University of Technology, Minna, Niger State
[3]Department of Microbiology, Federal University of Technology, Minna, Niger State

*Correspondence Email: infoamosndarubu@gmail.com: Phone: 08032920909

ABSTRACT

Microbial proteases are the most widely used proteases in a number of industries. Due to their numerous applications in many industrial processes, the demand for proteases has experienced upsurge industrial demand. The use of cheap materials such as agro-wastes biomass with nutrients capable of supporting the growth of microorganism such as could help in meeting industrial proteases demand. The ability of pineapple and watermelon peels comparing to that of glucose as carbon sources to support proteases production by *Bacillus licheniformis* isolated from cow rumen ingesta was evaluated. In the current study, *Bacillus licheniformis* isolated from cow rumen ingesta at abattoir dump site was screened for the production of protease enzyme by culturing it onto skim milk agar to observe distinct zone of hydrolysis due to enzyme production. It was inoculated into enzyme production broth under submerged fermentation conditions at 37 ˚C for 48 hours. The optimum pH of the proteases produced with agro-wastes biomass was observed at pH 9, while that of glucose was at pH 6. The optimum temperature of all the proteases produced was found to be at 30 °C. However, the activities of proteases produced with agro-industrial wastes were significantly higher ($p<0.05$) than that of glucose under optimum pH and temperature conditions, though protease produced with pineapple peels had highest activity. The Km of the proteases produced with pineapple peels, watermelon peels and glucose were 1.64, 0.95 and 1.24 mg/mL while their Vmax were 277.78, 270.27 and 222.22 U/mL, respectively. The lower Km values of proteases produced from pineapple and water melon peel suggests that both agro-wastes biomass could be used as an alternative substrate to glucose. Therefore, these agro-wastes and *Bacillus licheniformis* should be considered in the low-cost production of protease enzymes for industrial application.

Keywords: Partial characterization, Protease, *Bacillus licheniformis, A*gro-wastes biomass and Cow rumen ingesta.

INTRODUCTION

Industrial enzymes such as proteases have a wide range of industrial applications and are of worldwide commercial importance (Ramadan, 2019). Proteases are of greatest biotechnological

Tsado A. N., Egwim E. C., Oyeleke S. B. & Shittu O. K. (2021). Production and Partial Characterization of Proteases Produced by *Bacillus lichenformis* Grown on Pineapple (*Ananas comosus*) and Watermelon (*Citrillus lanatus*) Peels as Carbon Sources. 2nd Faculty of Natural Sciences Annual Conference. IBB University Lapai held between 30th August to 2nd September 2021. Pp 282-289

interest, with a multitude of industrial applications in detergent, food, pharmaceutical, leather, textile, paper industries and in peptide synthesis (Farhadian *et al*., 2015, Uttatree *et al*., 2017). Proteases hydrolyze bonds in protein molecules to yield smaller chains (peptides) and units (amino acids). They include a complex group of hydrolase enzymes comprising about 60% of global enzyme sales (Uttatree *et al*., 2017 and Elumalai *et al*., 2020). While proteases are produced by animals, plants and microorganisms, microbial proteases are favoured for industrial applications because of their extracellular properties and high production rate (Elumalai *et al*., 2020). Microbial proteases are produced either by submerged or solid-state fermentation, and are stable under acidic and alkaline conditions and tolerate surfactants, heavy metals and oxidizing agents (Singh *et al*., 2017). Most microbial proteases are produced by bacteria. These bacteria grow fast, require only a short fermentation period, and secrete extracellular proteins into the medium (Orts *et al*., 2019 and dos Santos Aguilar and Sato, 2018).

The wide range of proteases applications in numerous industries owe to their ability to degrade wide varieties of raw materials to produce new and beneficial products (Yildirim *et al*., 2021). Recently, the demand for proteases in many industries have increased and lesser amount are produced owing to the high cost of production due to the substrates used for the production of the protease. In order to minimize the cost of production, the use of agro-waste materials as source of carbons in place of expensive carbon sources such as molasses, corn starch and glucose could bring significant commercial success in biotechnological enzyme production. Diverse agricultural waste materials such as wheat bran, soybean meal, cottonseed meal, rice bran, maize oil cake, linseed oil cake, coconut oil cake, mustard oil cake, groundnut oil cake, millet, lentil bran, orange peel, banana peel, and apple peel have been used for protease and amylase production by fermentation (Dar *et al*., 2015). These agricultural wastes can be used as substrates for enzyme production due to their availability, higher biomass and higher nutritional constituents (Gois *et al*., 2020). Thence, this study focused on producing proteases from *Bacillus lichenformis* grown on pineapple and watermelon peels in comparison with glucose as carbon sources.

METHODOLOGY

Media/Chemicals/Reagents

The media, chemicals and reagents used in this study were purchased from Panlac Chemicals and products of Sigma-Aldrich (St. Louis, Missouri, United States) Nutrient agar (NA), glucose, 0.87% sodium saline, 1% $CaCO_3$, 70% and 95% alcohol, crystal violet, gram's iodine, saffranine, glycerine, 3% KOH, Malachite green, carbolfuchsin stain, hydrochloride acid (conc. 3ml), methylene blue chloride (0.3g) beef extract (0.3%), zinc chloride, potassium iodine (0.1g), powdered zinc metal, yeast extract, MgSO4, K2HPO4, NaCl, methyl red, tryptone, potassium phosphate, sodium citrate, agar powder, bromomethyl blue, (NH4)H2PO4.

Collection of samples

Refuse dumpsite sample was collected in a sterile polythene bag and immediately transferred to the laboratory for the isolation of bacteria.

Methods

Isolation of microorganism

One gram (1gm) of refuse dumpsite sample was mixed with 9ml of saline solution (Master dilution) and 1ml of solution was serially transferred to tubes containing 9 ml saline each so that for each transfer the suspension was diluted 10 times. Each tube was shaken vigoroussly. 0.1ml solution was spread to petri plates containing sterilized nutrient agar and saboroud dextrose agar for bacterial and fungal isolation. The pure isolates were stored in bottles for further studies.

Screening for proteolytic activity

The isolates obtained from refuse dumpsite were spread on petri plates containing milk agar medium (pH7) and incubated for 24h at 37°C and 5 days at 25°C for bacterial and fungal isolates respectively. A clear zone of skim milk hydrolysis indicated protease producing organism. Colonies showing proteolytic activity were selected for protease enzyme production (Rehbar and Batool, 2017).

Identification of Proteolytic Bacteria Isolated from Soil

The selected potential strain was then identified by morphological and biochemical characteristics by using microbiology laboratory manual (Idu *et al*., 2019).

Production of Protease Enzyme by Submerged Fermentation

Protease production was carried out by inoculating protease producing isolate into a basal medium (NH_4Cl-0.5%, NaCl-0.5%, $CaCl_2$-0.2%, $MgCl_2.6H_2O$-0.2%, K_2HPO_4-0.4%, KH_2PO_4 0.3%) containing 0.7% peptone and 0.5 % as nitrogen and carbon source respectively. The mixture was adjusted to pH 7.5 and maintained at 37°C on a shaker at 250 rev/min for 96 hours. Samples were withdrawn and centrifuged every 12 hours and the supernatant was regarded as crude protease enzyme (Arunachalam and Saritha, 2009).

Determination of Protease Enzyme Activity

The activity of protease was assessed in triplicate by measuring the release of trichloroacetic-acid soluble peptides from 0.5% (w/v) casein in Tris-HCl (pH 9.0) at 60°C for 10 min. The 1mL reaction was terminated by adding 0.5mL of 10% trichloroacetic acid. It was left for 15 min and then centrifuged at 14000 g for 10 min. One unit of enzyme activity was defined as the amount of enzyme required to release 1 μg of tyrosine/min under standard conditions (Li *et al*., 2017).

Determination of Kinetic Parameters of Protease Enzyme

Factors affecting proteases activities such as pH, temperature and substrate concentration were determined.

RESULT

Table 1: Biochemical tests for the identification of the selected isolate

Test	Inference
Gram reaction	+
Shape	Rod
Catalase	+
H_2S	NA
Starch hydrolysis	+
Glucose	+
Mannitol salt agar	+
Citrate test	+
Urease test	+
Methyl Red	NA
Vogue Proska	+
Indole	–
Lactose	+
Slant	NA
Butt	NA
Isolate	*Bacillus licheniformis*

Keys: + = positive, - = negative, NA= Not applicable

Table 2: Proximate composition of pineapples and watermelon peels

Agro-wastes	**Proximate parameters**					
	Moisture content (%)	**Ash content (%)**	**Fat (%)**	**Fibre (%)**	**Protein (%)**	**Carbohydrates (%)**
Pineapple peels	11.62 ± 0.48^{b}	8.16 ± 0.25^{b}	2.06 ± 0.08^{a}	19.21 ± 1.39^{b}	7.42 ± 0.76^{a}	51.53 ± 2.73^{a}
Watermelon peels	6.11 ± 0.89^{a}	4.23 ± 0.23^{a}	2.14 ± 0.10^{a}	8.21 ± 0.95^{a}	10.25 ± 0.86^{b}	69.06 ± 2.45^{b}

Values are presented as mean**±**standard deviation of three replicates.
Values with different superscripts are significantly different at $p < 0.05$

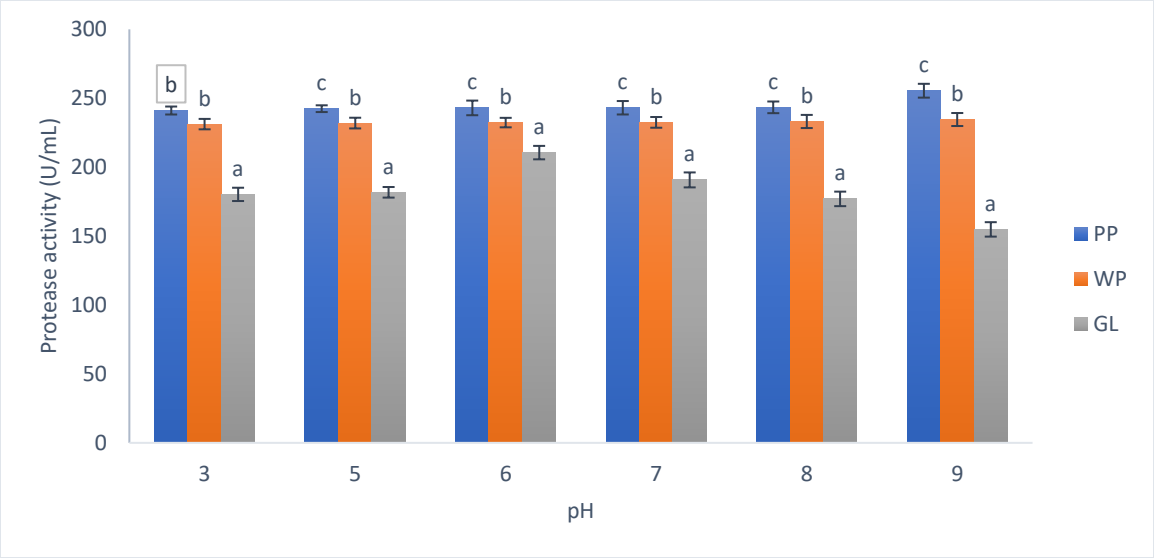

Figure 1: Effect of pH on the activities of proteases produced by *B. lichenformis* grown on pineapple and water melon peels as carbon sources

Bars presented mean ± standard deviation of three replicates. Bars with different alphabets at each pH are significantly different at $p < 0.05$.

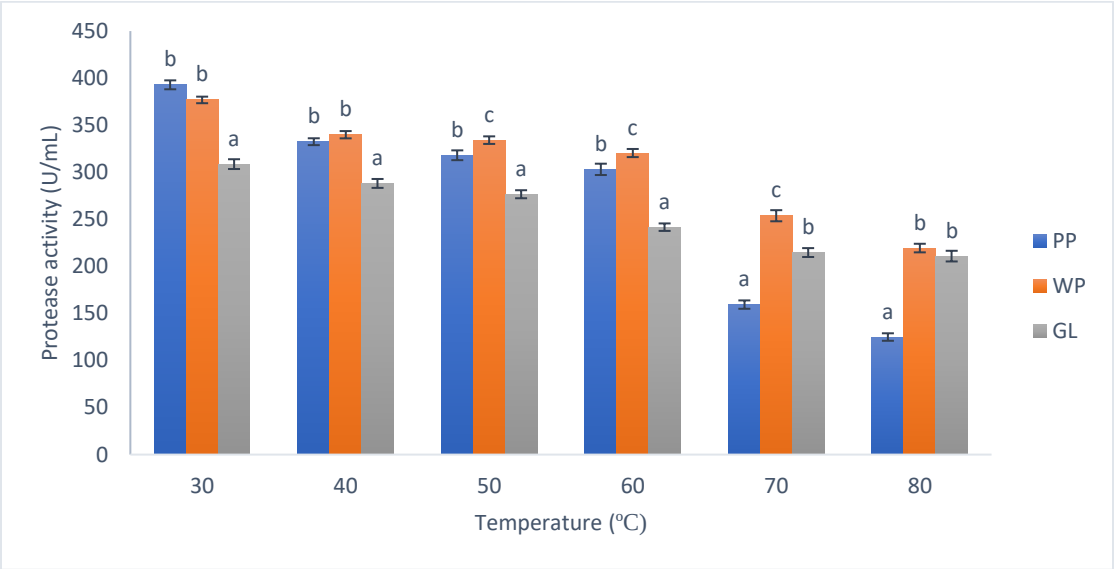

Figure 2: Thermal profile of proteases produced from *B. lichenformis* grown on pineapple and watermelon peels as carbon sources

Bars presented mean ± standard deviation of three replicates. Bars with different alphabets at each pH are significantly different at $p < 0.05$.

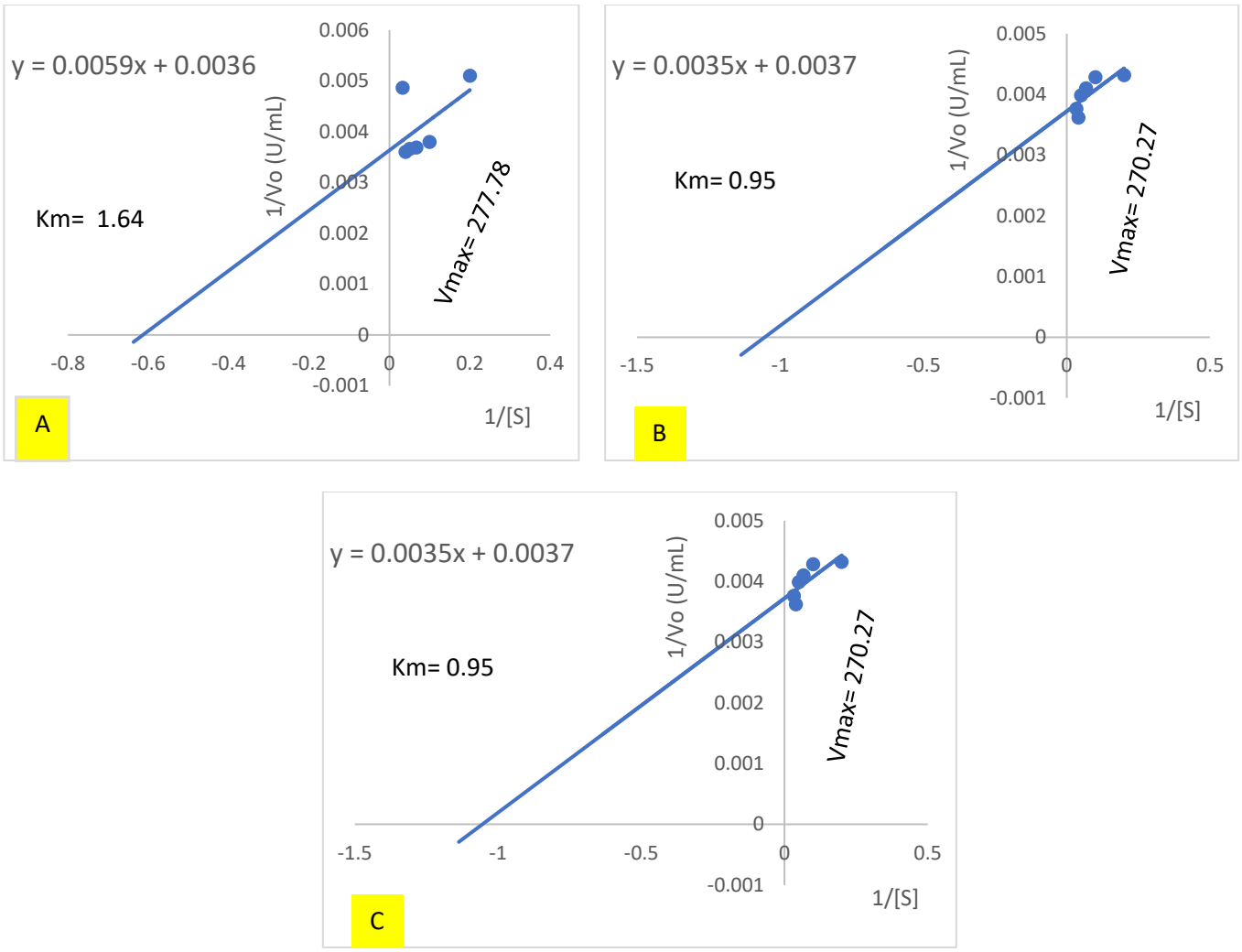

Figure 3: Double reciprocal plots of proteases produced from *B. lichenformis* grown on pineapple peel, watermelon peel and glucose, respectively.

A, B and C represent double reciprocal plots of proteases produced from *B. lichenformis* grown on pineapple peel, watermelon peel and glucose, respectively.

DISCUSSION

The isolate obtained from cow rumen ingesta was subjected to a series of biochemical identification tests which revealed the isolate to be *Bacillus lichenformis* (Table 2). The ability of the isolate to hydrolyze skimmed milk was as a result of its ability to produce protease enzyme. Also, the presence of such proteolytic isolate at the site may be due to the presence of protein-rich wastes usually disposed at the site (Hamid *et al*., 2019). Therefore, from this finding, it can be concluded that the domestic dumpsite harbours a number of proteolytic enzyme-rich bacteria which can be used in many industries.

A number of studies have reported agro-industrial wastes to support the growth of microorganisms by providing them with the nutrients required for their growth. These microorganisms are of industrial importance because they are used for the production of a

number of industrial products including enzymes (Sadh *et al*., 2018). Hence, the ability of the agro-industrial wastes to support the growth of the isolate implies that they were able to supply the bacterium with required nutrients for growth and thus able to support protease production. This invariably implies that the wastes can be used in place of glucose for protease production. In order for a protease to be used in numerous industrial processes, such enzyme must be active and stable under certain pH and temperature conditions. The same optimum pH value was observed for proteases produced by *B. lichenformis* grown on pineapple and watermelon peels (pH 9) (Figure 1) while that of glucose was observed at pH 6 (Figure 1). The difference in pH may be as a result of difference in the constituents of the carbon sources. The optimum temperature conditions of all the proteases produced were observed at 30 °C (Figure 1). Under optimum pH and temperature conditions, the activities of the proteases produced with agro-industrial wastes were significantly ($p>0.05$) higher than that of glucose. Since the agro-industrial wastes have been shown to support microorganisms' growth by providing them with required nutrients for growth (Sadh *et al*., 2018). Therefore, it is rational to infer that the higher protease production with agro-industrial wastes than glucose could be as a result of their nutritional constituents (Table 2).

Vmax measures the rate of enzyme-catalyzed reactions while the Km is defined as substrate concentration in which velocity is half Vmax. Thus, higher Km implies that the enzyme will have to act on higher amount of substrate to reach half Vmax, which in turn implies higher product formation (dos Santos Aguilar and Sato, 2018). The Vmax values of proteases produced with pineapple peels, watermelon peels and glucose were 277.78, 270.27 and 222.22 U/mL (figures A.B and C), respectively while their Km values in the same order were 1.64, 0.95 and 1.24 mg/mL (Figures A, B and C), respectively. The higher Km of proteases produced by *B. lichenformis* grown on mango peel implies that the pineapple peel supported production of a more active enzyme that can enhance product formation than the glucose and watermelon peel as carbon source, although, the affinity for the substrate is lower (Anpalagan *et al*., 2020). dos Santos Aguilar and Sato (2018) also reported Km value (1.60 mg/mL) for protease produced by *B. lichenformis* grown on agro-industrial wastes.

CONCLUSION

From the results obtained in this study, it may be concluded that both pineapple and watermelon peels are good carbon sources for *B. lichenformis* and can be used in place of glucose, although, pineapple peel is preferable. The presence of *B. lichenformis* reveals that domestic dumpsite could be an important source of proteolytic bacteria with numerous industrial applications.

Conflict of interest

The authors thereby declare no conflict of interest.

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Enzymatic Degradation of Human Hair by Purified Papain, Extracted from the Leaves of Matured Female Pawpaw (*Carica papaya*)

***Ejoba R. & Ayeni G.**

Department of Biochemistry, Faculty of Natural Sciences, Kogi State University Anyigba, Nigeria, PMB 1008

*Corresponding author email: gideon4.ayeni@gmail.com; Tel: +234-8067657044, +234-7068317885

ABSTRACT

The removal of human hair for social, religious and other cultural reasons dated back to the early civilization, have been revalidated with empirical approach. Depilation is simply the removal of human hair using depilants. Most of the depilants used are chemicals, knives which had adverse effects on the human skin such as rashes, eczema and burnt. Consequently, this leads to the search for enzymes that can perform human hair degradation by avoiding damage to human. The research aimed to find out the human hair degrading ability of the enzyme papain. Briefly, human hair digestion was done by dissolving 1.0 g of the hair in 100 mL of phosphate buffer at the pH 6.8, with the purified papain at single dose (1.5 mL). Observation and reading were taken at an interval of one hour at a varying concentration. Reading of the degraded hair was done with the aid of haemocytometer and microscope. The resultants solution, with a decreasing enzyme concentration, showed a degrading ability of papain on human hair. Degradation of human hair in this regard is directly proportional to the time taken and concentration of the enzyme, papain. This work gives an insight of the possibility of using the enzyme papain as depilant for human hair.

Keywords: Papain, Proteolytic enzyme, Depilant, *Carica papaya*.

INTRODUCTION

Human hair removal for social, cultural, religious have been in practice dated back to the ancient period. Different local (crude) method using various implements were employed in the time past for the removal of human hair (depilation). These include blade, knives, powder among others. These depilants have an adverse effect on human skin such as rashes burnt, eczema, pimples, etc (Natow, 1986; Yablonsky and Willians, 1986; Golberg 2003). Papain (EC 3.4.22.2) is a protein hydrolyzing enzyme that have been use in several biochemical research such as tendering meat, clarifying beer, removal of hair from hides, cleansing agents. It is a cysteine protease usually obtained from the latex of the papaya plant (Carica papaya) (Dordoni *et al.*, 2014). Papain have been used in several biochemical researches, with its routine applications such as in tendering meat, clarifying beer, cleansing agent, removal of hair from hides (Anilu, 2012). The enzyme, is abundant in the leaves and latex of matured female pawpaw (*C.*

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papaya). Papain cleave enzymatically to the disulphide bond of the keratin protein present in human hair, with concomitant liberation of the cells.

Toxicity associated with Papain such as curing allergies, degradation of adversity arising from its medicinal effects have been reported (Yamasaki *et al*, 1984; Shenenberger, 2002).

The aim of the research work is the enzymatic degradation of human hair by papain. The papain cleave the disulphide bonds present in the keratin, a protein present in human hair, leading to the liberation of the cells, which are now counted. Owing to the difficulty and time involved in depilation process using mechanical approach. However, evolution of biotechnological procedure are now in place by taking advantage of the enzymatic degradation action of purified papain for the removal of human hair (Shaving) (Damrongsakkul *et al*, 2007; Nienhaus *et al*, 1982).

METHODOLOGY

Sample Collection

Fresh pawpaw was obtained by plucking directly from pawpaw tree in the Anyigba, Dekina Local Government Area, Kogi State, Nigeria**.** Extraction papain proceeded with cutting mature of pawpaw by vertical incision. The sample was dried under early morning sun facilitating the flow of latex from the cut region of pawpaw. The latex was collected in a glass container. The latex was immediately used for the purification of papain in its native form following a reported procedure of Monti *et al*, (2000).

Purification and Digestion of Human Hair with Papain

Monti *et al*. (2000), procedures of papain purification and digestion was adopted. In this method, a purified papain solution was prepared by dissolving 0.05 g in 1.5 mL of distilled water and mixed with 0.5 g of sodium sulphite in 60 mL of water. The pH was adjusted to 6.8 using HCl and the prepared solution for 15 minutes. The insoluble material was removed by centrifuging at 3,000 rpm for 10 minutes. Human hair was added to 1.0 g of the hair to 30 mL of the prepared solution and allowed to stand in a warm water bath. The temperature was maintained at 40 ^{o}C for a proper digestion for 5 hours. This experiment was carried out at varying concentrations of the purified enzyme solution (1.5 mL, 3.0 mL, 4.5 mL, 6.0 mL and 7.5 mL). The number of human hair degraded was taken at zero time while other counting was taken at every one hour interval using the haemocytometer.

Cell Count of digested Protein

A clean covership was passed to the counting chamber of the Haemocytometer, until the Newton rings begins to appear. Micropipette was used to pick 0.02 mL of the digested human hair solution. This was poured in the gap between the counting areas. The solution was allowed to drop in the trough of the counting chamber and was left to settle down for 2 minutes. The counting chamber was placed under a high power microscope and the cells counted. The counting was done 16 squares. After each counting, the surface of the counter and the coverslip were washed thoroughly in ethanol and detergent solution, before using it again for the next counting.

RESULTS

Using the optimum pH 6.8, obtained for the enzymatic digestion, the results are as follows:

Table 1: Number of Cell Count on Substrate without Enzyme and Substrate at with Single Enzyme Dose (1.5 mL)

Time of digestion in hours	Number of cell count for substrate with no enzyme	Number of cell count for substrate with single enzyme dose (1.5mL)
0	16.12	17.10
1	18.50	64.21
2	22.80	68.54
3	19.00	73.90
4	21.11	86.70
5	23.56	90.65

Table II: Number of Cell Count on Substrate with Double Dose of Enzyme (x2) and Triple Doses of Enzyme (x3)

Time of digestion in hours	Number of cell count for substrate with double dose of enzyme (x2)	Number of cell count for substrate with triple dose of enzyme (x3)
0	15.50	14.29
1	67.38	70.54
2	75.90	79.09
3	81.32	84.25
4	88.07	91.02
5	95.16	98.04

Table III: Number of Cell Count on Substrate with Dose Level of x4 and x5 Lose of the Enzyme

Time of digestion in hours	Number of cell count at dose level (x4)	Number of cell count at dose level (x5)
0	15.32	14.05
1	70.06	73.23
2	78.43	79.40
3	84.31	88.72
4	95.17	97.21
5	106.89	109.78

DISCUSSION

The degradative action of papain are thought to be by the cleavage of the disulphide bond of protein present in the human hair, utimately leading to the liberation of cells (Monti *et al*., 2000). The number of cell count when there is no papain was low compared to when papain was added at a single dose (1.5 mL). This increment was a function of time. It was also observed that the increase is a concentration dependent, in relation to the quantities of papain used, a measure of enzymatic degradation of the human hair.

The data generated showed that papain obtained from fresh pawpaw has the capacity to remove human hair by enzymatic degradation through the removal of outside portion (cuticle) of the hair before the inner portion leading to cells liberation. Thus human hair degradation by papain outside required a specific pH (6.8) for a medium containing enzymatic action. Degrading activities of papain increases as its concentration on the substrate (human hair) increases as presented in the findings. Previous reports showed that papain was effective in degrading native collagen using structural analysis studies of digested collagen by infrared spectroscopy and chemical analysis of decayed dental tissue (Vatic *et al*, 2020). More so, papain have been shown to be stable in the presence of detergent (Tween-20 and Triton X-100) and significantly elicit biological activities at a low concentration than any other protease that were previously reportedly used for clearing wound debris, removing human hair and maintain the integrity of the intact cells (Srdan *et al*, 2021). In addition to the proteolytic activity of the study enzyme, Gomez *et al*, (2002), studies on pawpaw fruit showed that the amino acid sequences and sugar moieties under irradiation demonstrate high solubility coefficient and enhanced taste at the end of the irradiation process, an inherent feature that differentiate probably, papain, from other proteases.

CONCLUSION

Nonetheless, this work gives a pointer to the effectiveness of degrading ability of human hair by the enzyme papain and the possibility of commercial production of the enzyme, as depilant.

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Conflict of Interest

Authors declare that no conflict of interest in this study.

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Expand RSA Algorithm Cryptography Built on "Multiple Nth" Prime Figure

[1]Mohammed B. H., [1]Olumide O. & *[2]Abdulrahman A.

[1]Department of Computer Science, University of Abuja, Nigeria
[2]Department of Computer Science, Ibrahim Badamasi Babangida University Lapai, Niger State, Nigeria

*Corresponding author: abdulq2009@yahoo.com, 08056701631

ABSTRACT

Cryptography system are research that offers security services that help to protect information from unauthorized users. It upholds strong confidentiality and integrity of a stored data. Application of double key cryptosystem offers a vast change in area of cryptography. This application uses private and public keys cryptography. The public key converts the text to cypher text while private key which is the secret key is use to convert the text to a plain text respectively. This research proposed an Expand RSA (ERSA) Algorithm Key cryptosystem based on *n* discrete prime number system. The prime number upsurges difficulty in factoring randomly picked "*N*" prime, which rises the intricacy of the RSA algorithm used. In this method, four randomly selected variables are generated; each from large factor of "N". The information undergoes a cypher text-decryption process; this provide strong security. Research using random prime numbers proved that the key generation period and its complexity analysis of encryption and decryption show is stronger unlike the traditional RSA algorithm. This method is proficient, extremely and are laborious to decode.

Keywords: RSA cryptography, cypher text, Private Key, plain text, Cryptosystem, Public Key Security.

INTRODUCTION

To have an effective and low cost storage of data and files in the cloud as is getting popularity daily. Most private organizations and government looking at cloud services for their confidential information to be stored. Big companies like Microsoft, Amazon and others are good example of organization that provides service on the cloud. The problems of storing information on cloud is security and many cloud providers have given some method of protections to encrypt and decrypt information (AbdElminaam, 2018). Cloud computing are being apply both in public and private organizations to store massive quantity of data and information which are reachable from any part of the world. Application of cloud computing is majorly found in organization like private organizations, companies, military. Information stored on the cloud can be access through user authentication at various layer of security(Budiman & Rachmawati, 2018). The Cryptography system apply on various layers' security is reliant on the level of confidentiality required, though we cannot rely fully on these securities provided as hackers still access our information on cloud without our authority.

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The emergences of cloud computing system brought many benefits, likewise sum of weaknesses that come along with cloud computing. The major drawback is inadequate security provision to information in the cloud from unapproved entree. We have numerous security concerns in cloud computing atmosphere (Bhandari, Gupta, & Das, 2016). These bugs are challenges to cloud service providers and customers. These challenges come from network to data side. In order to secure the sensitive data from unlawful access, a suitable enhance encryption should be applied to cloud data storage. There are numerous security processes which have been futured for securing the cloud computing, some of these proposed security systems uses encryption procedure.

RSA algorithm is one of the security measure use by researcher to secure cloud, this has been working fin to protect unauthorized users. RSA algorithm has three main processes: Key generation, then encryption with the decryption system. The intention is to curtail the time complexity and space analysis throughout these progressions. More to this RSA algorithm, an expand manipulated encryption method has been introduced which is called Expand RSA (ERSA). This system of algorithm is to prevent attacks against timing, mathematical attacks with brute forces attack which are taking care with the help of using ERSA algorithm. In Brute Most of this attacker make attempts to presume on decipher the key by producing imaginable combinations. In traditional RSA, there is a high probability of conjecturing the combination until there exponent size scope are made complex and above 2048 bits, in the anticipated algorithm, the likelihood is reduced if the exponent scope size is 1024 bits or more. (Meneses et al., 2016), (Budiman & Rachmawati, 2018).

The goal line of our research is to utilize different cryptography ideas during correspondence alongside its application in distributed computing and to upgrade the security of cipher text or scrambled information in cloud. Following crafted by (Chourasia & Singh, 2016)) we proposed portrayal of E-RSA design key and encryption RSA that shields with additional effectiveness and trust in distributed computing framework.

Related Works

This segment contains the effort made by several researchers to improve cryptosystems especially in area of RSA algorithm. Our argument built on the existing algorithm which uses two prime numbers. We expand the numbers of prime number to NTH we call it Expand RSA (ERSA) cryptosystem and also modification of encryption keys before sending it to the end user.

Akash Kumar Mandal & Tiwari (2012) applied four primes numbers key generation procedure and these increasing periods needed to predict these primes number. While this method strengthens the security on cloud, the periods of creation of the proposed algorithm is longer than normal RSA cryptosystem and this researcher resisted it research to using prime numbers prom 3 to 20 for their work. The computation of encryption and decryption procedure are very compound and numerous issues are presented without visibly vindicated.

Dixit, Gupta, Trivedi, & Yadav (2018) inferred an algorithm as "dual RSA" using two approaches for RSA through shrinking the necessities for large capacity for keys. This utilization changed claims; the first is called daze signature while the second is called safety.

Chinnasamy & Deepalakshmi (2018) brought another strategy for recognizable proof of private key without utilizing the conventional manipulative style utilizing Pell's condition. Pell's RSA fortify the decryption key "d". This have not tackled the issue of programmers not accessing data as realizing that RSA was utilized for the encryption, they can even now anticipate conceivable prime number summoned the e and d. The decoding with the mark age which are upgraded by this strategy, alteration in the encryption and unscrambling that contains enchantment square shape and irregularity of encryption of text which is from plain content was proposed (Chaudhary *et al*., 2018). The primary shortcoming of this strategy is the need of extra period for building an enchantment square shape.

Our own enhancement for extend RSA which utilizes four prime numbers or more rather than two primes with the encryption controlled. The decoding succession is equivalent to unique RSA. Our examination certifications of cutting edge security by decreasing modulus and private example in particular exponentiation.

METHODOLOGY

An Expand RSA cryptosystem is prospect by means of “n” prime numbers. Four randomly selected numbers with and modular multiplicative reverse are applied to strengthen the security of normal RSA algorithm. The secret key which encrypt and also converting to plain text give Expand RSA (ERSA) strength to denial document to be hacked by unauthorized users. We analyze this research by utilizing JAVA programming language, as Expand RSA (ERSA) is executed utilizing JAVA, whole number library capacities for displaying and reproduction reason (HAMMAWA MB & UKWUOMA HENRY CHIMA, 2015). We can randomly introduce four prime numbers which might be enormous number to protected arbitrary capacity. Countless lengthy primes are applied by utilizing prime system creator which work in Java Bulky Whole number library to delivered secret key cipher text time. The Expand RSA (ERSA) classic does examine the key production coding and decoding period amid Conventional RSA and Extend RSA (ERSA) in light of these structured periods for unequivocal piece interval.

RSA (Rivest Shamir Adleman) Cryptosystem

RSA (Rivest Shamir Adleman) uses two prime numbers to generate it keys for public and private keys, these keys are used to manipulate text messages in a way that unauthorized users cannot understand the text messages. The e which is call encryption key is use to turn the message to a cipher text and the cipher text is been sent to the end receiver which he/she will convert it to plain text, that is readable format using d decryption. Below show the three steps in which keys are been generated and possible calculations on traditional RSA algorithm.

Generation of RSA Key

RSA_key_gen() Input:
Input prime A and B
*output the product of A*B=N*
Euler phi of N = (A-1)(B-1)
code key: {e, Decode Key: {d, N}

RSA ENCRYPTION RSA

In RSA algorithm text are been converted to meaningless text to unauthorized user by using e to encrypt our information before sending it to the end users. The following step are taken to covert plain text to cipher text:

1) Obtains the recipient public key (n, e)

2) Represents the plain text message as positive integer.

3) Compute the cipher text.

4) Sends the cipher text.

RSA DECRYPTION

In RSA decryption is done with the help of private key to get the plain text. The steps for decryption are given as:

1) Compute by using private key.

2) Extracts the plain text from integer representing.

Proposed Model

An Expand RSA algorithm is proposed to enhance traditional RSA algorithm as many attempt have act on the traditional RSA. People or unauthorized users has get to know two prime numbers are being used for the traditional RSA. Some times when sending the encryption keys hackers make attempt to break it to the encrypted text. We propose Nth prime numbers to strengthen and make it harder to predict any possible attack on this new modified RSA algorithm. Expanding the RSA algorithm help to make it difficult to hack in to the encrypted data which is to be sent to a receiver. The end user who have the decryption key can now decrypt it for better understanding of the text. With the help of this ERSA it will protect the data from hackers who are making attempt to crack the information which we are sending which is confidential.

ERSA KEY

This proposed algorithm has N^{th} prime numbers that are involve to strengthen the Expand RSA scheme. Our exploration ciphers and deciphers examine four randomly picked prime numbers, indiscriminately chose enormous. The prime numbers will randomly generate, in this case we generate four primes which the variables are "p", "q", "r", and "s". From here we get the product of these numbers as n. After that we calculate phi number, from that we get our e key. The keys advocate contains different parts (e, f, N) where "f" is made from encryption key "e". It gives greater unpredictability while the considering of "N". The "N" is covered up in to segment "g" and g are sent. Consequently, a trespasser with the realities of "g" got a bogus of "N" which is expected to calculated and get the estimation of four prime numbers and afterward "e". With security reason, the little length of each of the four picked prime is of a similar degree as if there should be an occurrence of customary RSA. Our new calculation is introduced underneath.

```
ERSA_ key_gen()
  */
  public void generatePublicPrivateKeys()
  {
  // N = p * q*r*s
  N = p.multiply( q ) ;

  // r = ( p – 1 ) * ( q – 1 ) * ( r – 1 ) * ( s – 1 )
  r = r.multiply( q.subtract
     (BigInteger.valueOf(1 ) ) ) ; //(p-1)(q-1)
```

ERSA ENCRYPTION WITH DECRYPTION

The cipher text is made with encryption and decryption keys are made with public and private key exponent. This “N” which is a product of randomize prime number sgiving it strong to factor and four arbitrary “e”, “f”, “d”, “g” are part of what built this encryption with problematic to hack the system. So, the procedurals in which we operate this algorithm goes:

* Generate Public and Private Keys.

```
// Choose E, coprime to and less than r
do
{
E = new BigInteger( 2 * primeSize, new Random() ) ;
}
While ( ( E.compareTo( r ) != -1 ) || ( E.gcd( r ).compareTo( BigInteger.valueOf( 1 ) ) != 0 ) ) ;
```

Figure 1 show the data flow diagram that fully explaining the interconnectivities of Expand RSA (ERSA) system. Four randomly distinctive prime integer is chaotically chosen as an input to process, the product is N while the Φ(N). The (e, f) is thoroughly looked over the unveil 1 < e < Φ(N). The (d, g) is determined to apply the secret key one cipher text and plain text are applied smearing secret and open key types.

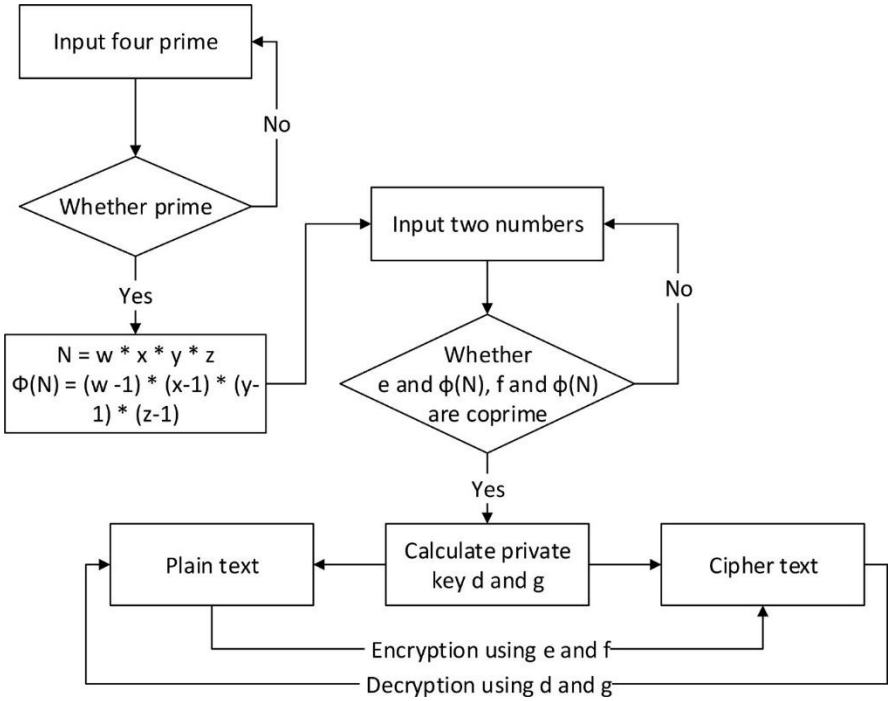

Figure 1. Flow chart of Expand RSA (ERSA) Algorithm.

To discuss our proposed, Expand RSA (ERSA) algorithm.

ERSA example

- **Key Generation**

w = 199, *x* = 211, *y* = 223, *z* = 227
N = *w* * *x* * *y* * *z*
= 199 * 211 * 223 * 227
= 2, 125, 525, 169
Ø (N) = (*p* – 1) (*q* – 1) (*r* – 1) (*t* – 1)
= 198 * 210 * 222 * 226
= 2, 086, 151, 760

Public Key

e = 563
f = (563 * 2) + 1
f = 1127

Private Key

563d (mod 2, 086, 151, 760) = 1
3, 705, 420.533
0.533 ≈ 1
d = 1,111, 918, 888
g = *d* – 1
g = 1,111,918,887

Encryption

$C = M^{(f-1)/2} Mod (g+1)$
$C = 1234^{((1127-1)/2)} Mod (1111918887 + 1)$
$C = 1406540448$

Decryption

$M = C^{d} Mod (g+1)$
$C = 1406540448^{1111918887} Mod (1111918887 + 1)$
$C = 1234$

RESULTS AND SIMULATION

The proposed Expand RSA (ERSA) algorithm is actualized utilizing JAVA running on an Intel Center (TM) i3-3540M CPU @ 4.60 GHz and 6.00 GB Slam. The algorithm (Customary RSA and proposed Expand RSA (ERSA)) have assorted noteworthy limitation influencing its degree of security quality and speed. Expanding the modulus length raise multifaceted nature of factorizing it. Thusly, additionally increment the length of the mystery key and this bode well the key. The conventional RSA and proposed Expand RSA (ERSA) boundary changes with time and others near noticeable quality.

Analysis on RSA performance

Expand RSA (ERSA) algorithm was research which factor bit sizes of information. The display of conventional RSA Algorithm [2] is trendy on Table 1. Likewise, the proposed Expand RSA (ERSA) framework of encryption period and decoding are show on Table 2.

The proposed Expand RSA (ERSA) is more mind boggling compare to traditional RSA. More drawn out secret keys period of proposed Expand RSA (ERSA) can be viewed as merit on the ERSA by way that the stretch to hack the cipher text is harder as a result of the unnecessary intricacy included.

Figure 2 portrays the encryption stretch assessment among RSA and proposed Extended RSA (ERSA) structure. It shows that, for the subordinate piece length of prime numbers, two algorithms take nearly a similar total of time. In any case, with the expansion of bit length, the hole between bends rises quickly.

Table 1. Performance of RSA.

Length of w, x	Time analysis for RSA algorithm		
(in bits)	Key generation (in ms)	Encryption (in ms)	Decryption time (in ms)
64	67.53	0.15	0.23
128	86.45	0.18	0.26
256	88.86	0.29	0.87
512	168.57	0.48	4.3
1024	530.80	1.56	23.16
2048	4189.37	3.29	126.72
4096	53,456	10.15	1100.20

Table 2. Performance of Expand RSA (ERSA).

Length of w, x, y and z (in bits)	Analyzing time for Expand RSA (ERSA) algorithm		
	Key generation time (in ms)	Encryption time (in ms)	Decryption time (in ms)
100	236	0.19	1.60
128	249.29	0.58	2.78
256	246.9	1.40	13.30
512	234.6	3.88	85.87
1024	1249.4	6.76	439.38
2048	7067.9	20.77	2362.77
4096	1241,900	55.79	18,999.46

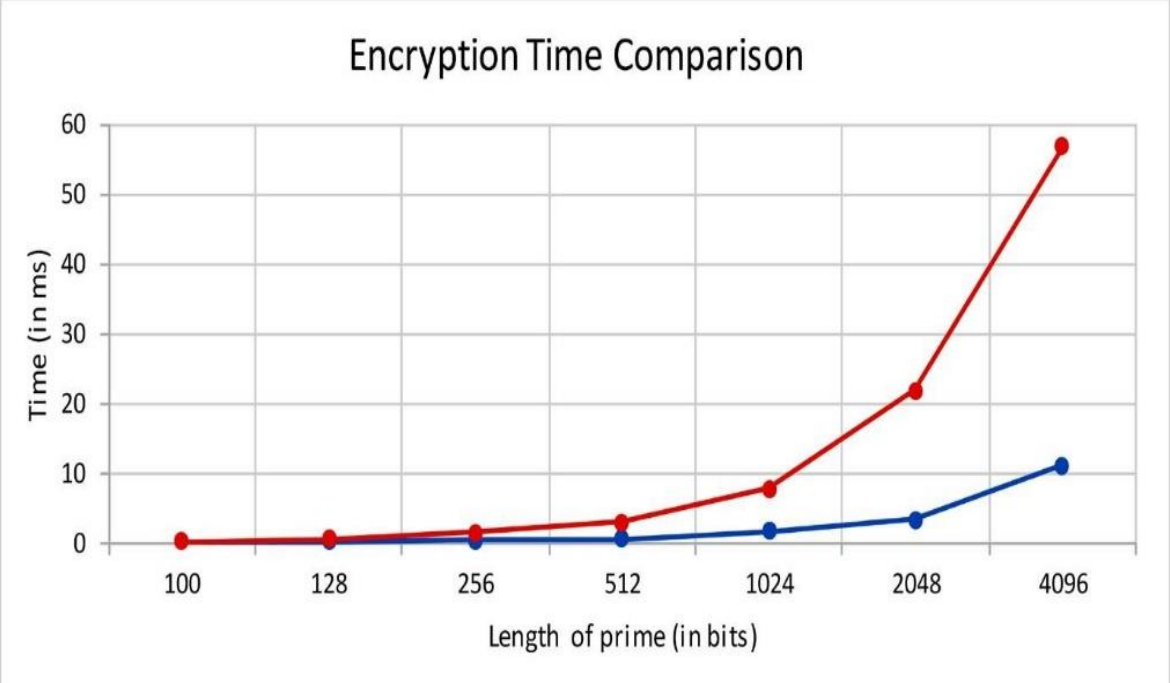

Figure 2. Encryption time comparison

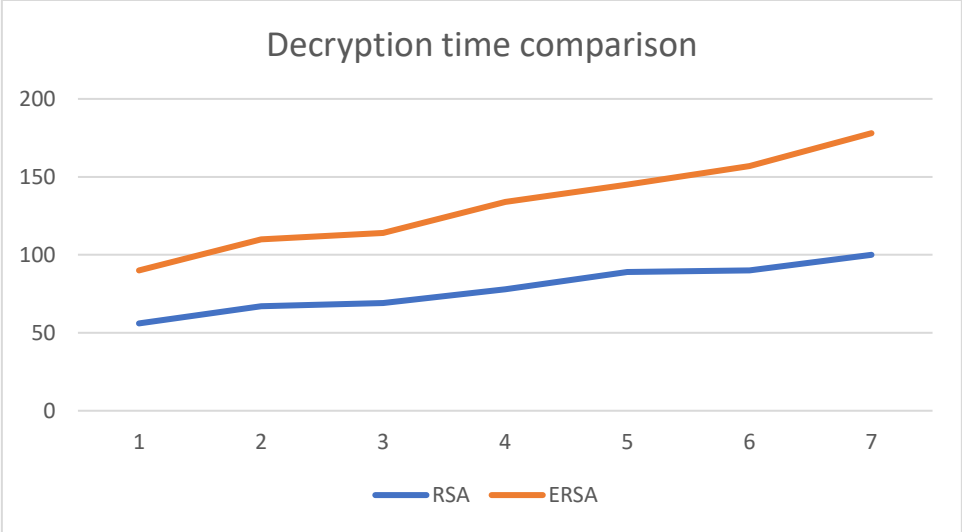

Figure 3. Decryption time comparison.

Looking at above sample, inputting primes numbers with bit length 2048, cipher period of Expand RSA (ERSA) is 18.77 ms while RSA is 4.26 ms.

Figure 3 demonstrations show decryption period comparison between traditional RSA and proposed ERSA system. It proves looks almost the same amount of time expended by traditional RSA and ERSA for the minor bit length of primes. With the rise of bit length, the variance between curves raises rapidly. For instance, input primes of bit length 4096, decryption period in ERSA is 18,999.46 ms and traditional RSA takes 1100.20 ms

Without difficulty the above graphs show that encryption and decryption period are of the high side than traditional RSA. The increase in stretch is flexible because it strengthens the security to a great degree in the proposed ERSA technique.

Complex Analysis

Analyzing the complexity of Traditional RSA algorithm with ERSA algorithm is analyze beneath.

Complexity of RSA Algorithm

In Arbitrary Complexity selected two prime figures.

- The verdict of a prime number above is $O\ s(*(\log 2p)2 *\ln p)$ [11].
- Likewise, the next number complexity stands as $O\ s(*(\log 2q)2 *\ln q)$.
  Complexity computation N:
- Computational of N stands as $O(\log 2p*\log 2q)$.

Using Euler phi of N

(N −1). The big of O calculating phi of N [10]: $O((\log 2(p-1)(q-1)^2((p-1)(q-1)1))$.

Complexity for arbitrary selection variables e which is the encryption key:

- The complexity of finding the random variable e the encryption key is:

$O(\log 2(p-1)*(q-+1)\ \gcd(e\ p,(-1)*(q-1)))$, as it is known that e and (N) are coprime to one another so $\gcd(e,(p-1)*(q-=1))$ 1, while $^{O}((\log 2\ \log 2(p-1)*(\log 2q-1)^2+1)$.

Complexity of Expand RSA (ERSA) **Algorithm**

The complexity will rise based on the number of the prime accepted for the proposed algorithm.

Complexity for Randomly picked prime numbers:

- (1^{st}) prime number is $O\ s(*(\log 2w)4 *\ln w)$.
- 2^{nd} prime number is $O\ s(*(\log 2x)4 *\ln x)$.
- 3^{rd} prime number is $O\ s(*(\log 2y)4 *\ln y)$.
- 4^{th} prime number is $O\ s(*(\log 2z)4 *\ln z)$.
  Big of O N:
- The calculation using complexity of N $O(\log 2w*\log 2x*\log 2y*\log 2z)$.
  Next is calculating of complexity using Euler phi value of N
- Using Euler phi value of N remains:

  $O((\log 2(w-1) * - * - * (x\ 1)\ (y\ 1)\ (z-1)_)^4$
  $*((w-1) * - * - * (x\ 1)\ (y\ 1)\ (z- -1)\ 1^)$.
  Complexity for random variables e and f:
- The random variable e remains

  $O(\log 2(w-1) * - * - * (x\ 1)\ (y\ 1)\ (z- +1)$

  therefore gcd $(e\ w\ (\ -1)* - * - *(x\ 1)\ (y\ 1)\ (z-1)))$, as we acknowledged that e and (N) are coprime to one another so gcd$(e\ w,(\ -1)* - * - *(x\ 1)\ (y\ 1)\ (z - =1))\ 1$,

  while the complexity is $O((\log 2\ \log 2(\ w-1)*(\ \log 2x-1)*(\log 2y-1)*(\log 2z-1)_)^4+1)$.
- Equally, Complexity of computing the random value f is $O((\log 2\ \log 2(\ w-1)*(\log 2x-1)*(\log 2y-1)*(\log 2z-1)_)^4+1)$.

  Linking the above complexity, it illustrates that Expand RSA (ERSA) is more complex than traditional RSA algorithm. The complexity will high liable to the number of primes picked for the algorithm.

CONCLUSION

Looking at large prime on ERSA keys, it shows the security is stronger than that of two primes. This also shows that the time taken to predicts the 5 prime take longer time. The encryption key relies on ERSA (ERSA) large factor appreciation "N" hence it needs long period of time. The larger the primes key, this also expands the period needs to hack the framework which brands the framework. The cipher text and plain text steps ERSA is straightforward weighed with the traditional RSA, consequently it isn't overhead on the framework. Encryption and extrication likewise take additional time than RSA algorithm. The achievement of this design is estimated with period of time taken for brute force attack. Restriction of this proposed pattern is it won't work appropriately except if "n" particular prime numbers are thought of. To improve the security of RSA calculation by including some additional components in encryption and decoding cycle can be a decent future work.

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Effects of Graded Levels of *Craseonyeteric thonglongyai* Dung on the Concentrations of Selected Anti Oxidant in the Leaf of *Cnidoscolus aconitifolius* (Euphorbiaceae)

*Lawal B. A., Amanabo M., Abu M. L. & Uthman A.

Department of Biochemistry, Ibrahim Badamasi Babangida University, Lapai, Niger State

*Corresponding author's contact: dimejide@gmail.com; Tel: +2348066777723

ABSTRACT

Antioxidants prevent oxidation caused by free radicals and sufficient intake of antioxidants protect against diseases. The nutritional and antioxidant contents of vegetables are affected by soil nutrient compositions. A pot experiment was carried out to evaluate the influence of *Craseonycteris thonglongyai* on the antioxidant constituents in the leaves of *Cnidoscolus aconitifolius*. The antioxidants analyzed include lycopene, β -carotene, tocopherol, carotenoid, ascorbic acid and chlorophyll. The leaves of *C. aconitifolius* were harvested at vegetative phase and the concentrations of lycopene, chlorophyll, β-carotene and total carotenoid were determined by spectrophotometric method while ascorbic acid content was analysed by titrimetric method. Results showed that the concentrations of chlorophyll and β-carotene in *C. aconitifolius* increased significantly ($p < 0.05$) in a dose dependent manner with *C. thonglongyai* dungs concentration. While tocopherol concentrations were not detected there was an increased the carotenoid and lycopene contents up to 50 g compost concentration. Treatment with graded level of *C. thonglongyai* dung significantly ($p > 0.05$) decrease the concentration of ascorbic acid in the vegetable with increase in the quantity of the dung applied. The study concludes that *C. thonglongyai* compost enhance the bioaccumulation of selected antioxidants in the leaves of *C. aconitifolius*

Keyword: Antioxidant, *Craseonycteris thonglongyai* dung, *Cnidoscolus aconitifolius*

INTRODUCTION

Cnidoscolus aconitifolius is a perennial shrub belonging to the family Euphorbiaceae. The plant is commonly grown in the tropic and sub-tropical region. People around the world, eat it as vegetable in soup (Ganiyu, 2005). It has a succulent stem with milky sap when cut. It is usually pruned to about 2meter for easier leaf harvest but usually it can grow to 6 meters. It is called '*Iyana Ipaja'* in the Southwestern Nigeria. The raw leaves contain high contents of toxic hydrocyanic acid, so it should be cooked before eating (Mordi and akanji, 2012). It has tolerant for drought and, also for heavy rainfall which makes *C. aconitifolius* adaptable for both dry and raining season. Leafy part of the plant is poisonous when ingested and causes irritation or allergic reaction from handling of the plant without protection such as glove (Mordi and akanji, 2012).

The plant leaves has many medicinal values and it is used in the treatment of various illness,

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alcoholism, diabetics, insomnia, gout, scorpion poison, skin disorders and venereal diseases and for its ability to strengthen nails, darken grey hairs and improving brain functions and memory (Sarmiento-franco *et al.,* 2002). The plant is also used as laxatives, diuretic and circulatory stimulant to improve digestion, stimulate lactation, and harden the fingernails when the shoot and leave are applied (Rowe, 1994).

The edible part of the *C. aconitifolius,* serves as important nutritional source of fibre, protein, vitamins (A and C), minerals (calcium, iron and phosphorous) Niacin, Riboflavin, and Thiamine for populations that cannot afford expensive foods rich in these nutrients (Yang, 1979). Nutritional evaluation of the leaf of *Cnidoscolus aconitifolius* analyses reveal high content of protein, fibre and low carbohydrate content (Jiménez-Aguilar *et al.,* 2017). The vegetable also contain the following nutrients; calcium, potassium, iron, ascorbic acid, B-carotene (Proviatmin A), B_6 (pyridoxine), B_9 (Folic acid), B_{12} (cyanocobalamin) (Johnston *et al.,* 2016; Awoyinka *et al*., 2006). High content of vitamin C is an indication that *Cnidoscolus aconitifolius* can serve as antioxidant.

Hence the study was designed to evaluate the influence of graded levels of *C. thonglongyai* dung on the concentrations of some antioxidant (β-carotene, carotenoid, lycopene, ascorbic acid and chlorophyll) in the leaves of *C. aconitifolius*.

Materials and Methods

Pot experiment was carried out in the Department of Biochemistry, Ibrahim Badamasi Babangida University Lapai, Niger State, Nigeria.

Soil sampling and analysis

The superficial (0 – 20 cm depth) soil sample was obtained from 3 different locations at the Main Campus of Ibrahim Badamasi Babangida University, Lapai, Nigeria. The soil was mixed and sieved to remove any impurity. The physical and chemical properties of the soil and C. thonglongyai dung were analyzed according to the method of Juo, (1979). The particle sizes were analyzed using hydrometer method; pH was determined potentiometrically in the water and 0.01M $CaCl_2$ solution in a 1:2 soil/ liquid using a glass electrode pH meter and organic carbon by Walkey-Black method (Juo, 1979). Exchange acidity (E.A H^+ and $Al3^+$) was evaluated by titration method. The exchangeable Ca, Mg, K and Na were leached from the soil sample with neutral 1N NH_4OAc solution before they were analysed. Sodium and potassium were determined by flame emission spectrophotometry while Mg and Ca were determined by EDTA versenate titration method (Juo, 1979). Total nitrogen was estimated by Macrokjedal procedure and available phosphorus by Bray No 1 method (Juo, 1979).

Source of *Cnidoscolus aconitifolius* cuttings and *Craseonycteris thonglongyai* dung

The Dung of *C. thonglongyai* was collected from a cave in Faso village of Edati local government area of Niger State. The cuttings of *C. aconitifolius* were acquired from Teaching and Research Farm of Faculty of Agriculture, Ibrahim Badamasi Babangida University Lapai, Niger State

Manure treatment and application

Dried dung of *Craseonycteris thonglongyai* were pulverized into powder and applied to cultivate *C. aconitifolius* in pot experiment at six different

levels, which were control (No application), 25, 50, 75, 100 and 125 g per 20 kg soil.

Planting, experimental design and nursery management

Two stem cuttings of C. aconitifolius were planted in 20 kg bag of soil containing different levels of *C. thonglongyai* dung and thinned to one plant per pot after sprouting. Completely Randomized Design (CRD) was used for the six treatments. Each treatment has ten (Ezeonu *et al.,* 2002) pots replicated 3 times making a total of 180 pots for the experiment. The plants were watered twice daily (morning and evening) using watering can except on rainy days in which the pots were not irrigated. The surrounding was kept clean regularly to avoid pest and the pots were lifted from time to time to avoid the roots of the plants from growing out of the pot.

Analytical Procedure

Carotenoid and lycopene contents in the samples were estimated by the method described by Zakaria *et al*. (1979). The concentration of chlorophyll was evaluated by the method of Whitney *et al.* (1990). The ascorbic acid content in the samples was determined by 2, 6-dichlorophenol method of Jones and Hughes (1983). The concentration of βcarotene in the leaves of the vegetable was determined by ethanol and petroleum ether extraction method as described Musa *et al*. (2010). The concentration of tocopherol in the leaves of the vegetable was estimated by the Emmerie-Engel reaction as reported by Rosenberg (1992)

Statistical analysis

Analysis of variance (ANOVA) was done using SPSS statistical package to determine the effect of graded levels of *C. thonglongyai* dung on the concentrations of the antioxidant in the leaves of *C. aconitifolius*. Duncan's Multiple Range Test (DMRT) was used for comparison of the means at $p < 0.05$

RESULTS AND DISCUSSION

Table 1: Physical and chemical properties of the soil (0 – 20 cm depth) used for pot experiment

Parameters	**Values**
Sand (%)	845
Silt (%)	69
Clay (%)	85
Textural class	Loamy Sand
pH (H_2O)	5.7
Organic Carbon (g kg^{-1})	4.57
Total Nitrogen (g kg^{-1})	1.82
Available Phosphorus (mg kg^{1})	16.30
Na^+ (cmol kg^{-1})	3.70
K^+ (cmol kg^{-1})	0.01
Ca^{2+}	3.68
Mg^{2+}	1.28
Exch. Acid	0.60
EC	87

EC = Exchangeable cations.
Values represent mean values of triplicate determinations.

Physical and chemical properties of soil.

The analysis of the soil used in the pot experiment is presented in table 1. The pH of the soil is 5.7 which shows that it is strongly acidic. The organic carbon, available phosphorous, exchangeable carbon are high while the exchangeable potassium is very low. However,

both total nitrogen and exchangeable magnesium is low in the analyzed soil.

The soil textural class is loamy sand. Silt and clay are found to be low while the sand is high in the experimental pot soil. The Exchangeable capacity (EC) is high with 87 cmol/kg. The low concentration of nitrogen, potassium, magnesium the analysed soil could be as a result of less organic matter. According to Riezebos and loerts, (1998); Jaiyeoba, (2003) report that organic matter content decrease with land open for agriculture. According to Brook, (1983) the most favourable pH for availability of most nutrients correspond roughly with the optimum range of 6-7 for most of the crop plants. Moreover, low pH or soil acidity converts some available soil nutrients in to unavailable form and also acidic soils are poor in their basic cations such as Ca, K, Mg and some micronutrients (e.g. Mo) which are as essential to crop growth and development (Wang *et al.,* 2006).

Clay and silt content of the soil was determined to be lower. This was related to the properties of the main material and it depended on the level of decomposition while the soil had a higher sand. The soil had a high pore volume due to the activities of soil insects and other organisms. A high pore volume caused the bulk density to decrease.

The CEC of a soil represents its ability to store and release cations such as NH4+ into the soil solution for plant uptake. This significantly reduces the potential leaching losses of plant-available N, thus enhancing the N supply capability of the soil. High crop yield will not be achieving in an infertile soil if organic manure are not use in increase the nutrient required by plant which could result to less quality farm produced. All that is required is to sustain the fertility of the soils and modify or improve on them (Agboola and Unamma, 1991). Kapkiyai *et al.,* 1998 indicated that soil organic matter (OM) content is a critical component of soil productivity and its maintenance is a sound approach to maintaining productivity of continuously cropped soils.

Table 2: Chemical properties of the *Craseonycteris thonglongyai* dung

Parameters	Values
pH (H_2O)	6.1
Total Nitrogen (g kg^{-1})	34.44
Available Phosphorus (mg kg^{-1})	93.00
K (cmol kg^{-1})	1.67

Chemical properties of *C. thonglongyai* dung

The chemical properties of *C. thonglongyai* dung is shown in Table 2. The total nitrogen, phosphorus contents of the dung are very high. The pH of the dung is slightly basic.

According to Melo *et al.,* (2008), the use of manure as organic fertilizer, depending on the application rates and frequency, may contribute to soil salinization, due to the electrical conductivity of these materials.

Dahm *et al.,* 2010; Nannipieri *et al.,* 2000 report on a high metabolic activity and a great importance of microorganisms foremost processes occurring in the soil environment, with the decomposition and mineralization of organic matter (Manure and other natural composts) being most important.

This meant that microbes are abundant in the soil of organic plots. This was also confirmed by

the activity of dehydrogenase in the soil, which was significantly higher in organic fertilized soil than in chemical fertilized soil. The activities of soil microbes can make the metabolism of soil-plant nutrition smooth and help soil release various nutrients (Burns, 1986; Dick, 1992). Reports have shown that soil microbes can also produce some kinds of plant growth regulators that are good for plant growth and the related physiological activities (Arshad and Frankenberger, 1992).

The benefit of the plants from organic fertilization may be attributed to an integration of improvements of biological, physical and chemical properties, improved and sustainable nutrition including micro-nutrients, and the consequent improvement in the plant physiological activities.

Effect of graded levels of *C. thonglongyai* dung on the antioxidant contents

Table 3: Effect of graded levels of *Craseonycteris thonglongyai* dung on the concentrations of some antioxidant contents in *Cnidoscolus aconitifolius*

	Graded levels of *Craseonycteris thonglongyai* dung					
Antioxidants	**0 g (Control)**	**25g**	**50g**	**75g**	**100g**	**125g**
Chlorophyll (mg/g)	0.17 ± 0.02^{a}	0.18 ± 0.04^{a}	0.31±0.01^{b}	0.30 ± 0.04^{b}	0.30 ± 0.01^{b}	0.34 ± 0.02^{b}
β-Carotene (µg/100g)	25602.92±21.49^{b}	30331.39 ±40.86^{b}	60045.22±59.04^{c}	17627.78±10.86^{a}	17004.90 ± 20.67^{a}	16109.90±60.67^{a}
Tocopherol (µg/100 g)	ND	ND	ND	ND	ND	ND
Ascorbic acid (mg/100g)	13.05 ± 0.97^{b}	12.00 ± 1.48^{b}	9.83±01.39^{a}	8.95 ± 0.98^{a}	7.53 ± 0.21^{a}	7.15 ± 0.27^{a}
arotenoid (mg/g)	5413.52±56.34^{b}	5336.32±16.46^{b}	8492.59±25.13^{c}	3477.28 ± 69.95^{a}	3247.95 ± 48.85^{a}	2897.87 ± 47.01^{a}
Lycopene (mg/g)	275.04 ± 59.73^{a}	405.20±79.13^{b}	1072.13±64.82^{c}	854.75 ± 40.63^{c}	393.95 ± 12.30^{b}	348.40 ± 12.30^{ab}

ND = not detected

Row mean values carrying the same superscript are not significantly different for each other (p>0.05)

The determination of effect of graded level of *C. thonglongyai* dung on Antioxidant concentration in *C. aconitifolius* leaves showed that the Chlorophyll content increased significantly (p<0.05) with application of the dung. However, the concentration of chlorophyll in the vegetable treated with 50, 75 and 125 g were significantly (p<0.05) higher when compared with vegetable treated with 25 g. The mean value of Chlorophyll in control, 25, 50, 75, 100 and 125 g were 0.17 ± 0.02, 0.18 ± 0.04, 0.31 ± 0.01, 0.30 ± 0.04, 0.30 ± 0.01, 0.34 ± 0.02 mg/g respectively (Table 3).

Moreover, treatment with 25 and 50 g had significantly (p<0.05) increase on the concentration of β-Carotene in the leaf of *C.* aconitifolius. Application with 75, 100 and 125 g

had no effect on the concentration of β-Carotene in the vegetable. The mean concentrations of 25602.92 ± 21.49, 30331.39 ±40.86, 60045.22 ± 59.04, 17627.78 ± 10 .86, 17004.90 ± 20.67, 16109.90 ± 60.67 µg/100 g were recorded for control, 25, 50, 75 g, 100 and 125g respectively (Table 3).

Table 3 indicated that tocopherol concentrations in the leaves of *C. aconitifolius* were not detected in the different graded levels of the dung application and control. Treatment with graded level of *C. thonglongyai* dung significantly ($p<0.05$) decrease the concentration of Ascorbic acid in the vegetable with increase in the quantity of the dung applied. Although, vegetable treated with 50 , 75 and 100 g were not significantly different from the plant treated with 125 g. Moreover, the control and 25 g application do not differ significantly, from each other.

The results obtained from the effect of *C. thonglongyai* dung on anti-oxidant content in *C. aconitifolios* showed that the dungs significantly ($p<0.05$) elevated the carotenoid content in the leaves of the vegetable. The concentration of carotenoid in the vegetable treated with 50 g of the dung did not significantly differ from the vegetable without dung (control), the vegetable treated with 50 g was significantly higher in carotenoid contents when compare to vegetable treated with 75, 100 and 125 g. The mean values of control, 25, 50, 75, 100 and 125 g were 5413.52±56.34, 5336.32±16.46, 8492.59±25.13, 3477.28±69.95, 3247.95±48.85 and 2897.87±47.01 mg/g respectively (Table 3).

Lycopene concentration in the different levels of application of *C. thonglogyai* in the leave of *C. aconitifolius* revealed that the concentration increase significantly ($p<0.05$) from the control to 50g treatment. The concentration of lycopene in the treated vegetable with 50g of dung was significantly ($p<0.05$) higher when compare to the concentration (393.95±12.30 and 348.40±12.30 mg/g) obtained at 100 and 125g treated vegetable respectively.

Ascorbic Acid (Vitamin C) supports synthesis of hormone, iron absorption and collagen, which is extensively used in cosmetics and pharmaceuticals. It acts as water soluble antioxidant, which plays a major role in free radical scavenging activity and activates immune system (Locato *et al.,* 2013).

There was significant difference ($P<0.05$) in the result obtained shows that the content of Ascorbic Acid in the leaves of *C. aconitifolius*. This significant lowering of Ascorbic Acid in this study was similar to that of Musa *et al.*, (2010) who reported similar result by applying Nitrogen fertilizer to *Corchorus olitorius.* Since Ascorbic acid production is connected to protein production and carbohydrate formation, when dung is applies resulted in the decrease in Ascorbic Acid level in the leave of *C. aconitifolius*. Virginia, 2001 also reported decrease in Ascorbic acid concentration in the leaves of Tree Spinach which could be as a result of high concentration of magnesium and potassium. Augustin, 1975; Seung *et al.,* 2000 confirmed that higher doses of nitrogen reduce levels of vitamin C in corn, as was previously stated by Ševlíková, (1976), Netherlands, (1996), Lisiewska and Kmiecik, (1996). Toor *et al*., (2006), and Premuzic *et al*., (2004) showed an increase in vita. Convincingly, continuous application in level of *C. thonglongyai* dung decreased the concentration of the Ascorbic acid in the leaves.

The highest concentration of β-carotene obtained at 50g of the application was

significantly different from the other leaves of application. This implies that 50g is the best level of application of *C. thonglongyai* dung for β-carotene. The increase in β-carotene content in the vegetable treated with *C. thonglongyai* dung could be as a result of elevation in the content and activity of chlorophyll and associated light absorbing pigments (including caroteniods) (Taiz and Zeiger, 2002; Havling *et al.*, 2006). Other study revealed that increase in potassium levels enhance concentration of β-carotene in vegetable leaves since the macro-nutrient speeds up the rate of acetic thiokinase activity for the catalysis of condensation of acetyl CoA units which form the initial steps in the biosynthesis of carotenoids (Fanasca *et al.*, 2006). While some studies have suggested that organic production increases plant antioxidant activity (Janzantti *et al.*, 2012; Vinha *et al.,* 2014).

The observed higher concentration of carotenoid and lycopene at 50 and 75g of *C. thonglongyai* dung for the treated leaves of *C. aconitifolius* justifies that moderate application of the dung is required. However, the increase in carotenoid could be attributed to the action of phosphorus on enzymes like phosphofructokinase, pyruvate kinase and precursors of pyruvate have been linked with the biosynthesis of carotenoid (Bramely, 2002; Black *et al.,* 2008). This study is in line with the report of Meysam *et al.,* (2017) who also reported that increase in level of carotenoid production may be as a result of increase in Phosphorous.

Lycopene is an excellent antioxidant but do not have pro-vitamin A activity. Decrease in the incidence of prostate cancer have been associated with high content of lycopene (Gann *et al.,* 1999)

Result of this study indicates that continuous increase in the quantity of the *C. thonglongyai* dung leads to an increase the concentration of chlorophyll content in *C. aconitifolius* leaves. The quantification of chlorophyll provides important information about the effects of various conditions and treatments on plant growth (Schlemmer *et al.,* 2005). Physiologically, there is significant change in the colours of *C. aconitifolius* leaves, the colour of the treated leaves become more greenish as a result of increased in the content of the chlorophyll. This was similar to report by El-Arwy *et al.,* (2016) who reported higher chlorophyll-A concentrations, that application of fertilizer enhanced the formation of the pigments and caused an increase in their concentrations either in salt stressed or unstressed plants.

El-Arwy *et al.,* (2016) similarly reported that organic fertilizer concentration also increased the pigment colour. Chlorophyll content is an indicator for crop growth and development, therefore accurately determining and assessing of chlorophyll concentration is essential (Bannari *et al.,* 2007).

CONCLUSION

This study concludes that the application of *C. thonglongyai* dung improve the bioaccumulation of antioxidants contents in the leaf of *C. aconitifolius* due to the increase in concentration of the investigated antioxidant in the vegetable. Therefore, it is suggested that growing of *C. aconitifolius* dung will help increase the antioxidants content in *C. aconitifolius* hence complement the defence mechanism of the body for the management and possible treatment of degenerative diseases

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Chemical Modification of Variety of Underutilized Nigerian Faro 15 Rice for the Production of Pharmaceutical Grade Starch

***[1]Yisa B. N., [1]Musa A. D., [3]Abubakar M. & [2]Egwim C. E.**

[1]Department of Biochemistry, Ibrahim Badamasi Babangida University, Lapai Niger State, Nigeria
[2]Department of Biochemistry, Federal University of Technology Minna, Niger State, Nigeria
[3]Department of Biological Sciences, Al-Qalam University Katsina, P.M.B 2137, Katsina State, Nigeria

*Correspondence Email: **bennmayisa@gmail.com**, Phone No: 07048223082

ABSTRACT

Introduction: Nigerian FARO 15 rice starch has been underutilized due to low edible qualities. Therefore, chemical modification of this variety for pharmaceutical use was contemplated, as native starch has shown to be integrally unsuitable for pharmaceutical applications. The aim of this study was to chemically modify underutilized rice variety using standard methods for possible use as pharmaceutical grade starch.
Methodology: The physicochemical properties of native and modified FARO 15 rice starch were analysed using standard methods. Scan electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), Thermogravimetric analyser and Derivative Thermogravimetric analyser were utilized to decide the morphological properties of native and modified starch. The comparative binding and disintegrant capacities of these starches in tablets detailing were examined utilizing paracetamol as model sedate. Paracetamol tablets were defined using wet granulation strategy and direct compression utilizing native starch, acetylated starch, and phosphorylated starch as binders. The tablet features such as smashing quality, friability, crumbling time and disintegration test were assessed utilizing friabilitor machine, deterioration machine and disintegration device.
Results: The results showed significant decreased ($P<0.05$) values in pH, moisture content, and gelatinization temperature for modified starches compared to the native starch. Significant increase observed in ash content, solubility, swelling capacity, browning temperature, charring temperature and amylose content of modified starches as compared to the native starch. Furthermore, significant changes were also observed in morphology of the modified starches compared to the native rice starch. The weight, crushing strength, friability, disintegration time and dissolution profile values for paracetamol tablets formulated with the modified starches were within the standard specified by British pharmacopeia and were significantly different from that of native starch ($P<0.05$).
Conclusion: In conclusion, the research reviewed critical changes in physicochemical and morphological properties of modified FARO 15 underutilized Nigerian rice starch variety which improves the quality of the starches for pharmaceutical usage.

Keywords: Federal Agriculture Research Oryza (FARO), sodium trimetaphosphate (STMP), acetic anhydride, Modification, photomicrograph, physicochemical properties

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INTRODUCTION

Rice one of foremost important but adaptable nourishment fixings values having included properties for endless industrial uses. Corn, potato, wheat, cassava and rice are the common sources of starch. The most developed cereal crop around the world is rice and integral to billions of people lives around the world (Van-Nguyen & Ferrero, 2006). Rice is created broadly for food and industrial uses. Rice serves as staple food for rural and urban ranges in Nigeria and has changed from being subsistent crop to cash crop. Federal Agriculture Research Oryza (FARO) rice may be modern breed obtained at Badegi, Niger State, Nigeria national cereal research institute and cultivated predominately within Bida and its environs of Niger State, Nigeria.

Pharmaceutical or food industries utilize starch to either influence or control features like; texture, moisture, consistency and shelf soundness. Starch binds or disintegrates, expands, clarifies, attracts or inhibits moisture. It produce either smooth or pulpy surfaces and soft or crisp coatings. Starch serve greatly as multifunctional settling in pharmaceutical industries (Miyazaki et al, 2006). Regardless of the sources of native starch, they are undesirable for industrial applications due to failure to withstand situations as uncommon temperature, adverse pH, high shear rate, tall capacity to retrograde, misforture thickness, syneresis affinity and control thickening due to cooking reduced pH (Singh et al, 2007).

To advance appealing valuable features and overcome its limitations, native starches are often modified. Modification is used to improve basic dejected physicochemical properties of native starch for industrial applications (Miyazaki et al, 2006). Chemical form of modification is used for the treatment of native starch with specific chemicals. In this way modified starch are sensible for canning food, surgical cleaning powder and other applications (Miyazaki et al, 2006). Long time after, significant advances have been achieved in getting non-conventional botanical source starch become valuable in their physicochemical properties (Nunez-Santiago et al, 2004).

Morphological and physicochemical properties for FARO rice variety has never been study and on these bases, this research aimed at investigating effect of chemical modification on the variety of underutilized Nigerian FARO 15 rice starch for the production of pharmaceutical grade starch.

MATERIALS AND METHOD

Sample Collection

FARO 15 rice used for this work was obtained from the National Cereal Research Institute Badegi, Bida, Niger State, Nigeria.

Extraction of FARO rice starch

The method of (Sodhi & Singh, 2003) was used for starch extraction. Put 20 grams of the flour into 200ml of 0.05M solution of sodium hydroxide for 3 hours and drenched at 20˚C. Drain the steep liquor and dilute with 0.05M sodium hydroxide solution. Continue the process until the supernatant becomes clear and test negative for Biuret test. Centrifuged at 3500 rpm and oven dry at 50˚C overnight. The dried starch sieved with 100 mm sieve and stored until examination at -4^{0}C in plastic pack.

Phosphorylation Treatment

Methodology of (Soares et al, 2013) was utilized with slight modifications. About 10 grams of sample was dissolved in 200 ml distilled water, 0.5 g sodium sulphate added and pH adjusted to 9.0 with 1M sodium hydroxide solution. Suspension pre-heated under continuous heating to 70ºC. Three (3g) of modifying agent sodium trimetaphosphate (STMP) was introduced and pH readjusted to 9 with 1M sodium hydroxide. Modification done for 2 hours with constant stirring at room temperature and pH finally reduced to 6 with dilute hydrochloric acid 25% (v/v). The sample was washed with 130 ml ethanol A.P., filtered, and dried at 60ºC for 48 hours.

Acetylated Treatment

The method of Adebowale et al, (2004) and Rincón et al, (2007) with slight modifications was used. Around 42.5% starch suspension was subjected to agitation for 60 min at 30°C and pH adjusted to 8.0–8.2 with 0.1M NaOH solution. Afterward a short time 3 ml of acidic anhydride was introduced drop by drop, keeping the pH at 8.0–8.4. After completion of the reaction, readjust the suspension pH to 4.5 with 0.4N HCl solution. Filter the suspensions, wash severally with distilled water and dry at 30^0C for 48 hours.

Physicochemical Tests

***pH Determination*:** Five grams of each sample was put into 15ml distilled water and mixed separately. Pouring distilled water was used to make up the slurries to 100ml. The slurries cool and pH was recorded. The examination triplicated for each sample.

Percentage of Moisture Loss: The strategy portrayed by AOAC (1990) was utilized to decide the moisture content of native, acetylated and phosphorylated FARO rice starches. Five grams each of starch was dried to constant weight at 105^0C. Weight reduction recorded.

$$\boldsymbol{Moisture\ Content}\ (\%) = \frac{\mathrm{W1 - W2}}{\mathrm{W1}} \times 100$$

Ash Content Determination

The method portrayed by AOAC (1990) was utilized to decide the ash content of the samples. One and half (1/2) of each sample was put into crucible of known weight. The crucibles heated in a furnace for 4hrs at 400^0C, crucibles removed and cooled to room temperature and reweighed.

$$\%\ \text{ash in sample} = \frac{\text{Weight of ash in sample}}{\text{Weight of sample}} \times 100$$

Acidity Level Determination

About ten grams each of sample was incorporate into 70%v/v alcohol separately using phenolphthalein solution as indicator. Rotary shaker used to shake the mixtures for one hour, 50ml filtrate from each sample was pipetted separately and titrated against 0.1M NaOH solution. The method triplicated for each sample.

Solubility: The strategy of (Reddy et al, 2013) was used to determine the solubility of each sample. Two grams each of the starch was dissolved in 10 ml cold distilled water and drained overnight. About 5ml of the clear supernatant was taken for each and heated to dryness on water bath.

$$\boldsymbol{Solubility}\ (\%) = \frac{\text{Dry supernatant weight}}{\text{Initial smple weight}}\ \mathrm{X}\ 100$$

Swelling Capacity Determination

Daramola & Osanyinlusi, (2006) procedure was used to determine swelling capacity. About 0.1 g

of each sample was weighed into a test tube, 10 ml distilled water added to each and the mixtures warmed at 50^0C for 30 min with ceaseless shaking on water bath. On conclusion, centrifuged each at 1500 rpm for 20 min, supernatant decanted and starch paste weighed.

Swelling capacity $= \frac{\text{Weight of starch paste}}{\text{Weight of drying starch paste}}$

Gelatinization Temperature Determination

The method of Nnamani & Attama (2003) was used. One gram of each sample was put into 20ml beaker separately and 10ml distilled water added. Thermometer inserted into each slurry and heated with hot plate to obtain their gelatinization temperature. Triplicated the procedure for each sample.

Browning and Charring Temperature Determination

Builders et al, (2001) method was utilized. Few quantity of each sample was separately put into a capillary tube, using a melting point apparatus title Electrothermal 9100; browning and charring temperatures were recorded. Triplicated for each sample.

Viscosity Determination

Viscosity was determined utilizing the procedure of (Thiewes & Steeneken, 1997). The viscosity of each starch mucilages concentration was done with rotational viscometer utilizing shaft 4 at 20 revolutions per minute at room temperature. Starch formulated through the suspension of 10 grams of each starch in equal volume of distilled cold water, 250ml distilled boiled water was added to each slurry, mixed properly and mixtures heated to 70^0C on thermostated water bath until translucent mucilage formed. The procedure was triplicated for each sample.

Amylose/Amylopectin content Determination

Colorimetric method of Williams et al, (1970) was used. One hundred mg of each sample was transferred into 100 mL volumetric flask separately. 1 ml of 95% ethanol and 9 ml of 0.1N NaOH were added separately, heat samples for 10 minutes to gelatinize on boiling water bath, cool and make up to volume with water. Pipette 5ml each into 100-ml volumetric flask, add 1 ml of 1N acetic acid and 2 ml of iodine solution and make up to 100 mL with distilled water. Shake for 20 min on a rotary shaker and take absorbance at 620 nm utilizing UV/VIS spectrometer. Replicate this procedure.

Morphological Properties

Scan electron Micrographs of each sample were obtained with a scanning microscope (JOEL 6060LV version). The starch samples images were captured within the amplification ranges of 300 and above and 15KV working voltage.

Fourier Transform Infrared (FTIR) Spectra

The method of Colussi et al, (2015) was used for FTIR. About 2 mg of each sample was weighed and mixed with 200mg FTIR review potassium bromide, pressed using a manual press for 20 minutes and pellets transferred into FTIR system. Spectral range determination run from 500-4000 cm^{-1}.

Thermagravimetric Analysis

Ten (10) mg of each sample was put into a TGA dish. Along these lines, the samples experience warming at 25^0C to 850^0C with warming rate of 10 K•min−1 and nitrogen gas streams at 20 mL•min−1. Record for each sample was taken and their charts drawn.

Formulation Study

Native and modified starches were used for formulating Paracetamol tablets utilizing wet granulation and direct compression technique.

Method of Data Analysis

The obtained were analyzed as mean± standard deviation utilizing SPSS factual computer program, form 23.0. The pointer level of significance difference was set at $p<0.05$.

RESULTS AND DISCUSSION

Table 1 Physicochemical Properties of Native and Modified FARO 15 Rice Starch

FARO 15	Native	Acetylated	Phosphorylated
pH	6.40±0.08[b]	5.38±0.01[a]	6.28±0.04[b]
Moisture content	9.07±0.26[c]	6.75±0.06[b]	5.17±0.19[a]
Ash content	6.66±0.16[a]	9.00±0.06[b]	6.24±0.56[a]
Acidity	6.27±0.28[a]	8.00±0.06[b]	8.00±0.06[b]
Solubility	7.90±0.18[a]	12.61±0.05[b]	11.12±0.18[c]
Swelling Capacity	5.86±0.39[a]	8.75±0.08[b]	8.75±0.08[b]
Gelatinization Temp	80.00±0.58[b]	73.67±0.88[a]	73.00±0.58[a]
Browning Temp	202.67±1.20[a]	212.00±1.15[b]	203.67±1.45[a]
Charring Temp	215 – 230±1.20[a]	235.00- 250 ±1.15[b]	234-251±1.45[b]
Viscosity	863.37±3.44[c]	695.47±7.66[b]	608.80±2.26[a]
Amylose Content	0.042±0.0006[a]	0.054±0.0003[b]	0.082±0.0009[c]

Values are Mean ± SEM of triplicate determinations. Values with different alphabet across a row are significantly different at $p<0.05$

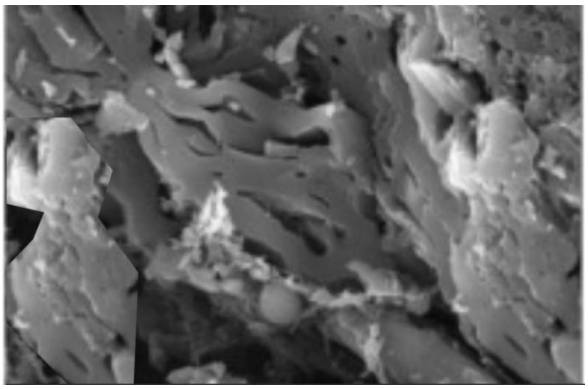

Figure 1a: FARO 15 Native Starch Scanning Electron Microscope image

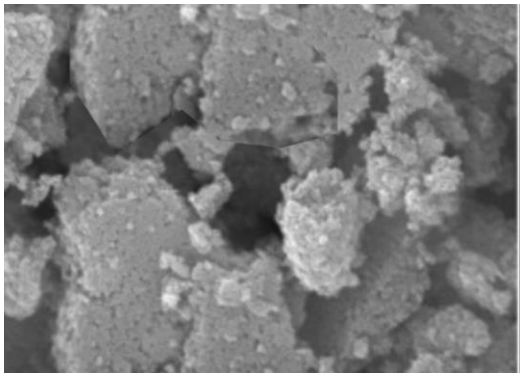

Figure 1b: FARO 15 Phosphorylated Starch Scanning Electron Microscope image

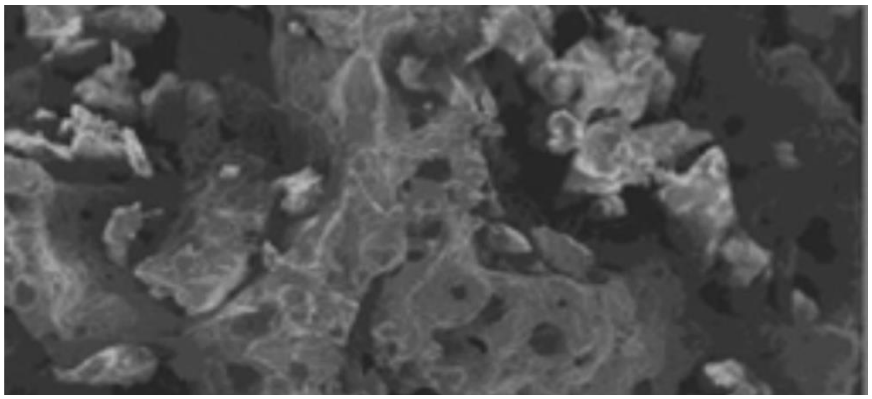

Figure 1c: FARO 15 Acetylated Starch Scanning Electron Microscope Image

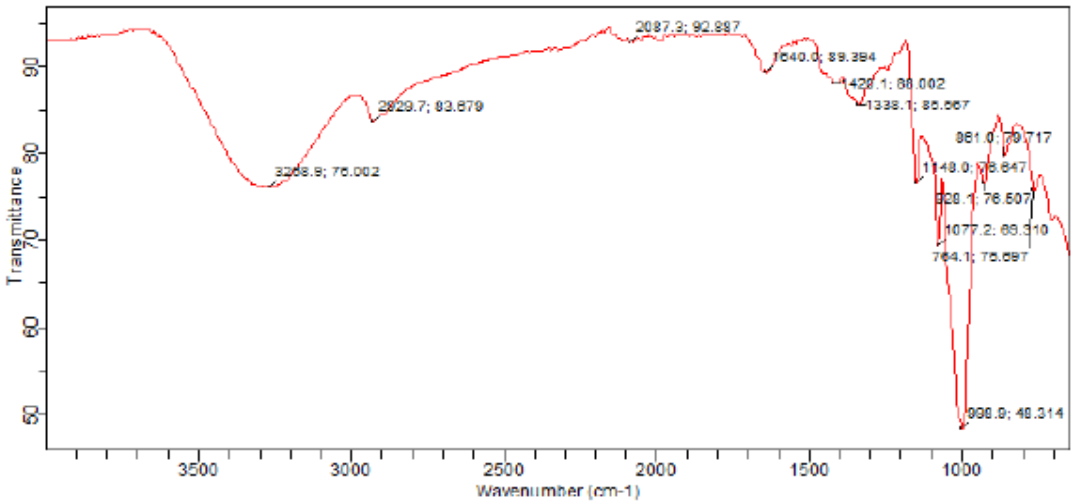

Figure 2a FARO 15 Native Starch FTIR Spectra

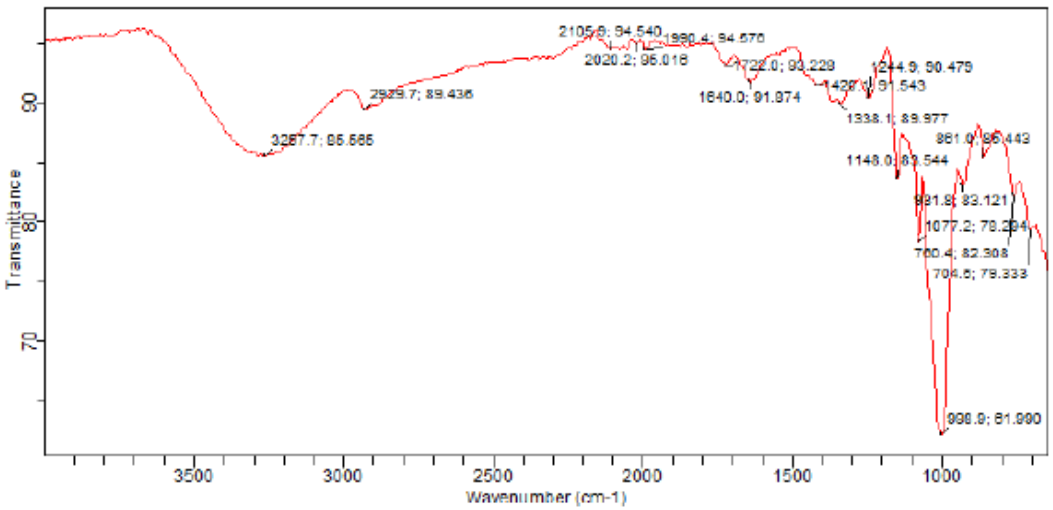

Figure 2b FARO 15 Acetylated Starch FTIR Spectra

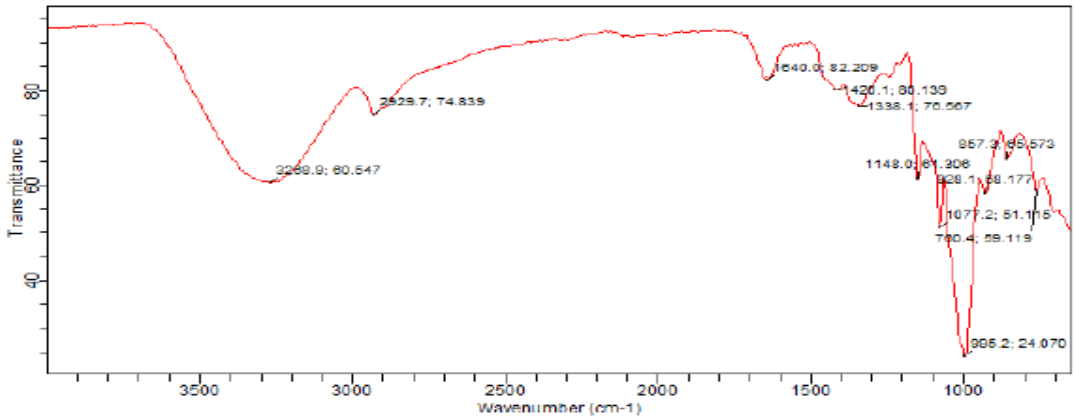

Figure 2c FARO 15 Phosphorylated Starch FTIR Spectra

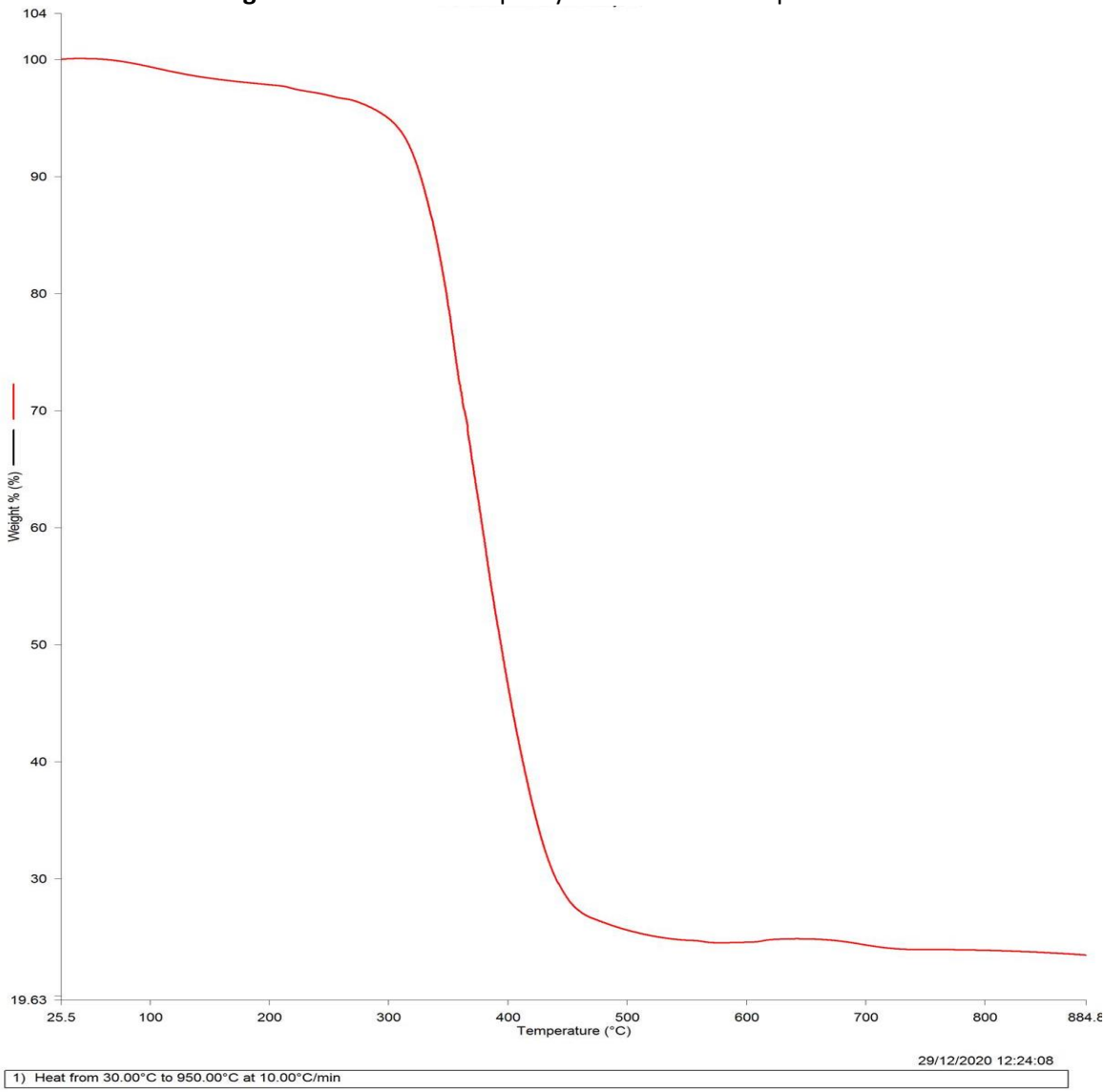

Figure 3a FARO 15 Native Starch TGA Plot Spectra

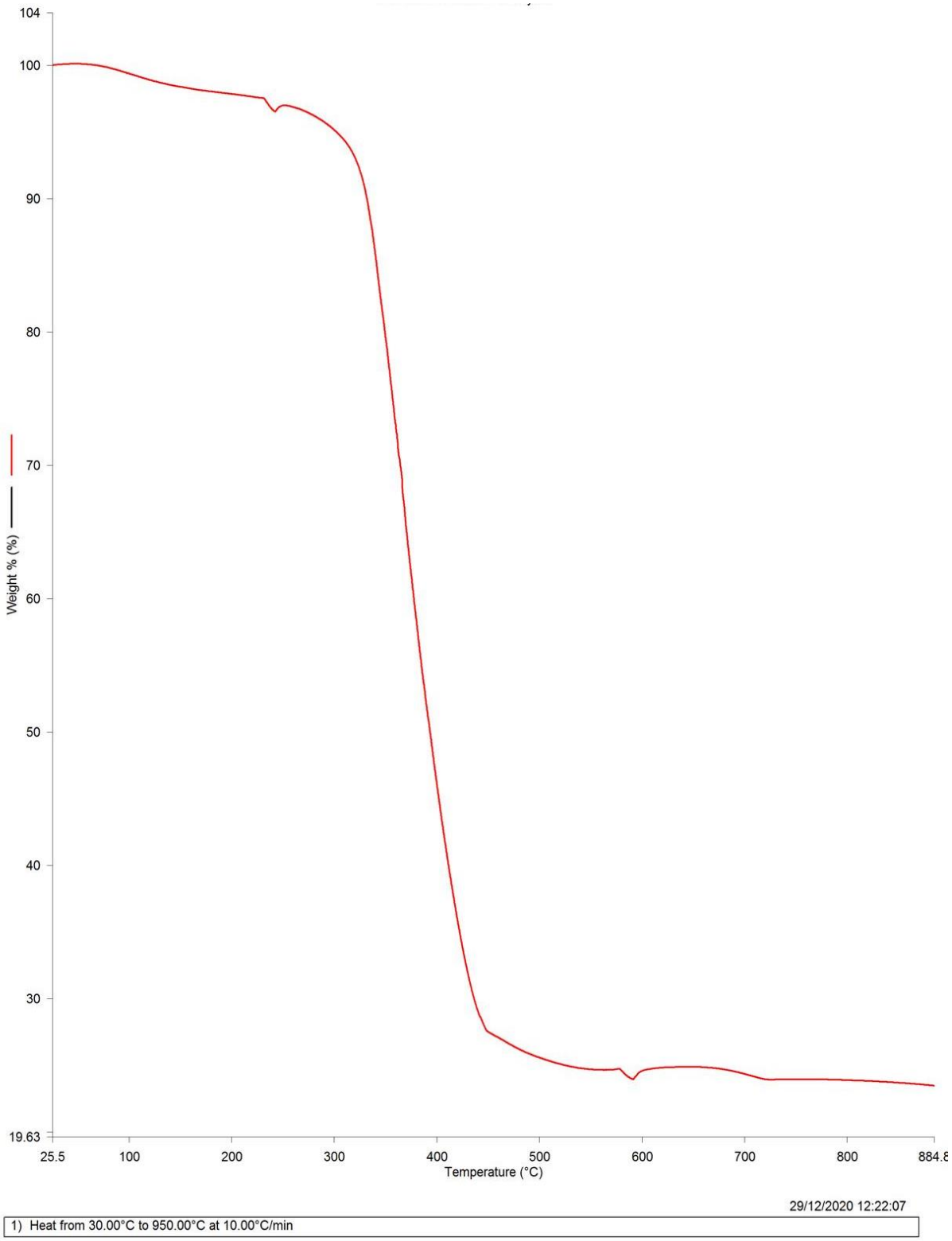

Figure 3b: FARO 15 Acetylated Starch TGA Plot

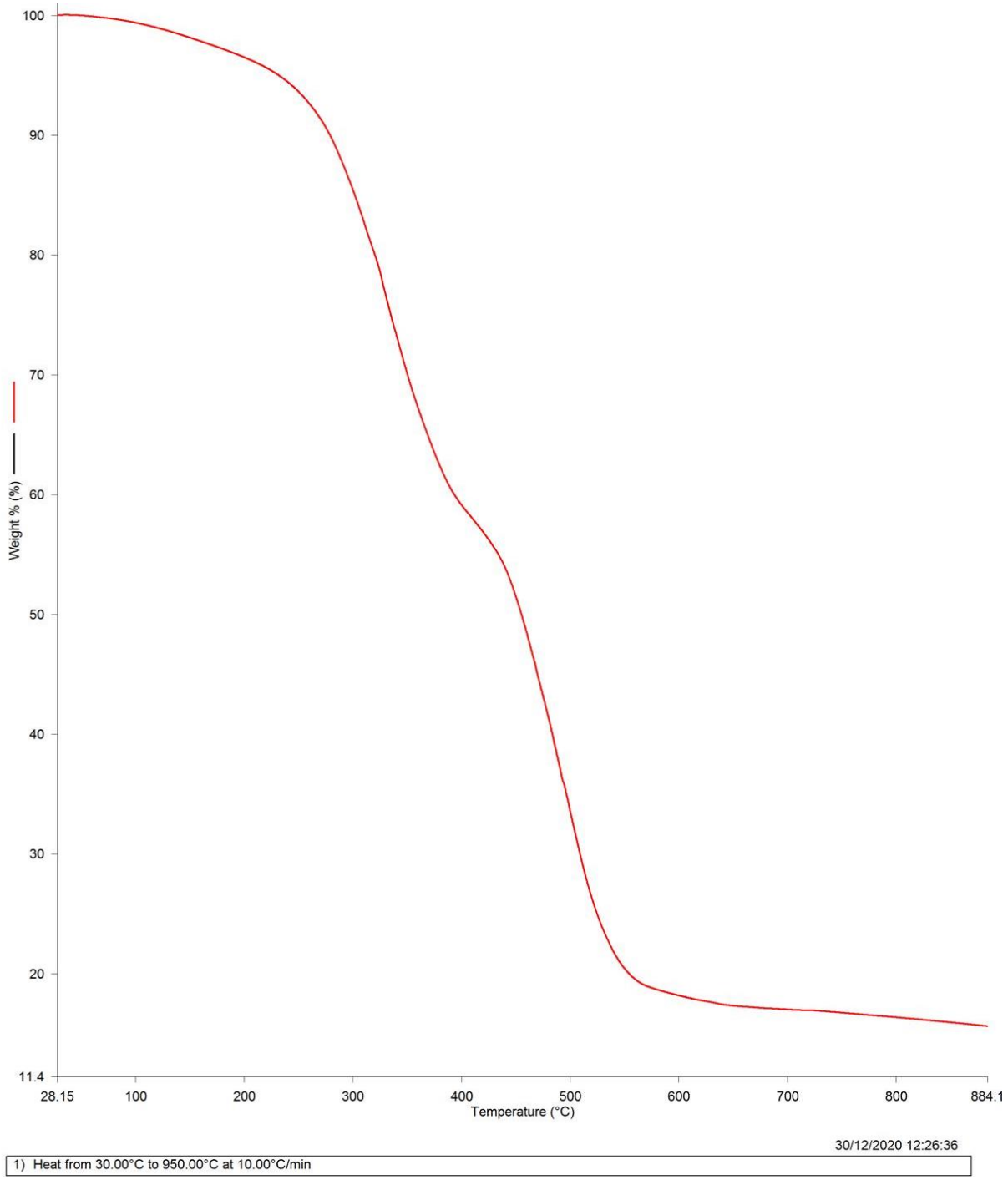

Figure 3c: FARO 15 Phosphorylated Starch TGA Plot

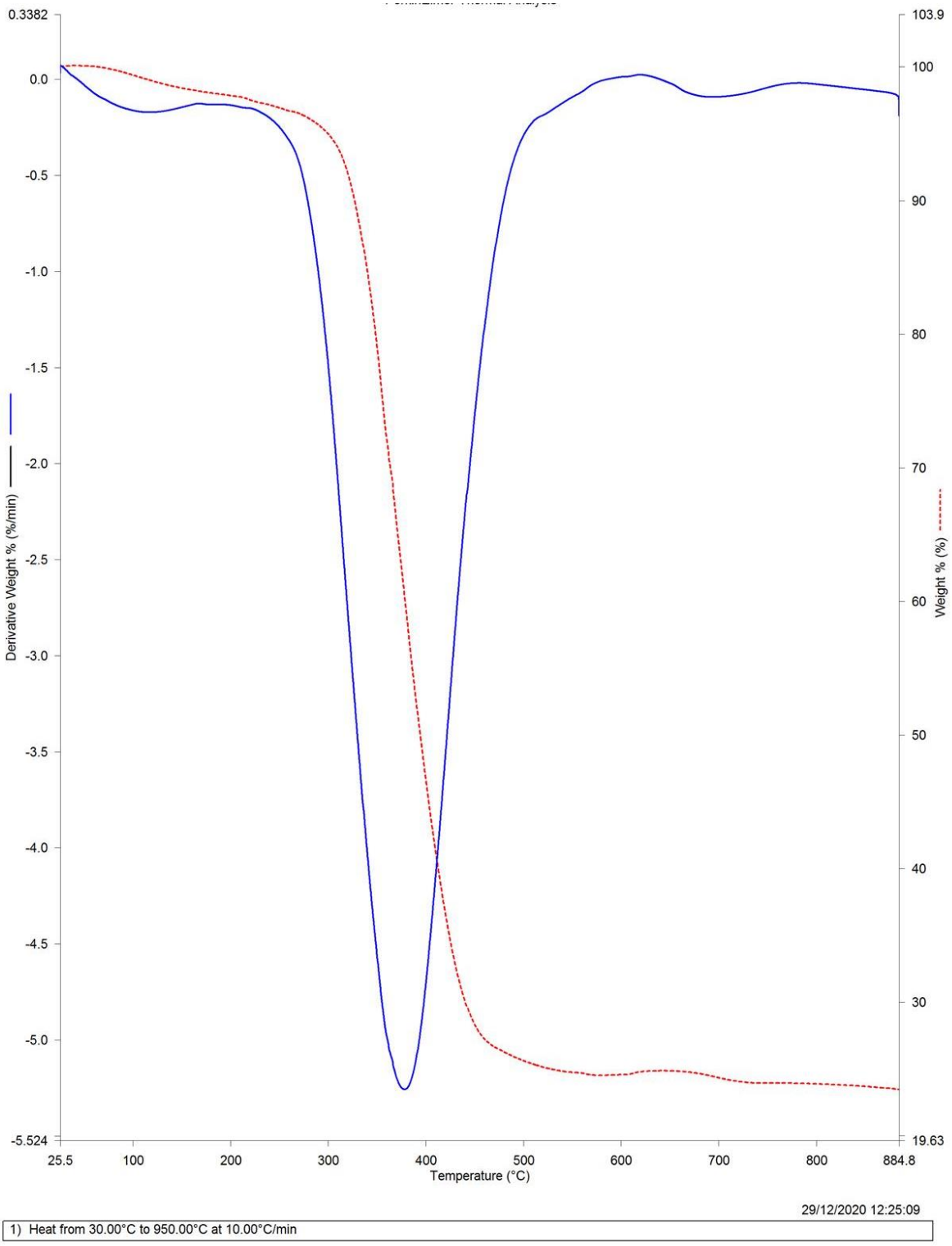

Figure 4a: FARO 15 Native Starch DTG Plot

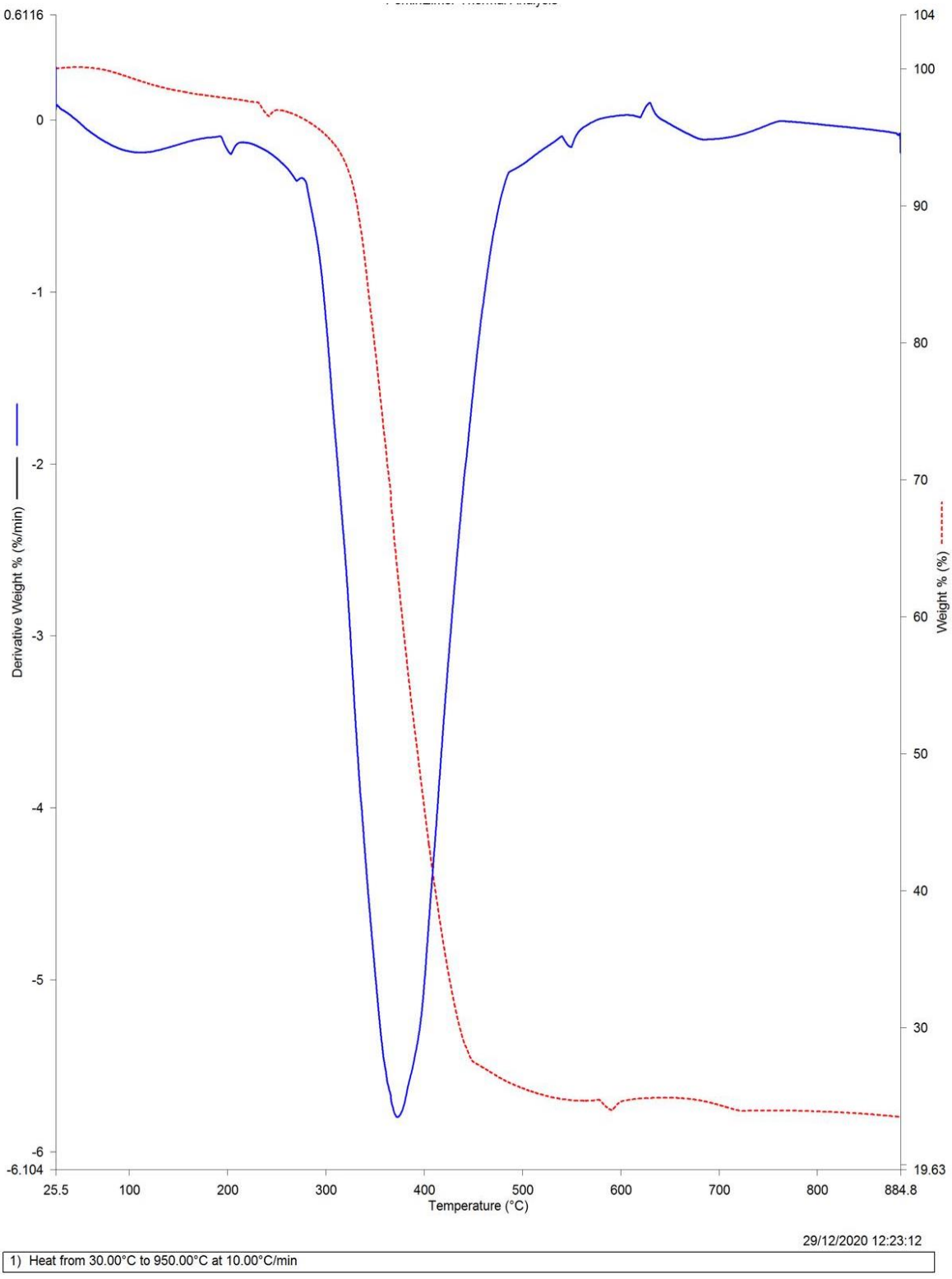

Figure 4b: FARO 15 Acetylated Starch DTG Plot

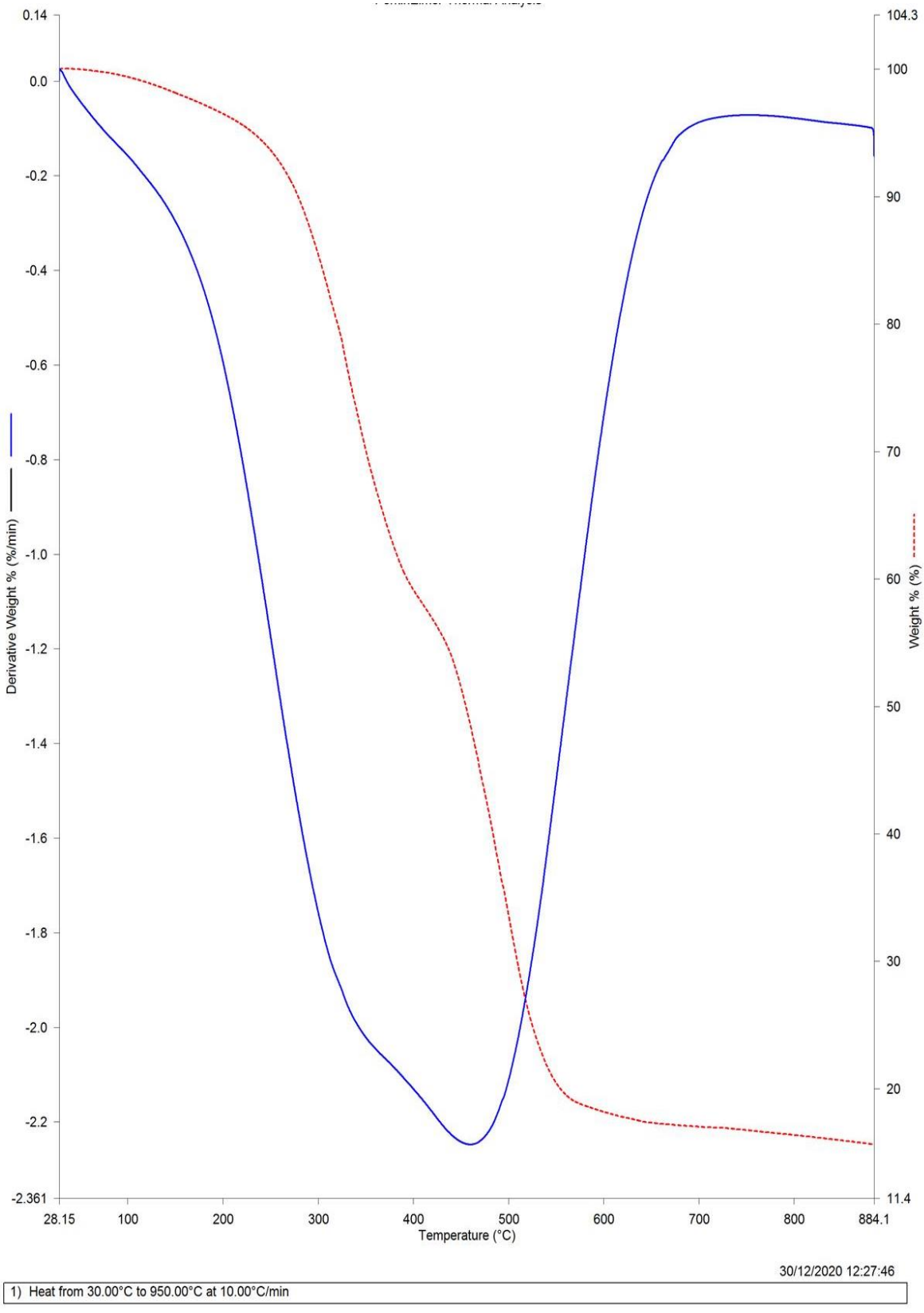

Figure 4c: FARO 15 Phosphorylated Starch DTG Plot

Paracetamol Production Certificate of Analysis for Native, Modified and Standard

Starch

SPECIFICATION (B.P)	PARAMETERS	FARO N15	FARO A15	FARO P15	MAIZE STARCH
625 (mg)	Max. Wgt	600±3.44	620±1.34	616±3.22	619±2.11
565 (mg)	Min. Wgt	565±3.22	565±2.23	565±3.22	568±3.11
NLT 3 (kg/cm^2)	Hardness	4.0±0.04	5.0±0.7	5.5±0.10	5.5±0.10
NMT 1 (%)	Friability	0.86±0.10	0.69±0.03	0.72±0.08	0.65±0.05
3.8-42 (mm)	Thickness	4.0±0.00	4.0±0.00	4.1±0.00	4.0±0.00
NMT 1-5 Mins (s)	Disintegration Time	128±0.10	153±0.05	123±0.11	121±0.15
NLT 70 (%)	Dissolution	79±2.12	83±2.13	83±0.04	81±0.00
95%-105 (%)	Assay	96±0.00	97±0.00	98±0.00	101±0.00
1.5 – 2.5 (%)	Loss on Drying of the Granules	2.23±0.00	1.75±0.00	2.03±0.00	1.86±0.00
NLT 65 (%)	Coarse of the Granules	65.80±0.00	66.18±0.00	67.36±0.00	66.71±0.00

DISCUSSION

The pH values of modified starches were found to be lower than that of the native starch as shown in table 4.1 and this is obviously because of the reaction with acetic anhydride and sodium trimetaphosphate, but all falls with the pH range of 4 – 8 obtained for most starches used in pharmaceutical, cosmetucs and food industries. Acetylated starch pH was significantly difference from phosphorylated and native starches at $p<0.05$. The pH of both native and modified FARO 15 rice starches recorded were within normal range of 4 – 8 as already detailed by Nuwmanya et al, (2011) and Omojola et al, (2012) utilized in most pharmaceutical, makeup and nourishment industry.

The results in Table 4.1 showed decreased moisture content in modified starches compared to native starch. Modified starches were significantly different at ($p<0.05$) from unmodified starch. The reduction in moisture content seen in modified starches might be due to alteration and change within the big molecular structure of the starch granules caused by acetic anhydride and STMP. The diminishing tread seen in this work is comparable to that detailed by Dolas et al, (2020), and Xia et al, (2011) on impact of adjustment of physico-chemical, utilitarian and basic features of cassava starch (Manihotesculenta Crantz). The reduction in moisture content of modified starches suggests a prolong shelf life stability during storage (Gope et al, 2016).

The ash content of modified starches increases than native starch as shown on table 4.1. Acetylated starch shows significant difference at ($p<0.05$) from phosphorylated and native starch. This result corresponds with that of Yousif et al, (2012) and Dolas et al, (2020) who observed that modified starches had higher ash content than native starch.

The solubility of modified starches were higher than that of the native starch as shown on table 4.1 Introduction of acetyl and phosphate groups into native starch structure debilitated acquainted powers between particles within starch granules that results in high solvency for modified starches. Significant different was observed in modified starches compared to native starch. Barimah et al, (2009) and Jairo et al, (2016) work on impact of acetylation and carboxylation on physicochemical properties of cassava starches shows dissolvability of cassava starch increments.

Table 4.1 shows that swelling capacity of modified increases than native starch. The increment observed in modified starches may be due adjustment within the glucose granular structure that makes up the starch which permits water to enter easily. Modified starches has a Significant difference at ($p<0.05$) than native starch. This tread was reported by Chatakanonda et al, (2000) and Bea-Young & Yoo (2010) on cross-linked starch with higher swelling capacity than native starch at increased temperature. Hence, modified starch are better choice as a distingrant in tablets formulation since tablets are formed and used at the temperature range where it has a better swelling. Also high swelling power results into high digestively and ability to use starch in solution suggesting improved dietary properties and the use of starch in different dietary industries (Nuwamanya et al, 2010).

The process whereby starch undergoes an irreversible change under heat and absorbs water with swelling thereby causing granules swell more and become a paste rather than a dispersion which it forms in cold water is called gelatinization. Decreased gelatinization temperature was observed for modified starches compared to native starch as shown on table 4.1 and this might be due to weakening intermolecular qualities and introduction of acetyl and phosphate groups into the starch structure. Significant difference was observed between modified and unmodified starch. This is comparable to work done by Jairo et al, (2016) and Adewoyin et al, (2017) on modification of yam and sweet potato where they observed reduction in their gelatinization temperature. The modified starch gelatinize at lower temperature than native starch, which is an indication of water absorption at this temperature is irreversible and industries that require their uses in gel form might find this modified starches very useful

Browning and charring temperature of modified starches were higher than the unmodified as shown in table 4.1 Acetylated starches has a significant difference at ($p<0.05$) from that of phosphorylated and native starches. This shows that modified starches indeed can be heated to higher temperature without change in colour or charring. This makes the starch better for industries that utilize them at higher temperatures. This is similar to Omojola et al, (2012) work on preparation and physicochemical characterization of icacina starch citrate – a potential industrial starch.

The viscosity values for acetylated starch was higher compared to phosphorylated and native starch as shown in table 4.1 The values were significantly difference at ($p<0.05$) level. The increase in acetylated starches may be attributed to the addition of new functional groups (acetyl), degree of hydrolysis and increased amylose recrystallization. Decrease in Phosphorylated starch viscosity compared to native starch may be due to the introduction of phosphate bunches through treatment with

sodium trimetaphosphate (STMP) that might have reduced or may completely prevent granule swelling which results in decreased in thickness. This is similar to work done by Chantaro et al, (2013) on viscosity behaviour for cross-linked starch on rapid viscosity analyzer (RVA) and work of Akpa & Dagde (2012) on modification of cassava starch for industrial uses.

Table 4.1 presents amylose content for native FARO 15 rice starches lower than that of acetylated but higher than that of phosphorylated starch and significant difference was observed between native and modified starches for FARO 15. The increase seen in acetylated starches may be ascribed to the interference of acetyl bunches with the functioning of amylose and amylopectin divisions of starch influencing assimilation of iodine during determination of amylose. In this way, the decrease in amylose content of the STMP modified FARO rice starch may be related to an expanded formation of dextrins and presentation of phosphate bunches within the course of corrosive treatment. These observations were similar to previous reports on increase in amylose content upon acetylation of starch from Canavalisensi formis, (Betancur et al, 1997) and work done by (Adewoyin et al, 2017), on phosphoric acid impact on physicochemical, useful, and basic properties of starch extricated from yam (Dioscorea rotundata) were they revealed decrease in blue value and amylose content.

Native and modified starches scan electron micrographs results shown on figure 1a, 1b, and 1c. Native starch micrograph shows clustered, smooth, and specific granular shapes compared to modified starches that shows rough and cruel surfaces. The changes observed on starch granules surfaces for modified starches could be due to acidic anhydride and sodium trimetaphosphate reactions. Adewoyin et al, (2017) previous research revealed similar surface roughness on maize and potato starch granules for phosphoric destructive alteration.

In this manner, granule shape of starch impacts the valuable characteristics of starches which influences their applications. Starch granule shape contributes to starch gelatinization, swelling and thickening rate.

Native and modified starches FTIR results are shown on figure 2a, 2b, and 2c. Native starch discernable peak observed at 3261.4 credited as O-H stretched bonds. The peaks came as a result of hydroxyl (O-H) groups and C-H bunches vibration. Other peak was observed at 2929.7 as C-H stretch (Alkanes), 1640 corresponds to C=C expand (alkenes) and C-O amplify. Other peaks between 1148 and 1077.2 corresponds to C-O amplify. At long last, another top band seen on FTIR chart at 708.2cm-1 for native starch as C-H bend. After modification, intensity of bands increases between 1148 and 1077.2cm-1, as signs for C-O bonds formed through the substitution of hydroxyl with acetyl and phosphate groups which was communicated by (Harvey et al, 2012). Intensity of absorption bands for O-H was at reduce rate for modified starches compared to native starch. Bands like C-O, C=C, and C-H for modified starches were at increased intensity when compared to native starches. Absorption bands intensity increase could be due to collapsing of the C-O, C=C and C-H bands resulting in changes within starch structures Xu et al, (2004) and Mulualem (2013).

Thermogravimetric analysis investigated starch-based materials utilized for industrial purposes degradation and thermal behaviour. Thermogravimetric graphs and derivative

thermogravimetric graphs are shown on Figure 3a, 3b and 3c for TGA while figure 4a, 4b and 4c for DGA of native and modified rice starches. The graphs shows similar trend, indicating thermal breakdown and mass loss for native and modified starches occurring at different phase representing particular event during heating. Thermal action occurs first between 100^0C and 150^0C for native and modified starch which results in mass loss due to moisture and water fragments elimination by evaporation. In this phase, higher moisture content leads to higher mass loss. Continuous heating leads to another lost in weight between 250°C and 460°C. At this weight lost, phosphorylated starch has higher temperature compared to acetylated and native starch within the region of 390^0C. This phase reduction in weight is related to depolymerisation and degradation of starch structure carbon chain. Nascimento, et al, (2012) states that starch thermal reactions usually occurs at about 300°C. Final weight loss occurs within the range 460^0C and 700^0C. Weight lost at these temperatures are not prominent compared to the one between 250°C and 460°C due to evaporation of less materials or water from native and modified starches which shows that modification hasn't altered thermal stability of modified of starches. Isotton et al, (2015), had similar report on effect of different plasticizers which they declared rate of degradation between 290.9^0C and 295.4^0C.

The values of the weight of paracetamol tablets formulated with native starch and modified starches ranges from 565 – 620±5% mg as shown on table 2 above. The values were within British pharmacopeia limit for particular tablet weight which states "for tablets weight > 500 mg, ± 5 % weight variety are permitted". It implies that tablets defined from native starch and modified starches as binder passes weight consistency test when compared to consistency weight results for tablet defined with maize standard starch. The die cavity uniform filling resulted from great stream properties of granules moreover improved by the addition of Glidant. Rawlings (2004) on suspicion expressed that variety within weight for individual tablets may be a substantial sign for comparing variety within sedate substance.

Index used to measure tablets hardness is called Crush strength. Van der Waal‟s attraction forces, frictional, mechanical powers, and strengths are due to arrangement of strong bond which serves as binders that held together the tablets (Musa et al, 2010). The comes about of smashing quality of tablets defined with native, modified and standard Maize Starches are presented in table 2 above. The results indicates that the values of crushing strength for all starches are within limit indicated by British Pharmacopeia which stated that smashing of tablet ought not be less than 3.0kg/cm^2. In any case, there's significant difference at ($P<0.05$) in the mean value of the crushing strength of native starch with that of the modified and maize standard starch. This is comparable to Ochekpe et al, (2013) who worked on the smashing quality of tablets defined with *Plectranthus esculantus* starch. This demonstrates that FARO 15 rice starches either as native or modified has superior crushing quality when utilized for tablet definition as a binder.

The degree of weakness of tablets is friability (FR); it gives a sign of the likely edge damage that would happen when the tablets are handled during packaging, transportation and dispensing. Significant difference at ($P<0.05$) within mean values of friability of native, modified and standard maize starches were observed. In any case, none of the starches has it friability esteem

over the limit indicated by British pharmacopeia (2015) which state that friability ought not to be more than 1% for each tablet. These shows that tablets defined from both native starch and modified starches has exceptionally great binding properties when compared to standard maize starch and other starches utilized ordinarily as binders and disintegrant.

Disintegration time outcome for tablets defined from native, modified and standard maize starches are presented in tablet 2. Acetylated starches is significantly difference from that of native starch, phosphorylated starch and standard Maize starch at ($P<0.05$). The high values observed in acetylated starches are as a result of hydroxyl bunches substitution with acetyl bunches that makes the starches more hydrophobic and anticipates hydrogen bonding arrangement between hydroxyl groups and water molecules. Phosphorylated starch deteriorates quicker than native starch and acetylated starch and this diminish within the sum of starch amylose content. The deteriorating time values for the four starches are within recommended ranges indicated by British Pharmacopeia (2015). These suggests tablets defined from both native and modified starches as binders passed deterioration test when compared to crumbling values of tablets defined from standard maize starch. These perpetually imply that FARO 15 rice starches either as native or modified frame are fabulous excipient to be utilized as binder or disintegrant in Tablets detailing. These outcome are comparable to discoveries of Apeji (2015) which includes a deterioration limit extending between 1-5 minutes.

Dissolution profile results for tablets defined from native starch, modified starch and standard maize starch as binder at distinctive time interim are presented on table 2. At 70 minutes, disintegration time for native starch and standard Maize starch are factually and significantly different from disintegration time for modified starches at ($P<0.05$). All tablets passed British Pharmacopeia (2015) disintegration test, which states that at least 70% of the drugs ought to be discharged after 45 min. The results shows disintegration for tablets defined from native starch, modified starches and standard Maize starch agrees with the crumbling – disintegration hypothesis which demonstrates that deterioration more often than not play a crucial part in disintegration handle which decides to an expansive degree the period of contact between strong and fluid media (Dare et al, 2006). Michael et al, (2015) presents a comparable result on impact of heat and chemical alteration on industrial and discharge properties for paracetamol tablets defined from corn, cassava and sweet potato starch which present disintegration values extending from 70-90% which is comparative to the disintegration values gotten from tablet defined with native and modified FARO 15 rice starches. This implies that both native and modified FARO 15 rice varieties starches are good binders and disintegrant in tablet formulation.

CONCLUSION

The research showed significant changes in physicochemical and morphological properties of the underutilized Nigerian FARO 15 rice starch for native and Modified Starch. Fourier Transform Infrared spectra shows considerable structural contrast between native and modified rice starches. Modified starch with sodium trimetaphosphate and acetic anhydride results shows impact on useful and basic properties like swelling capacity, solubility, amylose content,

few utilitarian bunches and starch granules surface unpleasantness. Paracetamol tablets produced from FARO 15 native and modified starches certificate of analysis shows physicochemical and morphological changes caused by modifications which has resulted in the improvement in the quality of this underutilized Nigerian rice starch. From the foregoing, FARO rice starch could be a cheap and good source of starch for pharmaceutical industrial.

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Conflicts of Interest

The authors declare no conflict of interest

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In vitro Activity of Methanol Extracts of Stembark of *Anogeissus leiocarpus on Plasmodium falciparum*

[*1]Ndayako H. H., [2]Abdulsalam M. S, [3]Bulus T., [4]Yunusa Y. & [5]Gara T. Y.

[1]Department of Biological Sciences, Ibrahim Badamasi Babangida University Lapai. Niger Stare,
[2]Department of Biological Sciences, Nigeria Defence Academy Kaduna, Kaduna State.
[3]Department of Biochemistry Kaduna State University Kaduna. Kaduna State.
[4]Department of Pharmacy, National Ear Care Centre, Kaduna, kaduna State.
[5]Department of Biochemistry Federal University of Technology Minna. Niger State.

*Corresponding Author E-mail: hauwannadyadya@gmail.com, Phone: +2348036102000

ABSTRACT

This study was carried out to evaluate the *in vitro* effect of methanolic extracts of stem bark of *Anogeissuss leiocarpus* (AL) on *Plasmodium falciparum*. Powdered stem bark and root of *Anogeissus leiocarpus* was subjected to cold maceration using 99.8% methanol. The crude extracts were sequentially fractionated using four solvents of increasing polarity. Preliminary Phytochemical screening of the crude methanolic plant extracts revealed the presence of saponins, tannins, flavonoids and cardiac glycosides. *P. falciparum* field isolate was cultivated *in vitro* using the Trager and Jensen Candle jar method. The plant extracts were tested against the ring staged synchronous *P. falciparum* field isolates by incubation in 96-well micro titre plate for 48hrs. Results showed that *Anogeissus leiocapus* extracts had the highest activity when used singly against *P. falciparum* with its crude methanolic extract having IC_{50} of 5.00 µg/ml, ethylacetate fraction with IC_{50} of 5.00µg/ml and benzene fraction with IC_{50} of 9 µg/ml. the other eextracts all showed moderate antiplasmodial acitivity with Aqueous fraction having IC_{50} of 17µg/ml and chloroform with IC_{50} of 20 µg/ml. Statistical analysis reveals increasing activity with increasing concentration which shows significant antiplasmodial activity at P value ≤ 0.01. The findings in this studies have shown that the crude methanolic extracts of AL have significant activity on the cultured field isolate of *Plasmodium falciparum*, Therefore the findings in this research is an appreciable effort to averting the challenges posed by parasitic resistance to the existing antimalarial drugs.

Keywords: Antimalarial drugs, Field isolate Malaria, *Plasmodium faciparum*, Wet partitioning

INTRODUCTION

Throughout history, man has suffered from infectious diseases caused by pathogenic microorganisms such as viruses, bacteria, fungi and parasites. Infectious diseases comprise clinically evident illness resulting from the presence and growth of the pathogenic microorganisms in the individual host (Neelavathi *et al*., 2013). A notable example of these infectious diseases, which is a major public health and developmental challenge in Nigeria and many other African countries, is malaria where transmission occurs all year round.

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The causative agent of malaria is a parasite in the blood called *Plasmodia.* Four species of this genus causes malaria in humans these are: *P. falciparum, P. vivax, P. malariae* and *P. ovale.* However, *P. knowlesi* associated with long tailed macaque (Lee *et al.,* 2011). Malaria contributes substantially to the poor health situation in Africa. About 90% of the world's 216 million cases and 655 000 annual deaths of malaria occur in the sub-Saharan African region (WHO, 2012). In Nigeria more than 3 million cases are reported annually. A significant proportion of such cases occur in children under the age of five (Ogunlana & Ademowo 2009). Treatment of malaria with potent, effective, available and affordable drugs nevertheless, remain crucial to the control and the eventual eradication of the disease in Nigeria and the sub-region as a whole.

Many antimalarial chemotherapeutic agents have been used to treat the infection but most of these agents are now not effective due to the widespread of multidrug-resistant malaria parasites (Asante and Asenso-Okyere, 2003). Africa is endowed with numerous plants that have been used traditionally to treat various diseases. And these plants are cheap, readily available and produce less adverse effects compared with conventional drugs.). This study focuses *Anogeissus leiocarpus,* also known as african birch of Combretaceae family and Gbanshi in Nupe dialect. The plant was selected through thorough consultations of the people around Bida Local Government in Niger state. This plant is used either singly or in combination locally to cure malaria like ailments such as headaches, high temperatures and seizures.

METHODOLOGY

Collection and authentication of plant materials:

The plant materials were collected in October 2015. The Plants were authenticated in the Department of Biologcal Sciences, Nigerian Defence Academy, Kaduna and the voucher specimens was deposited for future reference with voucher number 1413

Pre-treatment of the plant material:

The plant materials were initially screened for foreign materials such as sand and insects. A stiff brush was used to clean off the dirts from stems. then washed thoroughly in cold water to remove any trace of unwanted foreign matter.

Drying:

The parts collected were dried under shade for 13 days and when properly dried it was powdered using a grinding machine, then sieved to reduce the size. And then packed in an airtight container and stored at room temperature for further analysis.

Plant extraction:

Preparations of extracts of stem bark

The powdered plant parts (200g) was weighed using an electronic weighing balance and soaked in 1.2L of 99.8% methanol in transparent glass bottles. The setup were shaken intermittently to enhance the extraction process and left to stand for 48hrs. The filtrate were separated from the residue by sieving with muslin cloth then evaporated under reduced pressure 45(rpm) at 60^{o}C (in accordance to the boiling point of the solvent i.e. methanol) using a rotary evaporator (Ramalhete *et al.,* 2008) and then stored in well-closed plastic containers at low temperature (4^{0}C) until needed for further analysis (Sutherson *et al.,* 2007).

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Fractionation of the crude extract of *Anogeissus leiocarpus* Stembark

Fractionation of the crude extract was done by first dissolving 10g of the crude metanolic extract of the stem of A.L was first dissolved in 90ml/10ml distilled water and methanol in a beaker, then transferred into a separating funnel then Benzene being the solvent with the lowest polarity index was introduced to this suspension in ratio 1:1 as that of water. By using a separating funnel this mixture was separated in to two distinct layers with the aqueous layer which is less dense at the top. The benzene fraction was then separated out. This procedure was also performed on the other polar solvent in order of increasing polarity i.e. chloroform, ethylacetate respectively with polarity index: 2.8>4.1>4.4>9. The fractionation process followed the procedures by Fatope *et al.,* 1997 with little modification.

Preliminary Phytochemical screening of the crude extract:

The preliminary phytochemical tests was conducted using modified versions of the standard protocols by Njoku & Obi (2009) and Nobakht *et al.,*(2010).

ANTIMALARIAL BIOASSAY

Culture Technique:

The *in vitro Plasmodium falciparum* parasite cultivation was based on the method described by J ensen and Trager (Trager & Jensen, 1977, 1997, 2005). called the Candle Jar Method of Culture. Were A white candle was lit and placed within the desiccator containing petri dishes containimg the *Plasmodium* parasite in a complete medium, and the cover is put on with a stopcock open. When the candle goes out, the stopcock was closed. This is a simple and effective way to produce an atmosphere with low O_2 and high CO_2 content which is optimal for *P. falciparum* growth. The Candle Jar was then incubated at 37 ºC in an incubator.

Source of malaria parasite

Field isolates of *Plasmodium falciparum* were used in this antimalarial bioassay because of its accessibility and availability. Isolates were obtained from out-patients at Yusuf Dantsoho memorial Hospital Kaduna.

Collection of blood samples:

0.47ml CPDA-1 (citrate phosphate dextrose adenine) of whole blood (plasmodium infected) samples were collected from patients attending department of Yusuf Dan Tsoho general hospital, Kaduna in February, 2016. An ethical clearance/permit was obtained from the Ministry of Health, Kaduna state

Screening of Blood Samples

Microscopic identification of *plasmodium falciparum*.

For each collected sample received, thin smear was prepared and stained with Giemsa stain to confirm the presence of *Plasmodium* parasite, Parasitemia was determined and growth stage of parasites were observed.

Estimation of the percentage (%) parasitaemia.

An area of stained thin blood film where the erythrocytes are evenly distributed was observed using 100 x objectives (under oil immersion).Approximately 100 erythrocytes in this area were counted. Without moving the slide, the number of infected erythrocytes amongst the 100 erythrocytes was also counted.

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The slide was moved randomly to adjacent fields and counting was continued as mentioned above. An equivalent of 1,000 erythrocytes was counted. The counting was repeated twice for a total examination of three different parts of the slide, that is, 3 areas 1000 cells. The mean number of infected RBCs per 1,000 RBCs gives the estimated percentage (%) parasitaemia of thet particular blood sample.

Preparation of extract stock solutions: Crude methanolic extracts and the fractions of. *A. leiocarpus* (stem bark) were screened for antiplasmodial activity against the Laboratory adapted *P. falciparum* isolates. The plant extracts were prepared using culture media RPMI1640 and 0.2% of dimethyl sulphoxide (DMSO) to produce 2mg/ml stock solutions. The stock solutions were sterilised using a 0.4 millipore filter. Subsequently the stock solutions were diluted with culture media to produce six concentrations of the extracts (1, 10, 50, 100, 500, and 1000) µg/ml (Koudouvo 2011; Treger & Jensen 1976).

***In vitro* cultivation of *P. falciparum* isolates and susceptibility Testing**: The assay was performed in triplicate on a 96 wells microtitre plates. The sterile 96 wells tissue culture plate was predosed with 100µl of culture medium containing extracts at various concentrations followed by the addition of 100µl of sub cultured parasite diluted with the O^+ erythrocytes to about 0.5-1% parasitemia. A negative control was maintained with 100µl of *Plasmodium falciparum* culture,100µl of culture medium and positive control was maintained by the addition of a standard drug Arthemeter-lumefantrin at varying concentrations at (1, 10, 50, 100, 500, 1000) µg/ml. The Plates were then incubated in a candle jar in an incubator at 37^0C for 48hrs (Trager & Jensen, 1976). After incubation, contents of each well was harvested after carefully removing the culture media which is at the upper layer then Thin blood smears from each well was prepared on a slide and fixed absolute methanol then stained with 10% Giemsa stain at pH 7.3.number of infected red blood cells was counted with the aid of a compound microscope and The control parasite culture freed from extracts was considered as 100% growth. Antimalarial activity was assessed by parasitaemia determination, determination of the inhibitory concentration as well as IC_{50} (the concentration of extracts that is able to kill 50%.

Parasites growth inhibition per 100 red blood cells was counted in 10 microscopic fields. The control parasite culture freed from extracts was considered as 100% growth. The percentage inhibition per concentration was calculated using the formula:

[(% parasitaemia in control wells – % parasitaemia of test wells)/ (% parasitaemia of the control)] x 100] (WHO, 2001; Ngemenya *et al*., 2006).

The IC_{50} was determined by linear interpolation from the growth inhibition curves (Log of concentration versus percentage inhibition) generated from each parasite-extract interaction (Mustofa *et al.*, 2007).

Statistical Analysis:

The data collected was subjected to one-way Analysis of variance (ANOVA) and mean of % parasitaemia of the extracts were compared using Duncan Multiple Range Test (DMRT) at P≤ 0.01.

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RESULT

Crude methanol extract of *A. leiocarpus* had a low % yield 10.10%. This is shown in table 1. The preliminary phytochemical analysis of the stembark of *A*. *leiocarpus* as shown in Table 3 revealed that it contains flavonoids, tannins, saponin and cardiac glycosides. Sequential partitioning of the crude methanolic extract using four solvents of different polarities gave different percentage yields as shown in table 2. The highest percentage yield wad recorded with the aqueous fraction with 50.1% and the lowest % yield in the ethylacetate fraction with 3.8%. The crude methanolic extract of the stem bark of *A. leiocarpus* and the four fractions showed promising inhibitory effect against *P. falciparum*. The crude methanolic extract had the highest effect against the parasite with the lowest concentration 1µg/ml having % Parasitaemia of 53.40. Among the fractions of *A. leiocarpus* stembark Benzene had the highest effect and the lowest was found to be the aqueous fraction this is all shown in Table 4. It was also observed that the antiplasmodial activity is dose dependent the % parasitaemia decreasing with increase in concentration of the extracts.

Table 1: % Yield of Crude methanolic extract of *A.l*

Plant species	Initial Weight (g)	Final weight (g)	% Yield
A.L	200	10.2	5.10

Keys: A.L= *Anogeissus leocarpus*

Table 2: Percentage yields of fractions of methanolic Stembark of *A. leiocarpus*

Plant species	Solvent, (PI)	Initial Weight(g)	Final weight(g)	% Yield
Anogeissus leiocarpus	Benzene, (2.8)	10	2.96	29.60
	Chloroform, (4.1)	10	1.10	10.10
	Ethylacetate, (4.4)	10	0.38	03.80
	Aqueous, (9.0)	10	5.10	50.10

Key: PI= Polarity index

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Table 3: Phytochemical components detected in *Anogeisus leiocarpus stem bark* extract

	A. leiocarpus (stem bark)
Tannins	+ +
Saponins	+ +
Anthraquinones	_
Flavonoids	+ + +
Alkaloids	_
Cardiac glycoside	+
Steroids	+

Key: +++: Highly present. ++: moderately present, +: Presence in trace,_:Absent

Table 4. Antiplasmodial activity of Crude methanolic extract of stem bark of *A. leiocapus* and the various fractions: Benzene, Chloroform, Ethylacetate and Aqueous

	Concentration in µg/ml					
	1000	500	100	50	10	1
Plant Extracts		% Mean Parasite Growth ±SD				
Crude M AL	2.6±0.2^{b}	7.40±0.4^{b}	15.79±0.2^{b}	22.10±3.7^{b}	36.50±0.7^{b}	53.40±1.5^{d}
Benzene fraction	6.20±0.2^{c}	11.40±4.0^{ab}	31.00±2.0^{bc}	38.20±5.0^{b}	57.36±1.8^{c}	66.30±2.1^{b}
Chloroform frac	10.62±0.3^{d}	19.70±0.3^{ab}	23.60±0.2^{a}	64.14±1.0^{a}	77.93±1.6^{a}	89.00±4.0^{a}
Ethyacetate frac	19.54±0.2^{a}	24.10±0.5^{a}	38.30±0.9^{c}	43.60±0.2^{b}	49.80±1.0^{d}	65.17±2.1^{c}
Aqueous fraction	21.30±2.0^{a}	24.71±0.1^{a}	24.56±1.4^{a}	54.60±0.9^{a}	71.29±4.4^{a}	82.00±3.8^{a}

Keys: Crude M AL= Crude methanolic extract of *A. leiocarpus*. % mean parasite values followed by the same superscript in the same column are not significantly different at P<0.01. (Duncan Multiple Range Test).

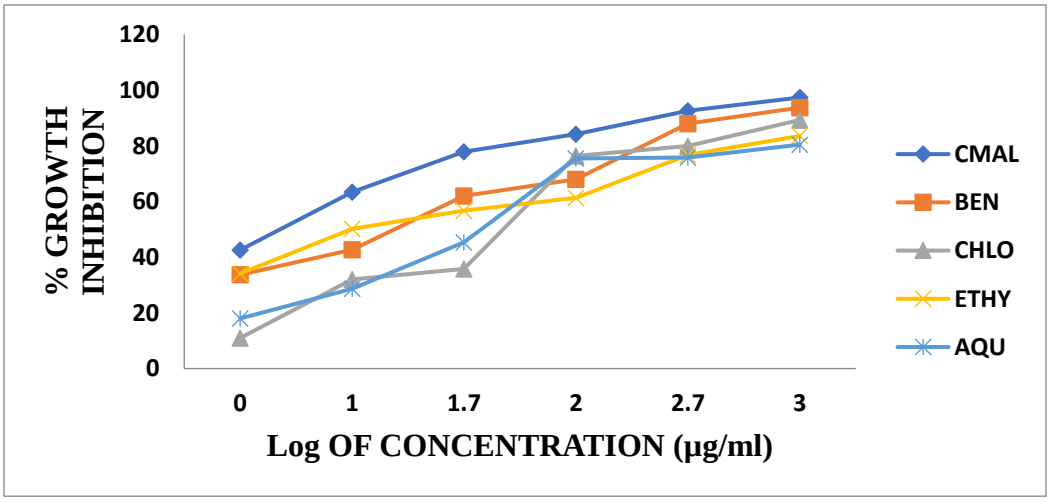

Fig 1. The percentage inhibition of the extracts of *Anogeisus leiocarpus* stembark showing dose-dependent antiplasmodial activity.

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DISCUSSION

The successful extraction of bioactive compounds from plants, according to Parekh *et al* (2005) is largely dependent on the type of solvent used in the extraction procedure. Effective extraction from the dried plant material was achieved using alcohol specifically methanol a slightly non-polar solvent used for herbal active components as this enhances the isolation of both polar and non-polar secondary metabolites (Shuibu *et al.,* 2008; Jordana *et al.,* 2010). *A. leiocarpus* had the highest % yield in the aqueous fraction following slightly by a non-polar solvent i.e. the benzene fraction with 29%, this is in agreement with Parekh *et al.,* (2005) who maintains that these observations can be explained due to the polarity of the compounds extracted. Phytochemical screens determine the overall chemical "fingerprint" or "chemical profile" of a plant extract (Bandaranayake, 2006) Phytochemical test for the seven compounds revealed that the stem bark of *A. Leiocarpus* contains Tanins, Saponins, flavonoids,cardiac glycosides but devoid of Anthraquinones and Alkaloids and this agreess with the findings of Elegami *et al.,* (2002). Previous studies have shown that the composition of Phytochemicals present in the plant has direct correlation with its pharmacological activity (Aremu, 2009). Thus the antiplasmodial properties of these p extracts may be attributed to these phytochemicals identified.

In this study five extracts were evaluated for antiplasmodial activity *in vitro* (Table 3 and Fig1). Among the five extracts, the lowest IC_{50} was observed with the crude methanolic extract of *A. leiocarpus* with IC_{50} of 5.0 µg/ml followed by the Ethylacetate fraction with IC_{50} of 7µg/ml. Benzene fraction had IC_{50} of 9 µg/ml then Chloroform and Aqueous fraction had 17µg/ml and 20µg/ml respectively. Literatures indicate that plant extracts with *in vitro* antiplasmodial activity with IC_{50} less than 10µg/ml as highly active.those with IC_{50} between 10µg/ml and 50µg/ml are classified as moderately active while the extracts with IC_{50} greater than 50 as inactive (Ramazani *et al*., 2010; Omoregie and Sisodia, 2014). Base on this classification the crude methanolic extracts, the benzene fraction and the ethylacetate fractions are all highly active against the parasite. The results is in line with the results of Okpekon *et al.,* (2004) who reported a high antimalarial activity when methanol extract of leaves of the plant was used against *Plasmodium falciparum* strain FCB1 with IC_{50} of 2.60µg/ml. Although this research was carefully prepared and has achieved its aim, there were some unavoidable limitations. First, the RPMI 1640 media was very susceptible to contaminations and even while they were stored in aliquots some had to be discarded due to contaminations and secondly, artefacts were seen on the microscopic slides when determining the parasitemia. Further studies such as the in vivo antiplasmodial assay in animal models should be conducted to investigate how liver metabolism affects the efficacy of the plant extracts.

CONCLUSION

The current studies have shown that the crude methanolic and fractions of stem bark of *A. leiocarpus* had significant activity on the cultured field isolate of *Plasmodium falciparum*. Thus, the results validate the traditional use of this plant as treatment for malaria. The findings will contribute to the ongoing efforts to eliminate malaria. This will be achieved when the bioactive compounds in these extracts are investigated, thus leading to the development of a novel antimalarialdrug.

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Characterization of Chemical and Enzyme Modified Cassava (*Manihot esculenta* Crantz) Starch

[*1]Chindaya M. F., [1]Musa A. D. & [3]Abubakar M. [2]Egwim. C. E.

[1]Biochemistry Department, Ibrahim Badamasi Babangida University, Lapai, Niger State, Nigeria
[2]Biochemistry Department, Federal university of Technology, Minna, Niger State, Nigeria
[3]Department of Biological Sciences, Al-Qalam University Katsina, P.M.B 2137, Katsina State, Nigeria

[*]Correspondence E-mail:marychindaya@yahoo.com; +23407036971248

ABSTRACT

Cassava (*Manihot esculenta* Crantz) is one of the main sources of starch production. The industrial application of cassava starch is limited due to its insolubility in water at room temperature and the increase in density after gelatinization, both of which restrict its functionality. In order to improve on these limitations, this study investigated the preparation of cold-water soluble starch via acetylation, alcohol-alkaline treatment and enzyme hydrolysis by standard methods, while physicochemical characterizations of the native and modified starches were also carried out using analytical methods. There was no significant difference ($p > 0.05$) in the gelatinization temperature and pH of the native and modified starches. The moisture content of the native and modified starches was all within the acceptable limit of 14%. A higher Amylose:amylopectin ratio was observed in the enzyme hydrolyzed starch (0.29±0.00), when compared to the acetylated (0.17±0.00), native (0.11±0.02) and alkaline-alcohol starch (0.08±0.00). Solubility in cold and hot water was higher in alkaline alcohol starch (94.68±1.15 and 97.19±1.15) followed by enzyme hydrolyzed starch (82.74±1.73 and 89.53±1.73) while the native and acetylated starch ranged between 1.42±0.12 to 3.53±0.06. Significant morphological and chemical structural changes were recorded in the Fourier Transformed Infrared spectroscopy (FTIR) result of the enzyme hydrolyzed starch. The thermal stability of the starches was examined using Thermal Gravimetric Analysis (TGA) and Differential Thermal Gravimetric Analysis (DTGA), while the structural change was examined using X-ray Diffraction (XRD). This study demonstrates that modification of native cassava starch could be useful in the enhancement of the physicochemical and chemical characteristics of starch to meet requirements for industrial applications.

Keywords: Acetylation, Alcohol-alkaline, Enzyme hydrolysis, Cassava (*Manihot esculenta* Crantz), Starch

INTRODUCTION

Starch is one of the major storage form of carbohydrate in plants, it is present in seeds, leaves, fruits, flowers, different types of stems and roots. Starch serves as source of carbon and energy for plants (Smith, 2001). The chloroplasts of green leaves and amyloplasts, organelles responsible for the starch reserve synthesis of cereals and tubers, synthesize it in plants during

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the dark phase of photosynthesis (Smith, 2001; Tester *et al*., 2004). The plant produces starch in the chloroplast at different times of the day and at different rates. The starch reserves formed by amyloplasts, on the other hand, are deposited over several days, if not weeks. During seed germination, fruit maturation, and tuber sprouting, starch is processed and cyclically mobilized (Alcázar-alay, *et al*., 2015). The endosperm is the primary site of starch synthesis and storage. The chlorophyll synthesizes it in the dark phase of photosynthesis in plants. The main sources of starch are cereals (40 to 90 %), roots (30 to 70 %), tubers (65 to 85 %), legumes (25 to 50 %) and about 70 percent of starch by dry weight is found in some immature fruits like bananas or mangos (Santana and Meireles, 2014).

Cassava is the third largest source of carbohydrate with Africa being the largest centre of production (Adenle *et al*., 2012). It is used as a staple food of many nations (Viduranga, 2018). It is eaten raw (sweet cassava) or cooked as starch flour, garri, and a host of other dishes in tropical countries like Nigeria (Tonukari, 2004). Strong paste viscosity, high freeze-thaw stability, high paste clarity (Gunorubon, 2012), excellent thickening characteristics, high purity level, good textural characteristics, cheaply accessible, and contains high concentration of starch are some of the notable properties of cassava for starch production over other sources of starch (Masamba *et al*., 2001). Raw cassava starch, on the other hand, has restricted industrial applications due to its insolubility in water at room temperature; additionally, the density of starch increases dramatically after gelatinization, limiting its versatility (Shi *et al*., 2011). As a result, the use of modified cassava starch is needed. Food processors have developed adaptable tools to meet the diverse needs of a number of food systems over time. The complex semi-crystalline structure of native starch necessitates the use of energy to gelatinize it (Jane, 1992). To alter the granular structure and transform native starch into cold water-soluble starch, physical modification has been used alone or in combination with chemical reaction. “This form of starch modification is new, and it's been used in microwave-cooked and instant foods like puddings, instant fillings, sauces, and dry mixtures that can be reconstituted with cold or ambient temperature liquids (National Starch and Chemical Corporation Brochure, 1988). Eastman and Moore (1984) were the first to manufacture cold-water soluble granular corn starch for gelled food compositions by subjecting slurried granular corn starch to high temperature and pressure in selected aqueous alcohols. Later, Rajagopalan and Seib (1992) prepared granular cold-water-soluble starches by heating starch slurry in a water–polyhydric alcohol mixture at ambient temperature. Jane and Seib (1991) patented a process for making granular cold water swelling/soluble starches using alcoholic-alkali treatments at the same time. This method works with waxy, high amylose, tuber, and regular starches. Chen and Jane (1994) used an alcoholic-alkaline treatment on starches to create granular cold-water-soluble corn, waxy corn, and high amylase corn starches. Then, using the alcoholic-alkaline form, granular cold-water-soluble banana starch was prepared and studied (Bello-Pérez *et al*., 2000). *Choi et al.* (2017). conducted the most recent research on granular cold water-soluble potato starch

Up to 70% of harvested cassava tubers in developing countries like Nigeria are processed

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into a toasted granule known as garri. Smallholder farmers/processors dominate the garri development industry in Nigeria, using simple implements for processing (Kolawole, 2014). Significant amount of native cassava starch is been wasted or not properly disposed during traditional garri production and this has led to soil and air pollution and loss potential revenue source to the economy. It is in line with bringing solution to these challenges that this study seeks the utilization of both chemical and enzymatic and modification of native cassava starch; a by-product in the production of garri from cassava tuber which is usually discarded especially in the rural areas as a raw material for industrial applications.

METHODOLOGY

Materials

Cassava starch was purchased from a local market at Lapai, Niger State. The chemicals used were sodium hydroxide (NaOH), Hydrogen chloride (HCl), potassium hydroxide (KOH), acetic anhydride, distilled water, iodine solution and phenolphthalein All chemicals used were of analytical grade without any further purification.

Methods

Preparation of alcohol-alkaline starch

To make alcohol-alkaline cassava starch, the method of Chen and Jane (1994) was modified slightly. At 35°C, 50 g of starch was suspended in 200 g of ethanol and mechanically stirred. 30 g, 45 g, 60 g, and 75 g of 3 M NaOH solution were added in various quantities, with effective concentrations of 0.39 M, 0.55 M, 0.69 M, and 0.82 M, respectively. With gentle stirring, the mixture was held for 15 minutes. A further 200 g of ethanol was slowly added, and the mixture was stirred for another 10 minutes. The resulting slurry was held at room temperature (25 ^{o}C) for about 30 minutes to allow the starch granules to settle. The starch was washed with fresh ethanol solution after the supernatant was decanted. The starch was then resuspended in ethanol and neutralized in absolute ethanol with 3 M HCl. The starch was then dehydrated with absolute ethanol after being washed with 60 % and 95 % ethanol solutions. The starch was dried in the oven for 3 hours at 80 degrees Celsius. The dried starch was carefully ground to a fine powder (250 m) using a bench top grinder (Micro Universal Bench Top Grinder, Retsch, Germany) and stored at room temperature.

Preparation of Acetylated Starch (AS)

The system proposed by Sathe and Salunkhe in 1981 was used. 100 g native cassava starch was distributed in 500 mL distilled water and magnetically stirred for 20 minutes. Using 1.0 M NaOH, the pH of the obtained slurry was adjusted to 8.0. The acetic anhydride (10.2 g) was slowly applied to the mixture, keeping the pH between 8.0 and 8.5. Following the addition of acetic anhydride, the reaction continued for 5 minutes. Using 0.5 M HCl, the pH of the slurry was eventually changed to 4.5. It was filtered, and then washed four times with distilled water before being air dried for 48 hours at 30 at 50.

Preparation of Enzyme-hydrolyzed Starch (EHS)

The method defined by Mc Pherson and Seib, (1997). A 40 percent w/v aqueous suspension of starch was added to “a double-walled reaction vessel at 6.5 pH and temperature 0f 85 ^{o}C. With the dosed enzyme (15 mL of -amylase extracted from sprouting maize) and constant stirring, the reaction was allowed to continue for 1, 2, 3, 4, 5, and 6 hours”.After that, the enzyme's activity

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was stopped by lowering the pH to 2.5 with 0.1 N HCl. The reaction medium was then neutralized by adding 0.1 N NaOH to raise the pH to 7. After settling, the resulting product was isolated "from the reaction medium. It was washed with purified water several times before being dehydrated with 100 mL of ethanol (95 % v/v). After decanting the ethanol, the dehydrated product was air dried and powdered". The initial and final weights were used to measure the percentage yield.

$$\textbf{\textit{Percentage yield}} = \frac{\text{Wt of starch before hydrolysis} - \text{Wt of starch after hydrolysis}}{\text{Wt of starch before hydrolysis}} \text{ x } 100$$

Characterization

The amylose content of the starches was determined using Williams *et al.* (1970) process. The iodine test was carried out using the method mentioned in (B.P. 2015). Acidity test, pH determination, solubility, moisture content, the swelling capacity was determined using the method described by Daramola *et al.* (2006), viscosity, the gelatinization temperature was determined by the method of Attama *et al.* (2003).

RESULTS AND DISCUSSION

Physicochemical characterization of native and modified starch (NMS)

Table .1 shows the results of physicochemical properties of native and modified starch. The gelatinization temperature, pH, moisture content, solubility in hot water, and viscosity at 6 rpm of the native and changed starch were not significantly different (P>0.05), whereas the iodine and acid test, swelling potential, amylose/amylopectin ratio, solubility in cold water, and viscosity at 30 and 60 rpm were significantly different (P>0.05).

Table 1: Physicochemical characterization of native starch, acetylated starch, alcohol-alkaline modified starch and enzyme modified starch

	Native starch	Acetylated starch	Alcohol alkaline starch	Enzyme starch
Gelatinization temperature (^oC)	102.00±2.31^a	100.00±1.15^a	96.67±1.76^a	100.00±1.73^a
pH	8.14±0.20^a	8.54±0.04^a	11.47±0.03^b	8.15±0.05^a
Iodine test	0.32±0.01^a	0.63±0.01^c	0.55±0.03^b	0.69±0.03^c
Acidity Test	0.27±0.03^b	0.27±0.03^b	0.00±0.00^a	1.00±0.10^c
Moisture content	13.24±0.69^a	12.43±0.11^a	14.74±0.69^a	13.79±0.69^a
Swelling capacity	0.05±0.01^a	0.047±0.01^a	1.01±0.12^b	1.01±0.06^b
Amylose/Amylopectin ratio	0.11±0.02^b	0.17±0.00^c	0.08±0.00^a	0.29±0.00^d
Solubility in cold water	2.27±0.11^a	1.89±0.06^a	94.68±1.15^c	82.74±1.73^b
Solubility in hot water	1.42±0.12^a	3.53±0.06^a	97.19±1.15^c	89.53±1.73^b
Intrinsic viscosity at 6rpm	20126.57±205.04^a	18892.14±35127^a	20176.43±106.17^a	18010.57±157.56^a
Intrinsic viscosity at 30rpm	6570.49±17.99^b	5779.66±23.46^a	5515.89±245.22^a	7502.54±43.08^c
Intrinsic viscosity at 60rpm	5256.64±37.39^b	5307.59±19.32^b	5005.55±65.08^a	5606.14±15.26^c

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Values are expressed in mean ± standard error of mean of triplicate determinations. Values with the same superscript on the same row have no significant difference at $p<0.05$

The food and non-food industries are currently grappling with the challenge of supplying high-quality foods and non-food items. The quality of a product like starch, which is used as a simple raw material in most industries, can only be determined by the end use. This demand is based on the functional and physicochemical properties of the product (Aviara *et al.,* 2010; Garsetti *et al.,* 2005). To fulfill industrial needs and requirements, native starch must be modified to enhance the starch's physicochemical and functional properties.

For native, acetylated, alcohol-alkaline, and enzyme modified starch, the gelatinization temperatures were 102°C, 100°C, 96.67°C, and 100°C, respectively. These values are within the set level of gelatinization temperature for both native and modified starch, which is between 96 and 100 ^{o}C (Alcázar-Alay *et al*., 2015). The gel temperature for both native and modified starch in this analysis is higher than Kumoro *et al*. (2010)'s gel temperature of 91.4 ^{o}C for native cassava starch and 83.9 ^{o}C for acetylated cassava starch, as well as 77 ^{o}C gel temperature for fumaric modified cassava starch (Akpa *et al*., 2012). In this study, the small difference in gelatinization temperature between native starch and alcohol-alkaline modified starch could be attributed to a slight structural rearrangement of the starch molecule. When starch is heated in the presence of too much water, it undergoes a process called gelatinization. This phase is characterized by an interval temperature corresponding to each starch species (Alcázar-Alay *et al*., 2015). Gelatinization takes place when water diffuses into the granule resulting in substantial swelling due to hydration of the amorphous phase leading to loss of crystallinity and molecular order (Jiménez *et al*., 2012). The starch structure becomes more random as a result of the water penetration (Gunorubon and Kekpugile, 2014). As predicted, the stronger the intermolecular bond between the starch molecules, the more heat is needed to break it, and thus the higher the gel temperature (Singh-Sodhi and Singh, 2005). The aim of the cross-linking treatment is to add intra and intermolecular bonds to the starch granule at random locations in order to stabilize and reinforce the granule (Gunorubon and Kekpugile, 2014). As a result of the cross linking, the gel temperature of changed starches rises. The result of the gelatinization temperature in this study shows that the modification of the native starch was not effective and did not add further cross linkage, hence retention of the gelatinization temperature.

The moisture content of the native and adjusted starch samples in this study ranged from 12.43 to 14.74 percent, which is below the permissible moisture content limit of 14 % in starch flour (Azeh *et al*., 2018). The moisture content in this study is consistent with Kumoro *et al*. (2010)'s report of 9.6 to 16 % moisture content for cassava native starch, as well as Akpa *et al.* (2012) moisture's content of 11.75 % for sodium acetate modified cassava starch and 15 percent for ammonium phosphate modified cassava starch. The amount of moisture in a food sample is important because it indicates the food's storage shelf-life (Azeh *et al.,* 2018). The current findings revealed that all samples had moisture content (percentage) that was within the maximum allowable range. High moisture content encourages microorganism formation,

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which can result in odors and off-flavors (Gunorubon and Kekpugile, 2014). Since both the native and changed starches in this study have low moisture content, they will be easier to store and will last longer.

The pH value of any "substance is the degree of acidity or alkalinity of that substance. Modified starches have been reported to be less likely to break down with extended cooking times and possess increased acidity or severe shear" (Langan, 1986). The result from table 1 shows that there is no significant difference (P>0.05) between the native starch and the acetylated and enzyme modified starch samples however the alcohol alkaline modified starch shows an increase in pH of 11.47 which is significantly different from the native starch. The alkaline and alcohol modifications may have caused the pH to rise. It is critical that the pH of starch tends toward neutrality in order to maximize its use in industries where product pH changes are undesirable. The high pH in this study, which ranged from 8.14 to 11.47, boosted carboxyl content and increased water solubility while lowering swelling strength (Isti *et al.,* 2018).

The result of amylose/amylopectin ratio was significantly different (p>0.05) across all starch samples. The lowest ratio (0.08±0.00) was recorded for alkaline alcohol starch, with enzyme modified starch having the highest ratio (0.29±0.00) (Table 1). This finding aligns with the report of Nuwamanya *et al.* (2010), who observed a similar result in native and modified cassava starch samples. Low amylose content is associated with increased relative crystallinity of starch due to reduced amorphous regions within the starch granule, so amylose content is significant in almost all starches (Tukomane *et al*., 2007). The aggregation of amylose, which acts as nuclei during the phase of amylopectin retrogradation, affects the retrogradation properties of starch, with high amylose starches having increased retrogradation tendencies (RodrguezSandoval *et al*., 2008). The enzyme's effect on starch has resulted in a substantial increase in the amyloses/amylopectin ratio of native starch from 0.11 to 0.29. The effect of amylose on pasting properties is due to the leaching of the amylopectin network during heating into the solution, which affects the viscoelastic properties of the starch (Charles *et al*., 2004). Due to the prolonged movement of amylose out of the amylopectin network during the gelatinization of starch, which leads to prolonged swelling of starch granules (Moorthy, 2002), an increase in the pasting temperature (Novel-Cen and Betancur-Ancona, 2005) is required to form a starch paste (Novel-Cen and Betancur-Ancona, 2005). The hydrophilic properties (ability to absorb or bind with water molecules) of the enzyme modified starch increase as the amylose/amylopectin ratio of the enzyme modified starch increases in this study due to the enzyme treatment.

The solubility of starches is affected by amylose content, lipid content, granule structure, the amylose-lipid complex, and amylose leaching (Sandhu and Singh, 2007). Temperature and other factors influence the solubility of starch. Most starch becomes more soluble as the temperature rises (Salcedo-Mendoza *et al*., 2017). In cold and hot water, alkaline alcohol starch has the highest solubility, followed by enzyme modified starch, and native and acetylated starch has the lowest solubility. The solubility of alkaline alcohol and enzyme-modified starch may be due to the functional group present in this starch, which has a higher affinity for water than acetylated starch (Salcedo-Mendoza *et al*., 2017). The starch's

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solubility is also due to the degraded hydrolysis of Amylose (amorphous region) in cassava starch, which results in better water absorption and solubilization of the starch granules. Furthermore, the decrease in amylose content and rise in peak maximum viscosity are closely related to the shift in solubility of modified starch relative to native starch. This shift is due to the granule's morphological and structural changes as a result of hydrolysis (Shariffa *et al.,* 2009).

While there was no significant difference ($p>0.05$) in the swelling potential of native and acetylated starch, there was a significant difference ($p<0.05$) between the native starch and the alkaline alcohol and enzyme modified starches. In comparison to the native and acetylated starch samples, the swelling potential of alkaline alcohol and enzyme modified starch was higher (Table 1). If the temperature rises, starch granules absorb more water, causing swelling (Charles *et al*., 2007). The swelling power of starches from various botanical roots, which have different swelling powers at different temperatures, is a significant parameter (Moorthy, 2002; Charles *et al*., 2007). It also has an effect on the quality of cassava roots for consumption as well as the use of starch in a variety of industrial applications (Moorthy, 2002). High swelling capacity leads to increased digestibility and the ability to use starch in solution, implying better dietary properties and the use of starch in a variety of dietary applications (Nuwamanya *et al*., 2010). Swelling potential refers to a starch's ability to store water; it's a critical parameter to remember in food processing (baking) (Isit *et al.,* 2018). Table 1 shows that both alcohol-alkaline and enzyme modified starch have a large increase in their water storage ability with 1.01 swelling capacity compared to 0.05 swelling capacity of native starch, but acetylated modified starch has a decrease in swelling capacity with 0.047. The swelling potential of both alcohol-alkaline and enzyme modified starch in this study is similar to the 1.23 recorded by Isit *et al*. (2018) for sodium acetate modified cassava starch, but it is higher than the acetylated modified starch. Both alcohol-alkaline and enzyme-modified starch would be more suitable for industrial use in terms of swelling ability (baking process).

A substance's viscosity is known as its resistance to flow, and it increases as temperature drops or decreases as temperature rises (Coulson and Richardson, 2004). A chemical modification may be used to increase or decrease the viscosity of starch paste (Gunorubon and Kekpugile, 2014). It has been stated that increasing cross-linking levels reduces or even prevents granule swelling, resulting in a decrease in viscosity (Gunorubon and Kekpugile, 2014). Table 1 shows the viscosity activity of native and modified starch samples at 6, 30, and 60 rpm. The results show that the viscosity of the starch samples did not vary significantly ($p>0.05$) at 6 rpm, but at 30 and 60 rpm, the enzyme modified starch had the highest viscosity as compared to the other starch samples. At 6 rpm, enzyme modified starch has the best viscosity improvement (reduction), whereas alcohol-alkaline modified starch has the worst viscosity. At both 30 and 60 rpm, alcohol-alkaline modified starch has the best viscosity change (viscosity reduction), while enzyme modified starch has the worst viscosity. Alcohol-alkaline modified starch would be better suited to industrial applications (baking process) that need viscosity improvement.

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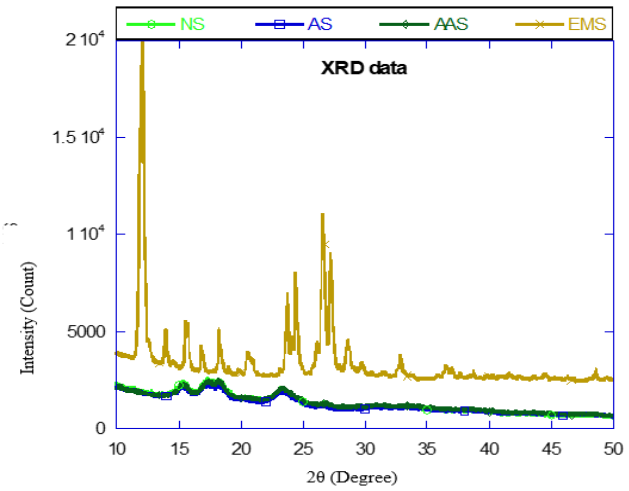

Figure 1: X-ray Diffraction Pattern of NMS

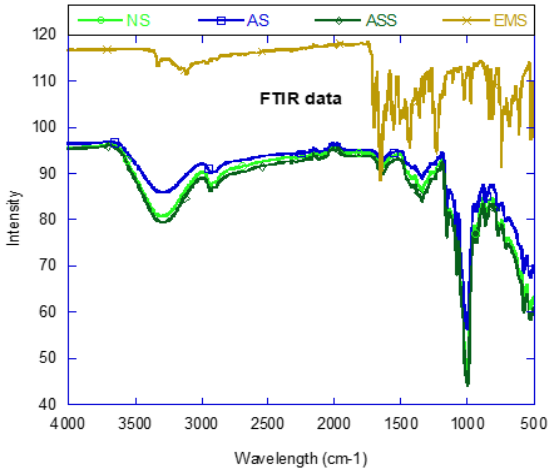

Figure 2: FTIR spectra of the NMS

Key: NS = Native starch, AS = Acetylated starch, AAS= Alcohol alkaline starch and ES = Enzyme starch

The X-ray diffraction patterns for NS, AS, and AAS are very similar (Figure 1), indicating that the modification has no effect on the X-ray pattern for AS and AAS. The broad peaks indicate that the crystalline particles are still surrounded by large amorphous material, indicating an amorphous behavior. That means the samples are mostly amorphous and have a low crystallinity percentage. Both native and phosphorylated cassava starch showed a similar X-ray pattern, according to Maria and Elevina (2003). However, the XRD pattern of enzyme modified starch (EMS) is different, with sharp peaks at 2 equals 120, 140, 160, 170, 220, 240, 250, 270, 280, and 330. These various peaks represent the various crystalline particles formed by the enzymatic modification. The presence of crystalline particles in the sample is indicated by the sharp peaks. The increase in the number of sharp peaks shows that the EMS is becoming more crystallin. In general, the EMS was more successful at increasing the crystallinity of the native starch (NS). By altering or breaking down the amorphous region, the enzyme was able to reduce the amorphous region in the sample and raise the crystalline percentage.

The FTIR results show that native starch (NS), acetylated starch (AS), and alcohol-alkaline starch (AAS) all have the same spectra pattern, which corresponds to an extreme band of 700 cm associated with the starch's water molecule deformation (Figure 2). The FTIR spectrum for the enzyme modified starch (EMS) sample, on the other hand, revealed a shift in the morphological and chemical structure of the sample, with peaks at 4000 - 3200 cm suggesting the existence of the hydroxyl (OH) functional group. This suggests that the enzyme targets the OH group through the oxygen atom, releasing the hydrogen atom and creating oxygen-enzyme (O-E) functionality, which may explain the increased peaks seen between 1600 and 1000 cm.

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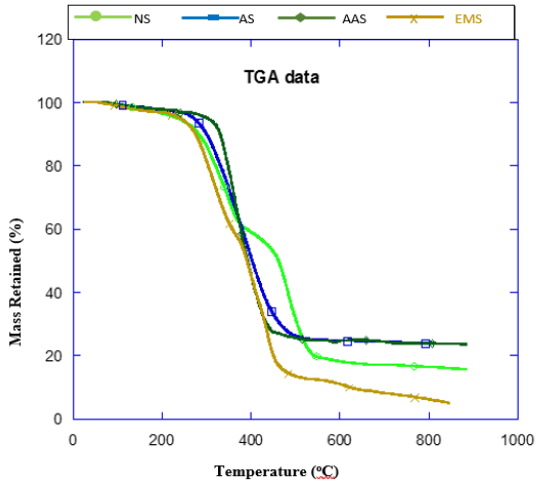

Figure 3: TGA thermogram of NMF

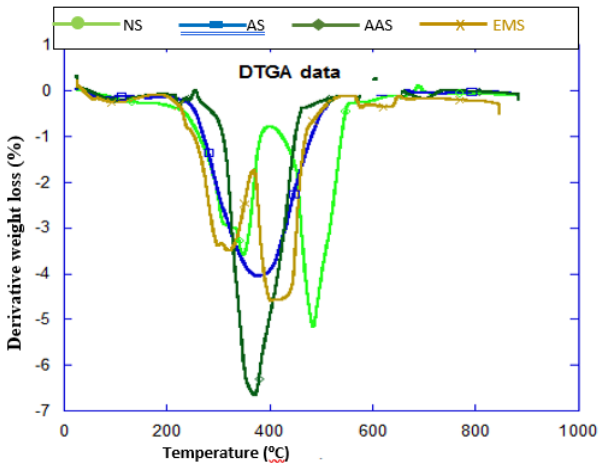

Figure 4: DTGA thermogram of NMF

Key: NS = Native starch, AS = Acetylated starch, AAS= Alcohol alkaline starch and ES = Enzyme starch

The TGA analysis (Figure 3) divides the thermal activity of native and modified starch into four stages of decomposition (Jeiffer *et al.,* 2018). The first stage of decomposition was observed between 100 and 160 °C, with both native and modified starches losing around 2% of their weight due to evaporation of the moisture content. The second stage of decomposition occurred between 290 and 350 degrees Celsius, with a 10% weight loss due to dehydration of the polymer chain. The elimination of carbohydrates is responsible for the third stage of decomposition. At 430 °C, NA has about 60% weight retention, AS has about 38% weight retention, AAS has about 30 % weight retention, and EMS has about 20% weight retention. The final decomposition of NS occurred at 570 percent with 20% weight retained, that of AS and AAS occurred at 450 °C with about 20% weight retained, and that of EMS occurred at about 800 °C with 10% weight retained. At the fourth stage, EMS has the highest thermal stability, followed by NA, with both AS and AAS having a very similar thermal stability range. This is in line with Jeiffer *et al.* (2018) findings that the amylose/amylopectin ratio increases the starch's thermal stability.

Figure 4 depicts the DTGA study of native and modified starch samples. Native starch (NS) produced two peaks on the thermogram, suggesting two distinct morphological stages during TGA study, similar to enzyme modified starch (EMS). During TGA study, however, acetylated starch (AS) and alcohol alkaline starch (AAS) samples each gave a single peak, suggesting uniformity in the morphological structure.

CONCLUSION

In conclusion, this research shows that modifying native starch can improve the physicochemical and functional properties of native cassava starch without changing its granular morphology. The solubility, amylose/amylopectin ratio, and viscosity of the native and modified starches vary significantly ($p>0.05$) according to physicochemical analysis. When compared to AS, both EMS and AAS have improved physicochemical properties. The chemical alteration had no impact on the granule

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morphology or surface functionalities of NS, AS, or AAS. XRD, FTIR, and TGA all confirmed this. However, when EMX was tested using XRD, FTIR, and TGA, it showed a different behavior, which could be due to the modification.

Conflict of Interest

There authors declared that there is no conflict of interest

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Detection of Genetically Modified Maize and Soybean in Some Food Products from selected Super and Local markets in Minna Metropolis, Niger state, Nigeria

[1,5]Musa A. D., [1,5]Ganiyu S. A., [2,5]Osuji C., [1,3,4]Egwim C. E. & *[1,2,5]Iloh A. C.

[1]Department of Biochemistry, Faculty of Natural sciences Ibrahim Badamasi Babagida University 911101 Lapai, Niger State.
[2]Biodiversity and Climate Research Laboratory, Biotechnology Advanced Research Centre, Sheda Science and Technology Complex, KM 32 Abuja Lokoja Express Way, Sheda Abuja.
[3]Biotechnology Centre, Federal University of Technology, Minna, Niger State.
[4]Department of Biochemistry, Federal University of Technology, Minna, Niger State.
[5]Biodiversity Education and Resource Centre (BERC), Abuja. Nigeria.

*Correspondence: Email: ac.iloh@shestco.org.ng, +2348036005958

ABSTRACT

Polymerase Chain Reaction (PCR) based method have been found to be an effective technique used in the detection of specific genes in genetically modified crops Hence, this study has adapted the technique in detecting the Cry1Ab maize gene and EPSPS soya bean gene in 72 samples made up of maize seeds and maize based products as well as Soya bean seeds and Soy based products from randomly selected five Super and five Local markets (Supermarkets: A, B, C, D, E. Local Markets: TM, KM, GM, BM, NM) in Minna. DNA yield (ng/µl sample) from samples ranged between 10.12±0.41 to 3389.81±2.37 indicates there is significance different while DNA purity (A260: A280) ranged between 1.20±1.36 to 2.08±0.04. 37.5% of all samples screened made up of 16 maize and 11 soya-based samples tested positive. GMO specific gene were found to be present in 31.5% and 52.4% of the total number of maize products and all soy-based products respectively. Out of 25 samples of maize seeds, 12 tested positive to the cry1Ab gene, indicating the presence of a genetically modified gene. Exactly 3 out of 10 samples randomly selected soya bean tested positive for the herbicide tolerance EPSPS gene in soya thus indicating the presence of a genetically modified gene. The highest number of positive samples was recorded in market TM while Markets KM and Super Markets A and B followed with four (4) samples each. The least record of samples was noticed in market BM, NM and supermarket D. Interestingly, in supermarket C, No GM product was recorded. The findings indicate that products made with GMO materials have entered the food chain at an alarming scale and requires urgent need of regulatory agencies assigned for GMO detection in Niger State, Nigeria.

Key words: Genetically modified organism (GMO), GM maize, GM soya, polymerase chain reaction (PCR), Niger State.

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INTRODUCTION

Many schools of thought have argued that genetic engineering is the technology for future especially in sub-Saharan Africa where there is agricultural and developmental pressure, despite numerous internal and external funding attempts (Arieff *et al.,* 2010). This according to Brambila-Macias and Massa 2010 is been complicated by issues around Population growth, increased vulnerability to food production as well as the increasing climate variability.

Currently, many different organisms are being used in today's genetic engineering research and development, including plants, trees, animals, insects, bacteria and viruses. In the agricultural sector, plants and crops are engineered to express a resistance to herbicides and specific pests (Hefferon 2015). However, the production of Genetically Modified Organisms (GMOs) raises the possibility of human health, environment and economic problems, including unanticipated allergic responses, the spread of pest resistance or herbicide tolerance to wild plants, inadvertent toxicity to benign wildlife, and increasing control of agriculture by biotechnology corporations (Lassen *et al.,* 2002)

This has led to the enforcement of labeling laws which, for example, is obligatory in the European Union (Alderborn *et al.,* 2010). Hence, platforms for the development of methodologies for GMO detection and quantification have risen all over the world in order to reveal the adventurous presence of GMOs in different matrixes and to comply with the respective regulations of labelling (Chaouachi *et al.,* 2013). The future of African genetically engineered foods and crops will rely heavily on the decisions taken by the African government to control this technology especially in GM detection in food products (Paarlberg *et al.,* 2010). As reported by Vidhya *et al.* (2011), The infiltration into the market of unapproved and spurious varieties of GM crop seeds and food products has been the cause of disputes over their acceptance among farmers because planting of these varieties leads to crop failures and enormous commercial losses. Hence a need to conduct well organized GM testing and detection that will identify GMOs and reveal and possibly stop the infiltration of spurious GM varieties into the market (Vidhya *et al.,* 2011). Again, a précised testing method will also inform country legislation that will guarantee freedom of choice for consumers (Vidhya *et al.,* 2011).

There is a need to enlighten the general public on what GMO and GM derived food products which can also be done when detection and traceability protocols are in place to help guide acceptance as a source of food.

As long as the genetically modified crops are gaining attention globally, their proper approval and commercialization need accurate and reliable diagnostic methods for the transgenic content (Salisu, *et al.,* 2017). Exponential amplification by the Polymerase Chain Reaction (PCR) remains a central step in molecular methods of GMO detection and quantification. In this study, we attempt the adaptation of the PCR based technique, targeting GMO specific sequences; 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) gene for herbicide tolerance in soybean and Bt resistance gene (Cry1Ab) in maize, to screen for the presence of GM material in processed and unprocessed maize and soya products in selected supermarkets and local markets across Minna metropolis, Niger state.

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METHODOLOGY

Sample collection

Finished and local food products obtained from maize and soya were randomly obtained from 10 selected supermarkets and local markets across Minna, Niger state. Food products such as corn flour, corn flakes, corn starch, custard powder, biscuits, soya plus, soya cheese locally made, pap, soya and maize seeds. The products were obtained from Five (5) supermarket stores tagged (A), (B), (C), (D) and (E). Samples were also collected from Five (5) local markets (TM, KM, GM, BM and NM) across Minna metropolis. Products that are generally in use by the public are strategically targeted for sampling. A total of 72 samples that were simply randomized were collected for this study (Table 1). All products were properly labeled for identification.

Table 1. Distribution of Sample Collection

	Location										
	Supermarket stores					Local Market					72
Sample type	A	B	C	D	E	TM	KM	GM	BM	NM	Total
Corn based biscuits	2	1	2	1	1	0	0	0	0	0	7
Corn/custard	0	1	0	0	1	0	0	0	0	0	2
Corn pap	0	0	0	0	0	1	0	0	0	0	2
Corn flour	1	1	0	0	0	0	0	0	0	0	2
Cornflakes/popcorn	2	1	0	3	1	0	0	0	0	0	7
Corn meals/snacks	1	1	2	1	0	0	0	0	0	0	5
Maize seeds	0	0	0	0	0	5	5	5	5	5	25
Soya based biscuits	0	2	1	2	0	0	0	0	0	0	5
Soya based drinks	1	0	1	0	0	0	0	0	0	0	2
Soya beverages /Cheese	1	0	0	0	0	1	0	0	0	0	2
Soya classic spread	0	0	0	0	2	0	0	0	0	0	2
Soya cooking oil	1	0	0	0	0	0	0	0	0	0	1
Soya seeds	0	0	0	0	0	2	2	2	2	2	10
Sweet corn	0	0	0	0	1	0	0	0	0	0	1

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DNA Extraction and Purification

The CTAB method described by Doyle and Doyle (1987) was used for genomic DNA extraction with slight modifications. 500 µl CTAB buffer was used for initial incubation, 750 µl isopropanol for DNA precipitation and 200 µl 70% ethanol for the two washing steps. Extracted DNA samples were finally dissolved in 200 µl TE buffer supplemented with 10 mg/ml RNase (2µl). DNA purity and quantified the extracted DNA samples was measured with the NanoDrop™ One/One C (ThermoFisher Scientific). DNA samples were stored at -20^{o}C until used for PCR.

Primer sequences

Herbicide tolerant EPSPS gene in soybean and pest resistance GM trait (Cry1Ab gene) were the two GMO specific DNA markers targeted in this study in line with previous studies (Datukishvili *et al.,* 2015). The primers and details of GMO sequences amplified are summarized in Table 2. Primers were purchased in lyophilized form from Eurofins MWG Operon (Germany) and reconstituted as directed by the manufacturer.

Table 2. List of PCR primers.

	Oligonucleotide primers		
Target Applicon bp	**Primers**	**Sequences**	
EPSPS gene	**EPSPS Forward**	**ACCGGCCTCATCCTGACGCT**	**256**
	EPSPS Revers	**CCGAGAGGCGGTCGCTTTCC**	
Cry1Ab gene	**Cry1 Forward**	**GCACCTCCGTGGTGAAGGGC**	**258**
	Cry1 Reverse	**AACCCACGGTGCGGAAGCTG**	

PCR detection of marker sequences

The Amplification of 5-enolpyruvylshikimate-3-phosphate synthase (*EPSPS*) gene for herbicide tolerance in soybeans and *Bt* resistance gene (*cry1Ab*) in maize. Was carried out in a PCR reaction mix (20 µl) which contained 12.5 µl OneTaq Quick Load PCR Master Mix (New England Biolabs; 20mM TrisHCL, 1.8mM $MgCl_2$, 200 µM dNTPs), 1 µl Taq DNA polymerase (25 units/ml), 0.5 µl bovine serum albumin (10 mg/ml), 5.0 µl template DNA and 2.0 µl each of forward and reverse primers. Amplification was carried out in triplicates using the Peltier- based Thermal Cycler (LABKITS, Hong Kong) themocycler. The PCR cycling condition involved an initial denaturation of 90^{o}C for 3 minutes, followed by 40 cycles of denaturation of 90 ^{o}C at 30 s, annealing at 65 ^{o}C for 30 s, elongation at 72 ^{o}C for 35 s and a final extension step at 72 ^{o}C for 5 mins, for EPSPS genes. Cry1Ab PCR cycling conditions were slightly different as initial denaturation was at 95 ^{o}C for 3 mins, followed by

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50 cycles of denaturation at 95 °C for 25 s, annealing at 62 °C for 30 s, elongation at 72 °C for 45 s and a final extension step at 72 °C for 7mins (Table 3).

Table 3. PCR conditions used for the detection of specific GM markers

Step	EPSPS	Cry1Ab
Initial denaturation	95 °C/3 mins	95 °C/3 mins
Denaturation	95 °C/30 s	95 °C/25 s
Annealing	65 °C/30 s	65 °C/30 s
Extension	72 °C/35 s	72 °C/45 s
Final Extension	72 °C/5 min	72 °C/7 min
Cycles	40	50

Gel electrophoresis

At 140v for 30 minutes, PCR products were checked for amplification of target genes using agarose gel (1%) electrophoresis. DNA bands were captured using the gel documentation system (Edvotek USA). On the gel documentation system, all of the results were visually analyzed.

Statistical analyses

Frequency distribution of GM markers across sample area was computed using IBM SPSS version 22.

RESULTS AND DISCUSSION

DNA was successfully isolated from products made from Maize and Soybean as well as maize and soybean seeds. DNA yield and DNA purity is represented in t**able 4**. The results showed that DNA yield (ng/µl sample) ranged between 10.12±0.41and 3389.81±2.37 while DNA purity (A260: A280) ranged between 1.20±1.36 and 2.08±0.04. The highest yield of DNA was noticed in the Soya seeds samples gotten from BM market (3389.81±2.37) while the lowest DNA yield was seen in Corn pap (10.12±0.41). Maize seed samples from the MT market had the best Purity (2.08±0.04) while the lowest purity was recorded in Maize samples gotten from KM market (1.16±0.86). Gel electrophoresis represented of total genomic DNA and PCR products for the extracted DNA is represented in plates **A to F.** Plates **A** and **B** Represents genomic DNA extraction for all 72 samples. **C** and **D** PCR products showing positive/negative for the cry1Ab gene in maize. **E** and **F** PCR products showing positive/negative for the EPSPS gene in soya beans. Percentage number of samples observed to show positive results for the detection of GMO specific genes is represented in **table 5**. 37.5% of all samples screened made up of 16 maize and 11 soya-based samples tested positive. 31.5% of the total number of maize products were positive to a GMO specific gene and 52.4% of all soy-based products.

The total number of positive samples to both cry1Ab gene in maize and EPSPS gene in soya beans is represented in F**igure 1.** Results showed that 11 samples of maize (Corn) seeds were positive to the test gene, 3 samples of Soya bean were positive while 1 of each other food products were positive for either the cry1Ab gene or the EPSPS gene. **Figure 2** represents the number of positive in maize food products including corn based biscuits, corn pap, sweet corn, corn flour, corn custard, corn flakes, corn seed and corn meal/snacks with eleven (11) maize (corn) seeds testing positive for cry1Ab gene while 1 sample each of corn based biscuits,

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corn pap, sweet corn, corn flour, corn custard, corn flakes, corn seed and corn meal/snacks tested positive for cry1Ab gene. **Figure 3** also represents the number of samples that were positive to the EPSPS gene in soybean. It was noticed that three (3) soya bean seed samples and one (1) of each soya based food products (soya based biscuits, soya based drinks, soya seeds and soya cooking oil) was positive for the gene. **Figure 4** shows the total distribution of samples showing either positive and negative for a GMO specific gene. Out of 25 samples of maize seeds, 12 tested positive to the cry1Ab gene indicating the presence of a genetically modified gene. 3 samples of the 10 samples randomly selected for soya bean tested positive for the herbicide tolerance EPSPS gene in soya thus indicating the presence of a genetically modified gene. Incidentally none of the Maize (Corn) base biscuits tested positive for any of the genetically modified gene while one (1) of the 2 maize(corn) based oat milk had the cry1Ab gene present. Two (2) samples from the Five (5) samples collected from Maize (Corn) meal was positive for the cry1Ab gene. Both Soya based spread samples collected was positive for the herbicide tolerance EPSPS gene. The frequency of occurrence of GM products in a sample location is represented in **Figure 5**, The highest number of positive samples was recorded in market TM while Markets KM and Super Markets A and B followed with four (4) samples each. The least record of samples was noticed in market BM, NM and supermarket D. Interestingly, in supermarket C, No GM product was recorded.

Table 4. DNA yield and purity

Sample type	*DNA yield (ng/µl sample)	*DNA purity (A260: A280)
Corn based biscuits	501.99±2.80	1.87±0.12
Soya based biscuits	273.18±1.99	1.72±0.15
Corn flour	1250.71±1.90	1.75±0.35
Corn pap	10.12±0.41	1.87±1.79
Maize seed (MT)	2797.29±5.28	2.08±0.04
Soya seed(MT)	2422.93±1.21	1.77±0.34
Maize seed (NM)	1097.67±1.13	1.89±0.03
Soya seed (NM)	1474.64±1.87	1.78±0.26
Maize seed (BM)	1957.98±6.53	1.89±0.11
Soya seed (BM)	3389.81±2.37	1.79±0.43
Maize (KM)	1664.35±3.12	1.16±0.86
Maize(GM)	892.52±1.69	1.89±0.03
Soya (GM)	3360.50±1.61	1.29±0.08
Corn s/custard	775.34±7.67	1.74±0.37
Cornflakes	12.03±0.40	1.20±1.36
Soya Classic spread	37.70±2.38	1.77±0.19
Corn based snacks	338.05±1.59	1.87±0.05

*Value ± Mean Standard Error with n = number of each sample.

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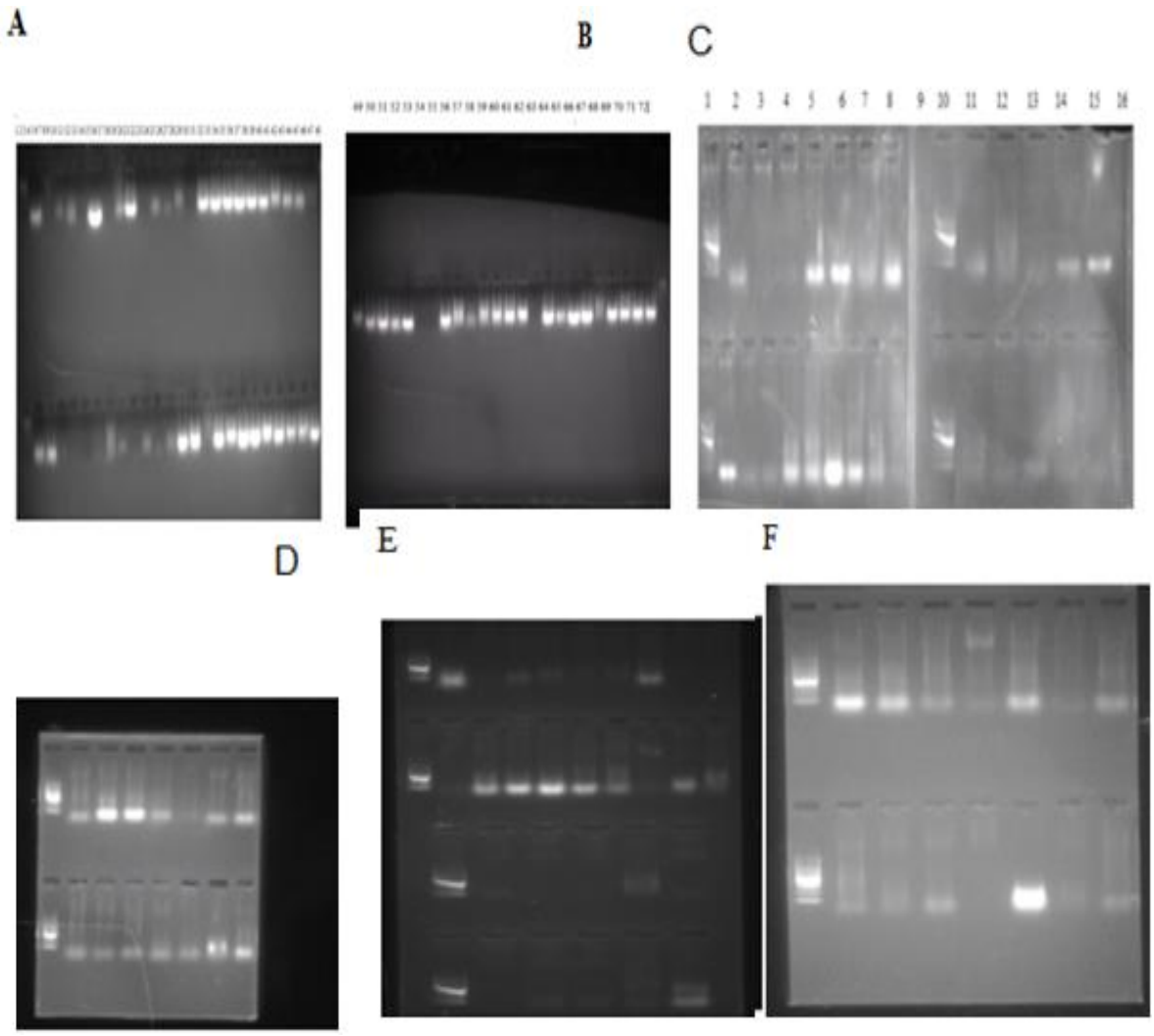

Plate 1 (A-F) Gel electrophoresis represented of total genomic DNA and PCR products for the extracted DNA. **(A)** and **(B)** Represents genomic DNA extraction for all 72 samples. **(C) and (D)** PCR products showing positive/negative for the cry1Ab gene in maize. **(E) and (F)** PCR products showing positive/negative for the EPSPS) gene in soya beans.

Table 5. Percentage (%) frequency of Positive samples.

Samples	% frequency of Positive sample
27 of 72 samples collected	37.5%
Maize Products	31.4%
Soya bean Products	52.4%

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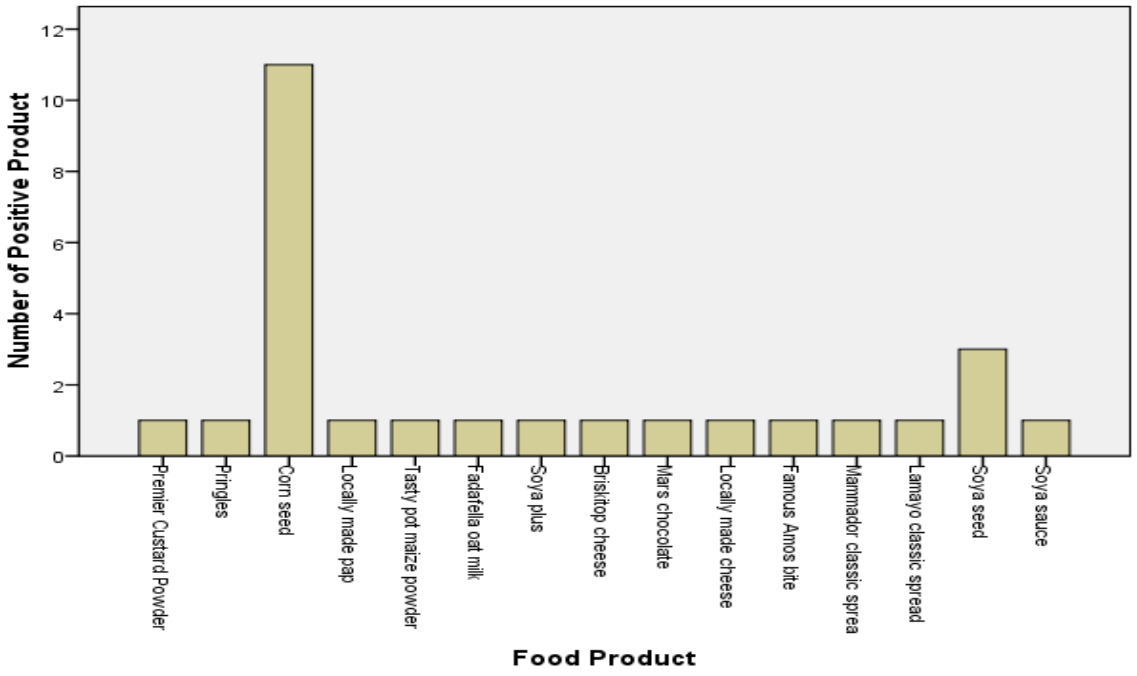

Figure 1: shows number of positive in all food products

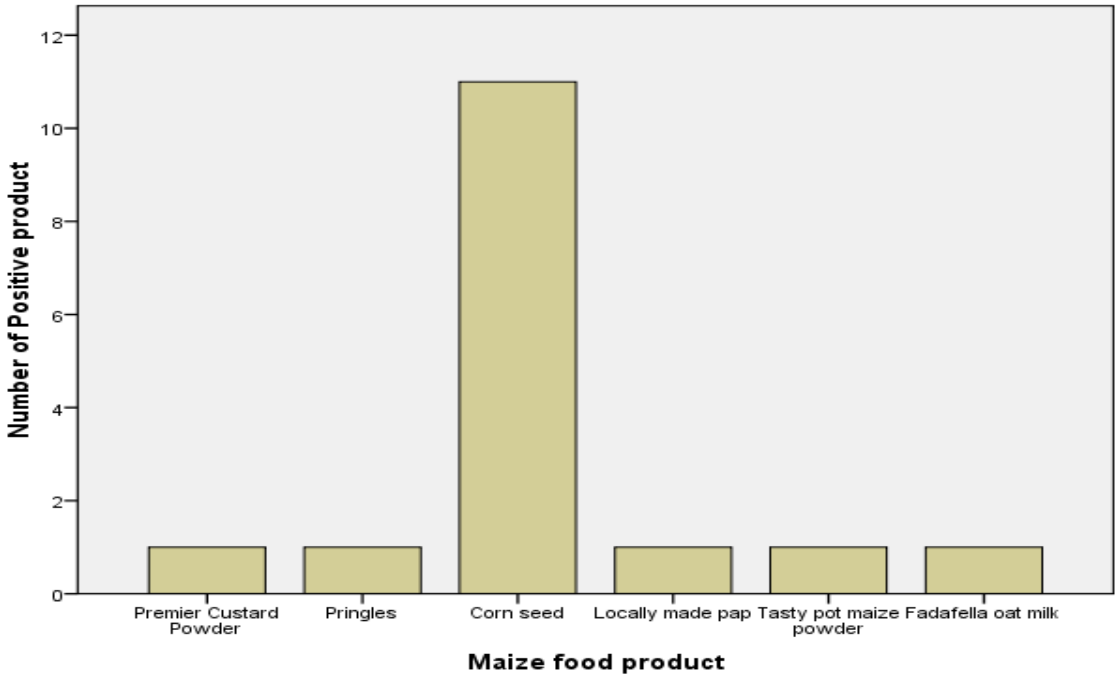

Figure 2: shows number of positive in maize food products including corn based biscuits, corn pap, sweet corn, corn flour, corn custard, corn flakes, corn seed and corn meal/snacks.

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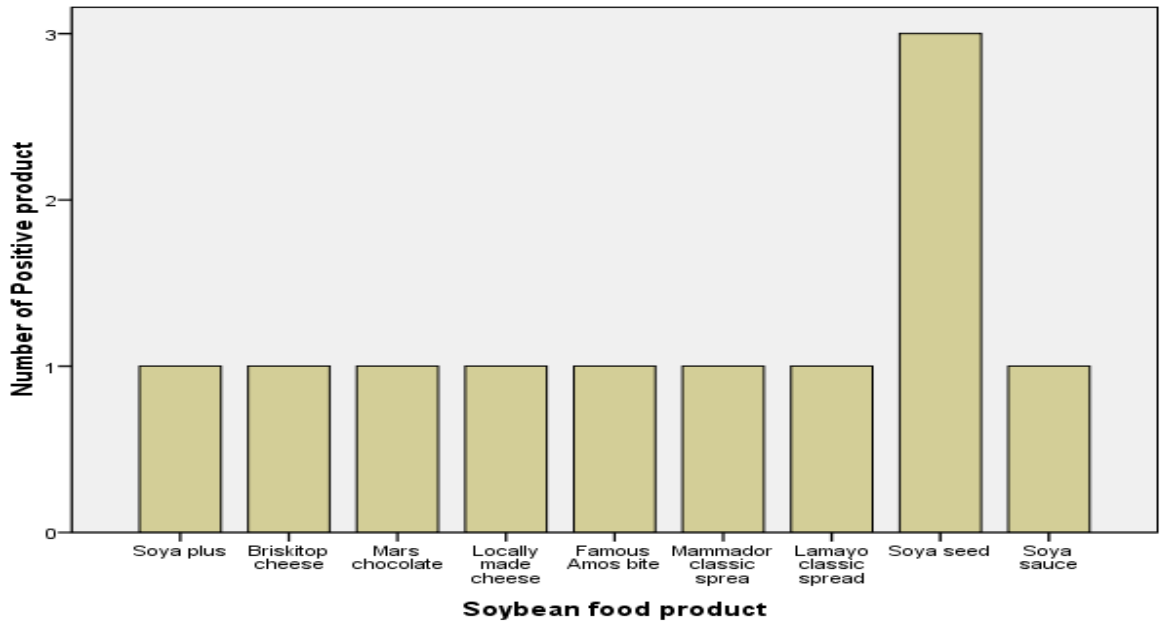

Figure 3: shows number of positive in soybean food products **i**ncluding soya based biscuits, soya based drinks, soya seeds and soya cooking oil.

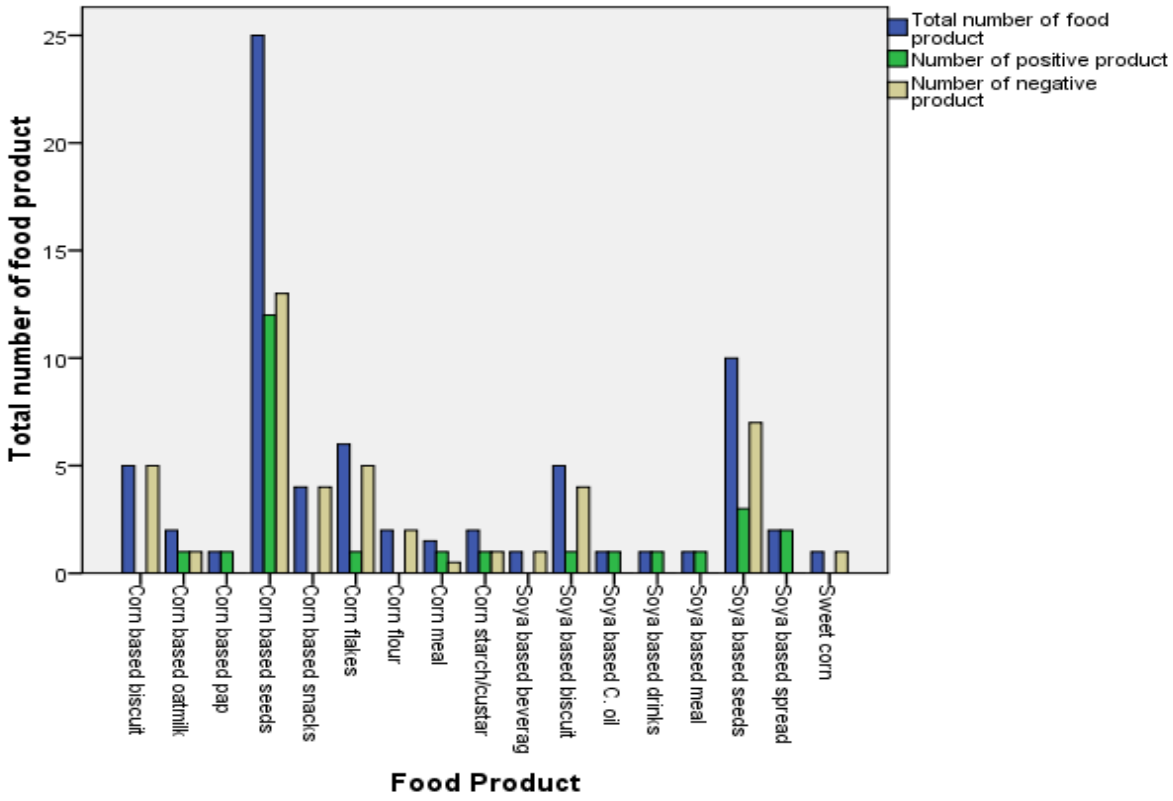

Fig 4. Distribution of samples showing positive and negative result for GMO specific gene

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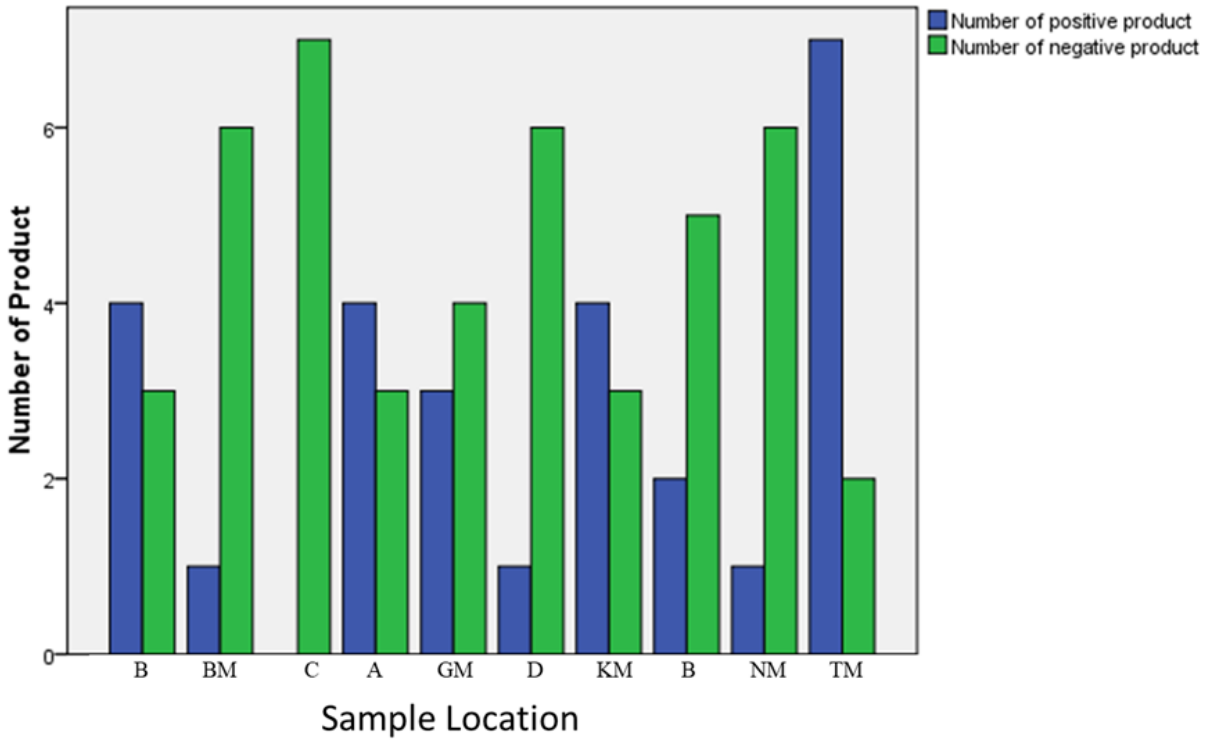

Figure 5: shows the frequency of positive and negative food product in different location

DISCUSSIONS

The quality of DNA extracted plays a very significant role in the determination of PCR methods for the detection of the presence of GMO (Tengel *et al.,* 2001; Ahmed, 2002). A good amount of DNA was extracted from all samples used in this study, except for corn pap, cornflakes and soya classic spread. DNA yield obtained ranged between 10.12±0.41 to 3360.50±1132.61. The A260/280 ratio for all the samples ranged between 1.20±1.36 to 2.08±0.04, which indicated that the extracted DNA was with less impurities that would inhibit PCR. These result obtained are in line tallies previous report on studies which extracted DNA from processed food products (Mandaci *et al.,* 2014; Greiner and Konietzny, 2008; and Cardarelli *et al.,* 2005). The most widely and efficient method used for extraction is CTAB DNA extraction method; it is used for the extraction of pure plants' DNA and plants derived food products due to its ability to provide effectively separate plant DNA from polysaccharides (Jasbeer *et al.,* 2008). Using the CTAB methods for DNA extraction indicates that DNA yield was relatively higher especially for the local products (maize seeds and soya seeds) compared to values reported from similar studies (Mandaci *et al.,* 2014). The indication for this high quality of DNA from the raw seeds is an indication that the samples were yet to be processed. However, low DNA yield observed in corn pap, cornflakes and soya classic spread might be due to important food processing factors such as pH and temperature, which affect DNA quality and quantity in processed food materials. Ahmed (2002), revealed that heating and other processes associated with food production can degrade DNA. This was also reported by Iloh *et al*., 2018, where DNA could not be gotten from processed soya oils. Again it was reported that

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pH and temperature could lead to DNA degradation which makes PCR analyses of the DNA thus rendering PCR analyses impossible (Gryson, 2010; Okpara *et al.,* 2016). Twenty-seven samples were observed to show positive results when detected for GMO specific genes. Indicating 37.5% of all samples screened in this study (Table 5). This is made up of 16 maize and 11 soya-based samples. These results shows that 31.4% of all maize-based products and 52.4% of all soy-based products indicates GMO specific genes were presence in the samples. Arun *et al.* (2013) has also reported the presence of genetically modified maize and soy in mildly and highly processed foods. This study showed consistency in the detection of both EPSPS genes and Cry1Ab genes for all GMO-positive samples. The percentage of maize (31.5%) and soya (52.4%) samples incorporating EPSPS gene and Cry1Ab genes is higher when compared to 28% previously reported by Okpara *et al.* (2016). This relative increase is due to the fact that 72 samples were collected within Minna metropolis, Niger state for this study, unlike Okpara *et al.* (2016) who screened 61 samples collected from the southern part of Nigeria. In order to contain agronomically desirable characteristics, transgenic maize and soya have been purposely genetically engineered. These characteristics that have been developed are the herbicide resistance (ESPS genes) and a gene that codes for the toxin *Bacillus thurigiencis* (Cry1Ab genes) thereby shielding plants from insect pest (Koch *et al.,* 2015). In this study, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) gene, which is expressed by roundup ready soybeans, is one of the first series of GM glyphosate-resistance soybeans produced and cultivated globally (Clarke *et al.,* 2013) was significantly detected which is in line with previous studies that the introduction of herbicide-resistant, genetically modified (GM) soybeans has allowed for increased productivity levels and a smaller workforce, enabling the crop's rapid expansion in production and product base (Northwestern University 2018, Martin *et al.,* 2019). This study showed 52.4% soy-based products was screened and harbors EPSPS gene. Interestingly, maize seeds gotten from the local markets were observed to contain more of the Cry1Ab genes. This raises questions to where the local traders were getting their products from. The indication that seeds from local markets and product from super markets contain GM materials raises the need for more vigilance by the relevant agencies to check the influx of unapproved GM materials.

CONCLUSION

PCR-based methods used in this study has successfully shown the presence of GM foods in Minna, Metropolis, Niger state. GMO detection using PCR techniques is an internationally validated and highly sensitive GMO screening strategy (Ahmed, 2002; Forte *et al.,* 2005; Meriç *et al.,* 2014). The widely recognized markers for this form of screening are the GMO-specific DNA markers used. Based on these findings, it can therefore be inferred that goods produced with GMO materials have reached the food chain in Minna, Niger state on a very large scale.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Application of Geographic Information System to Property Management

Dauda Y., Saidu U. A. & *Mohammed J. K.

Department of Estate Management and Valuation, The Federal Polytechnic, Bida, Nigeria

*Corresponding author email/phone: muhammad.jibrinkatun@fedpolybida.edu.ng, 08068868547

ABSTRACT

Property management decisions require consideration of location, geography and space. There is a constant need of technological advancement and training in the industry and Geographic Information System (GIS) is not fully explored extensively in tackling the problems of property management and related issues in Nigeria. This research examines the application of GIS in the management of properties by Usman Maishera and Associates in Bida, Nigeria. Census sampling technique was adopted where all the 103 properties managed by the estates firm were sampled. The street guide map of the study area was updated and spatial data for the properties were collected using Avenza map mobile collector. Conventional geo-database was created for properties managed by the firm. It was found out that conventional geo-database is a good tool in solving most of the property manager's challenges. It was therefore recommended amidst others that there is need for a sensitization programme for property managers around Bida, for them to switch to the use of the conventional geo-database methods instead of the manual for property management.

Keywords: GIS, Property management, Geo-database, Spatial query, Estate firms.

INTRODUCTION

Estate property management is a complex management process that primarily deals with locations/geographical area in an extensive manner and thus inherently spatial in nature (Paul *et al.*, 2019). Property management as an area of specialization within the real estate industry has emerged as a managerial science. Today property managers must possess the communication skills and technical expertise required for dynamic decision making (Kyle, Spodek and Baird, 2013). Property managers have been adopting manual method of property records and management for decades (Akeh, 2016). The manual method is accompanied by many challenges. For example, Babalola and Kardam (2011) found that information retrieval using manual approach was time-consuming and could sometimes take days before information is retrieved. Additionally, the long term usage of such records often leads to mutilation and damage. There is also the difficulty in information sharing among users with the manual system of record keeping. Chiemelu and Onwumere (2013) reported that manual property administration has become inefficient, time consuming and prone to abuses whereby some property managers siphon revenues generated because of inefficient land transaction

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records hence the need for the development of an integrated property/land information system. It was asserted that the problems associated with the manual or paper-based system of information management will continue to exist unless a comprehensive and automated system is put in place (Akeh, 2016; Akeh and Mshelia, 2018). Geographic Information System (GIS) is one of the automated approaches to address some of these problems.

GIS is a powerful set of tools for capturing, storing, querying, analyzing, and displaying spatial data from the real world for a particular set of purposes (Mohammed, 2020). GIS is a useful tool for nearly every field of knowledge from archaeology to zoology. In the built environment, GIS provides important information including current and past on planned housing, competition in the area, accessibility to the site, traffic counts, gravity models, other sites available, and zoning. This is all incorporated into easy-to-understand reports and map presentations. In real estate, GIS has been applied in several aspects such as property management, spatial property value assessment, property taxation, and several aspects of real estate (Kwiecień, Krajewska and Szopińska, 2018). For example, Faruk et al. (2016) examine the application of GIS in the administration and management of tenement rate in Bida Local Government Area where it demonstrates how GIS can be used to managed property rating and that GIS can aid policy formulation and decision making (Spatial Decision Support System) in terms of property rating. According to (UN-Habitat, 2013), GIS can be applied in property taxation by identification of ownership data by land plot, analysis of tax revenues by land use within various distances from the city centre and projecting tax revenue changes due to land-use changes. Another application of GIS in real estate is the Land Information System (LIS). LIS is a specialised application of GIS technology that is concerned with issues of land ownership, land planning and land management. The major aspect of LIS is the land titling registration which will not only bring effectiveness in land administration and management but will go a long way in ensuring the reaping of maximum benefit from the land by individual owners and boosting the real estate revenue base of the various tiers of government (Peter and Martin, 2003; Nuhu and Tunde, 2012).

GIS can be used by facility managers for space management, visualization, planning, emergency and disaster planning and response, as well as many other applications (Faruk, Mohammed and Mohammed, 2016). GIS can be used throughout the life cycle of a facility – from site selection, design and construction to use, maintenance and adaptation, and ultimately through closing, repurposing, and reclamation (IFMA Foundation, 2010). GIS is also applied in infrastructure assessment (Ajayi, Kemiki, *et al.*, 2015) where areas that need certain infrastructure are easily detected. Spatial assessment of rental price was also conducted using GIS (Ajayi, Nuhu, *et al.*, 2015). Similarly, several spatial and temporal assessment of rental prices were carried out using GIS geospatial techniques (Mohammed and Sulyman, 2019; Cichociński and Dąbrowski, 2013). There are several other studies on housing satisfaction, housing quality, real estate development, and real estate market analysis using GIS applications (Hovhannisian, 2017). GIS has proven to be a good ICT tool for problem-solving in real estate.

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Olaniyi et al. (2006), conducted a study on the application of Geographic Information System in the effective management of Estate and its resources. While the goals included capturing of geometric and attribute data about the estate features, such as road, parcel building, electric poles, wells, land use, ownership and address, design of a database for the estate, perform spatial analysis and product presentation. Thus, GIS has potential to handle spatial and non-spatial data in a single platform which makes it a favourable choice for estate property management system (Paul *et al.*, 2019). Qian (2013) opined that GIS-based property management system is to provide information services for real estate administrative department. Studies have also shown that GIS is important in keeping property records, transactions and maintenance by government agencies (Zaihua and Fengquan, 2013). Aforementioned studies were centered on application of GIS in management of housing estate in a single location where other properties managed by estate firms which are scattered around were not considered. However, detail study on the application of GIS to property management by estate firms has not been examined. It is on this backdrop that this study aimed at applying GIS in the property management by Usman Maishera and Associates estate firm in Bida, Nigeria.

METHODOLOGY

The study area is Bida town, a traditional north-central town of Nigeria located on latitude 9° 5' 0" North and longitude 6° 1' 0" East (see Fig. 1). The town is the second-largest settlement in the Niger State of Nigeria with a total built-up area of 67.45km^2 and about 255,008 population based on the last national population census (Mohammed and Sulyman, 2019).

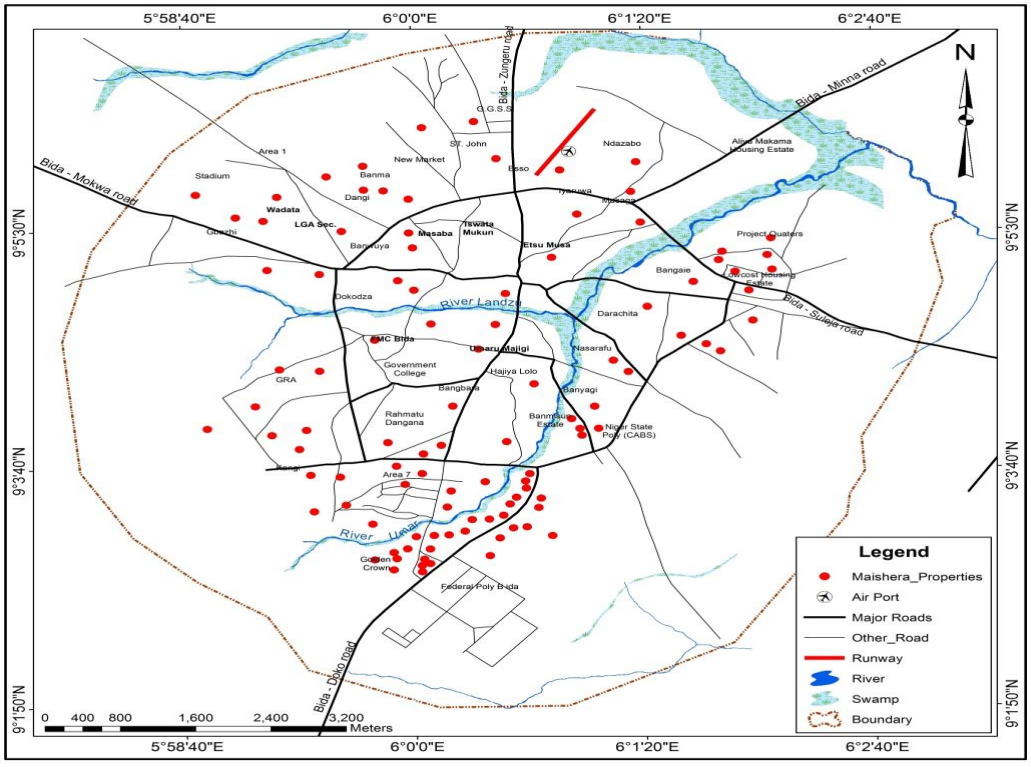

Fig. 1: Map of Bida Town (Field survey, 2020)

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Census sample technique was adopted where all the properties managed by Usman Maishera and Associates in Bida town were sampled. Data needed for this research was collected through checklist, interview, satellite image and physical assessment. The street guide map of Bida was updated using recent satellite images of the town and field survey. The position of the properties in terms of X and Y coordinates were taken using Avenza mobile application which provide spatial information about the positions of the properties in study area. The main attribute data for this work was name of client, tenant name, tenant address, tenant mobile number, type of property, rental value, property title, status of properties and coordinates of the properties were stored in ArcMap GIS relational database called theme table. Spatial query was conducted to offer a method of data retrieval in other to test the integrity of the spatial relational data component. The real power of the database is the ability to view data according to a pre-set order, with spatial query, questions about the attributes table and the graphic objects were asked using the query expression algorithm. For the purpose of this research, spatial query was carried out to determine rental value of properties, vacant properties, among others. Query for each set of housing attributes in all the three selected research areas were conducted.

RESULTS AND DISCUSSION

Geo-database

Conventional geo-database was built using ArcGIS 10 with integrated MS Access applications. It contains rows and columns, geographical coordinates of the locations of properties and other attributes of the properties such as tenant name, tenant address, tenant mobile number, type of property, rental value, property title, status of properties and so on. The geo-database was built using the identified properties with respect to their coordinates. This is the representation of a human conceptualisation of reality as shown in Figure 2. Conventional geo-database is a combination of attribute data tables which contains non spatial information about shape files (spatial data). The conventional geo-database is used to run spatial query, identified a property, a set of data for geo-statistics and also geospatial analysis. With GIS, property managers' task is made easy and fast, this is because property management involves large data and Geo-database has provision for such large data which cannot be easily managed using manual system.

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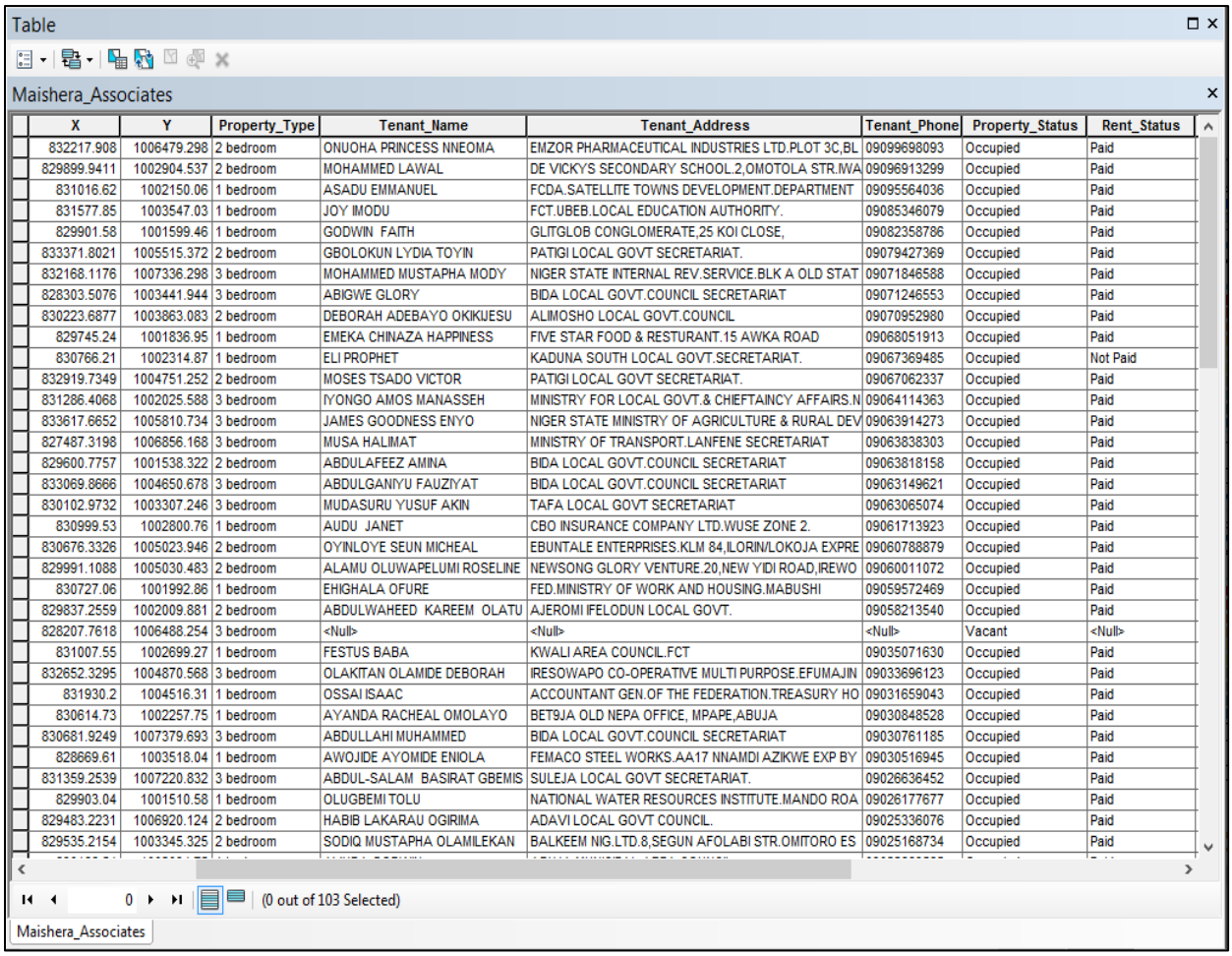

Table

Maishera_Associates

X	Y	Property_Type	Tenant_Name	Tenant_Address	Tenant_Phone	Property_Status	Rent_Status
832217.908	1006479.298	2 bedroom	ONUOHA PRINCESS NNEOMA	EMZOR PHARMACEUTICAL INDUSTRIES LTD.PLOT 3C,BL	09099698093	Occupied	Paid
829899.9411	1002904.537	2 bedroom	MOHAMMED LAWAL	DE VICKYS SECONDARY SCHOOL.2,OMOTOLA STR.IWA	09096913299	Occupied	Paid
831016.62	1002150.06	1 bedroom	ASADU EMMANUEL	FCDA.SATELLITE TOWNS DEVELOPMENT.DEPARTMENT	09095564036	Occupied	Paid
831577.85	1003547.03	1 bedroom	JOY IMODU	FCT.UBEB.LOCAL EDUCATION AUTHORITY.	09085346079	Occupied	Paid
829901.58	1001599.46	1 bedroom	GODWIN FAITH	GLITGLOB CONGLOMERATE,25 KOI CLOSE,	09082358786	Occupied	Paid
833371.8021	1005515.372	2 bedroom	GBOLOKUN LYDIA TOYIN	PATIGI LOCAL GOVT SECRETARIAT.	09079427369	Occupied	Paid
832168.1176	1007336.298	3 bedroom	MOHAMMED MUSTAPHA MODY	NIGER STATE INTERNAL REV.SERVICE.BLK A OLD STAT	09071846588	Occupied	Paid
828303.5076	1003441.944	3 bedroom	ABIGWE GLORY	BIDA LOCAL GOVT.COUNCIL SECRETARIAT	09071246553	Occupied	Paid
830223.6877	1003863.083	2 bedroom	DEBORAH ADEBAYO OKIKIJESU	ALIMOSHO LOCAL GOVT.COUNCIL	09070952980	Occupied	Paid
829745.24	1001836.95	1 bedroom	EMEKA CHINAZA HAPPINESS	FIVE STAR FOOD & RESTURANT.15 AWKA ROAD	09068051913	Occupied	Paid
830766.21	1002314.87	1 bedroom	ELI PROPHET	KADUNA SOUTH LOCAL GOVT.SECRETARIAT.	09067369485	Occupied	Not Paid
832919.7349	1004751.252	2 bedroom	MOSES TSADO VICTOR	PATIGI LOCAL GOVT SECRETARIAT.	09067062337	Occupied	Paid
831286.4068	1002025.588	3 bedroom	IYONGO AMOS MANASSEH	MINISTRY FOR LOCAL GOVT.& CHIEFTAINCY AFFAIRS.N	09064114363	Occupied	Paid
833617.6652	1005810.734	3 bedroom	JAMES GOODNESS ENYO	NIGER STATE MINISTRY OF AGRICULTURE & RURAL DEV	09063914273	Occupied	Paid
827487.3198	1006856.168	3 bedroom	MUSA HALIMAT	MINISTRY OF TRANSPORT.LANFENE SECRETARIAT	09063838303	Occupied	Paid
829600.7757	1001538.322	2 bedroom	ABDULAFEEZ AMINA	BIDA LOCAL GOVT.COUNCIL SECRETARIAT	09063818158	Occupied	Paid
833069.8666	1004650.678	3 bedroom	ABDULGANIYU FAUZIYAT	BIDA LOCAL GOVT.COUNCIL SECRETARIAT	09063149621	Occupied	Paid
830102.9732	1003307.246	3 bedroom	MUDASURU YUSUF AKIN	TAFA LOCAL GOVT SECRETARIAT	09063065074	Occupied	Paid
830999.53	1002800.76	1 bedroom	AUDU JANET	CBO INSURANCE COMPANY LTD.WUSE ZONE 2.	09061713923	Occupied	Paid
830676.3326	1005023.946	2 bedroom	OYINLOYE SEUN MICHEAL	EBUNTALE ENTERPRISES.KLM 84,ILORIN/LOKOJA EXPRE	09060788879	Occupied	Paid
829991.1088	1005030.483	2 bedroom	ALAMU OLUWAPELUMI ROSELINE	NEWSONG GLORY VENTURE.20,NEW YIDI ROAD,IREWO	09060011072	Occupied	Paid
830727.06	1001992.86	1 bedroom	EHIGHALA OFURE	FED.MINISTRY OF WORK AND HOUSING.MABUSHI	09059572469	Occupied	Paid
829837.2559	1002009.881	2 bedroom	ABDULWAHEED KAREEM OLATU	AJEROMI IFELODUN LOCAL GOVT.	09058213540	Occupied	Paid
828207.7618	1006488.254	3 bedroom	<Null>	<Null>	<Null>	Vacant	<Null>
831007.55	1002699.27	1 bedroom	FESTUS BABA	KWALI AREA COUNCIL.FCT	09035071630	Occupied	Paid
832652.3295	1004870.568	3 bedroom	OLAKITAN OLAMIDE DEBORAH	IRESOWAPO CO-OPERATIVE MULTI PURPOSE.EFUMAJIN	09033696123	Occupied	Paid
831930.2	1004516.31	1 bedroom	OSSAI ISAAC	ACCOUNTANT GEN.OF THE FEDERATION.TREASURY HO	09031659043	Occupied	Paid
830614.73	1002257.75	1 bedroom	AYANDA RACHEAL OMOLAYO	BET9JA OLD NEPA OFFICE, MPAPE,ABUJA	09030848528	Occupied	Paid
830681.9249	1007379.693	3 bedroom	ABDULLAHI MUHAMMED	BIDA LOCAL GOVT.COUNCIL SECRETARIAT	09030761185	Occupied	Paid
828669.61	1003518.04	1 bedroom	AWOJIDE AYOMIDE ENIOLA	FEMACO STEEL WORKS.AA17 NNAMDI AZIKWE EXP BY	09030516945	Occupied	Paid
831359.2539	1007220.832	3 bedroom	ABDUL-SALAM BASIRAT GBEMIS	SULEJA LOCAL GOVT SECRETARIAT.	09026636452	Occupied	Paid
829903.04	1001510.58	1 bedroom	OLUGBEMI TOLU	NATIONAL WATER RESOURCES INSTITUTE.MANDO ROA	09026177677	Occupied	Paid
829483.2231	1006920.124	2 bedroom	HABIB LAKARAU OGIRIMA	ADAVI LOCAL GOVT COUNCIL.	09025336076	Occupied	Paid
829535.2154	1003345.325	2 bedroom	SODIQ MUSTAPHA OLAMILEKAN	BALKEEM NIG.LTD.8,SEGUN AFOLABI STR.OMITORO ES	09025168734	Occupied	Paid

0 (0 out of 103 Selected)

Maishera_Associates

Figure 2: Attribute Data Table for Maishera and Associates Properties

Source: Author's field survey, 2020

Figure 2 is an attribute data table for Maishera and Associates properties showing point shape files that represent properties with their non-spatial information. It is one of the important aspects of geo-database against the traditional manual property record keeping. In the manual system all information are paper based and kept in files, which implies that 100 properties requires 100 files for record keeping. However, in the GIS system as demonstrated in Figure 2, all records are kept inside a single attribute table linked to the spatial data on the map (Akeh, 2016). This also makes the GIS system less expensive as less or no papers and files and less office spaces are required for the property management activities.

Spatial Query

The query builder allows for easy selection of features based on their attributes. The query builder comprises of syntax used as expression for selecting features and their attributes. By default, ArcGIS 10 highlighted the queried object(s) in magenta. The attributes that were queried were highlighted in magenta on the maps as shown in Figure 3 and 4.

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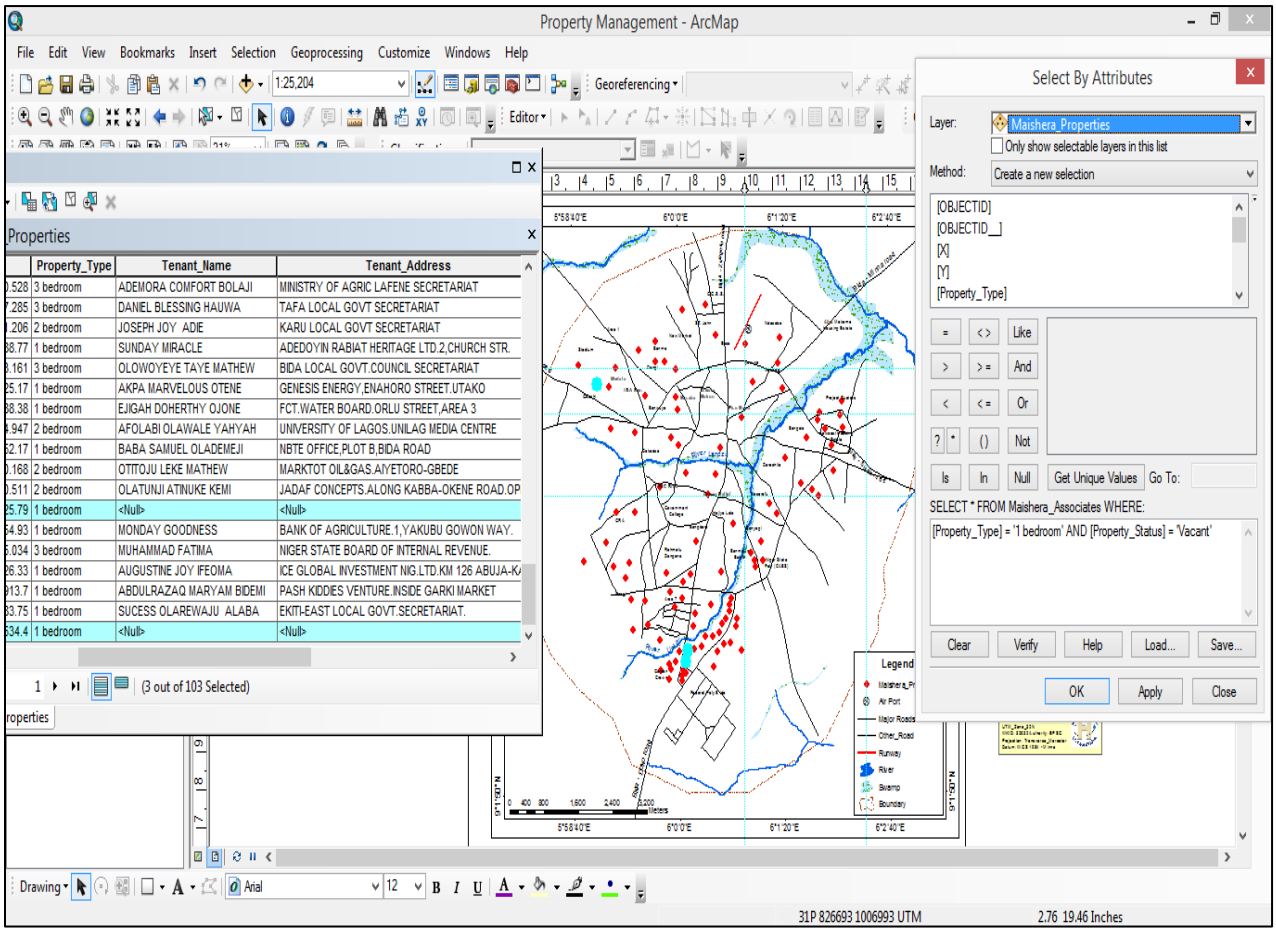

Figure 3: Query for 1 Bedroom Vacant Properties

Source: Author's field survey, 2020

Figure 3 shows a query for 1 bedroom properties that are vacant in the study area. This was done by the use of query dialogue box. The query dialogue box contains all the data contained in the attribute table. Query syntax used was *[Property_Type] = '1 bedroom' AND [Property_Status]= 'Vacant'*. After applying the command, the result shows vacant properties that are 1 bedroom in magenta colour both on the map and the attribute table.

This tool is imperative to property managers because it allow them to easily retrieve both spatial and attributes data on a particular type of properties and vacant or occupied properties within the portfolio of the estate firm. The spatial database accommodates large volume of both spatial and attribute data which can easily be retrieve using the query system (Mohammed, 2020). This is one of the reasons that it is important for property managers to embrace the GIS technology in conducting their activities.

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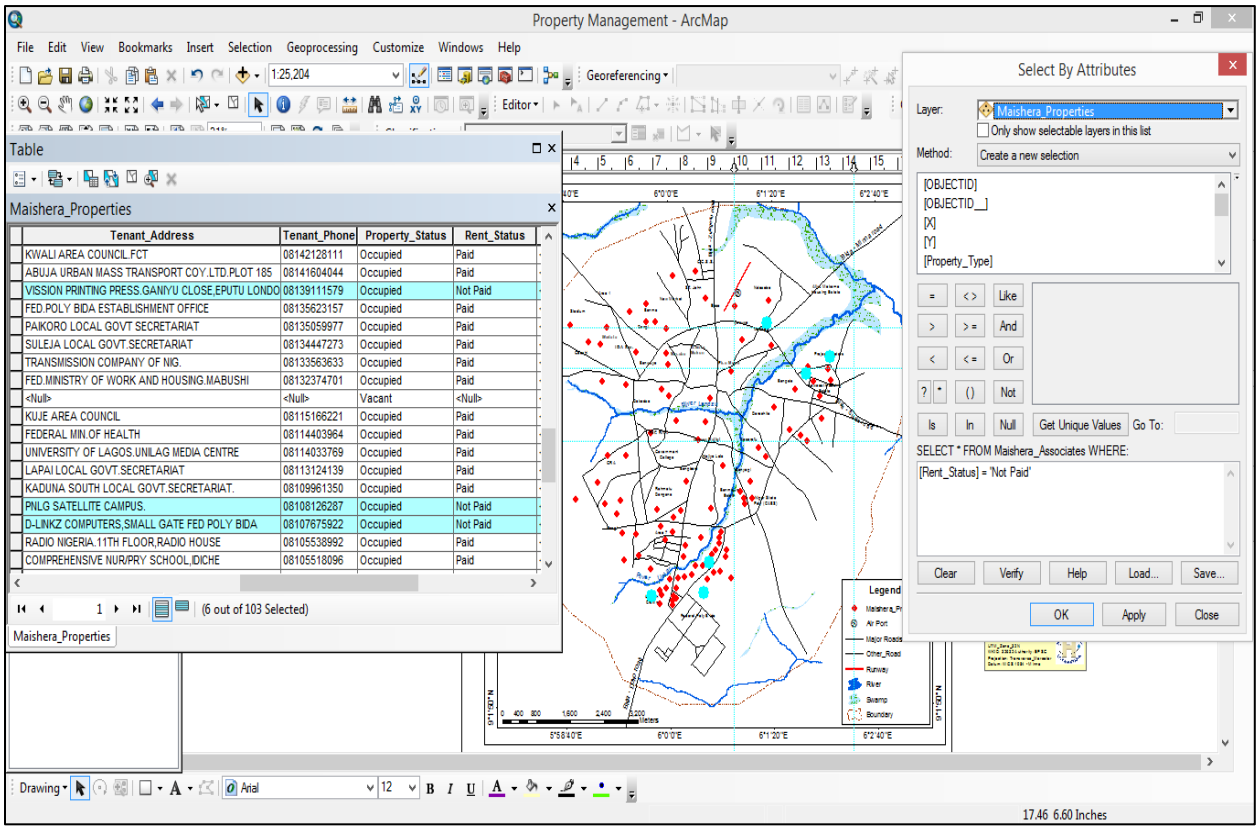

Figure 4: Query for Tenants that Have Not Paid Rent

Source: Author's field survey, 2020

Query for tenants that have not paid their rent in the study area is presented in Figure 4. This was also done by the use of query dialogue box. The query syntax used was *[Rent_Status] = 'Not Paid'*. After applying the command, the result shows properties that tenants have not paid rent in magenta colour both on the map and the attribute table.

As earlier mentioned, this will equally help to get information quickly as regards the property type when need arises. It equally helps the firm to know how many tenants have not paid their rent. It also helps to know the expected rental values for a particular period of time under the watch of Usman Maishara and Associates. Conducting this type of task using the manual system takes longer time and needs more man power. The manual system also requires large space and shelves for keeping files and paper records of the properties (Akeh and Mshelia, 2018). GIS-based property management system provides information services for real estate firms and administrative departments (Qian, 2013).

Selecting Spatial Data Using 'Identify Tool'

Since geo-database is a linkage between spatial data and attribute data, spatial data can be identify using the 'identify tool' in ArcGIS 10 to figure out the information related to that spatial data such as rental value, owner of property, house number, gender of the owner of property and so on. This can be achieved by selecting the spatial data using identify tool which displays the attributes of the selected spatial data in a dialogue box (see Figure 5).

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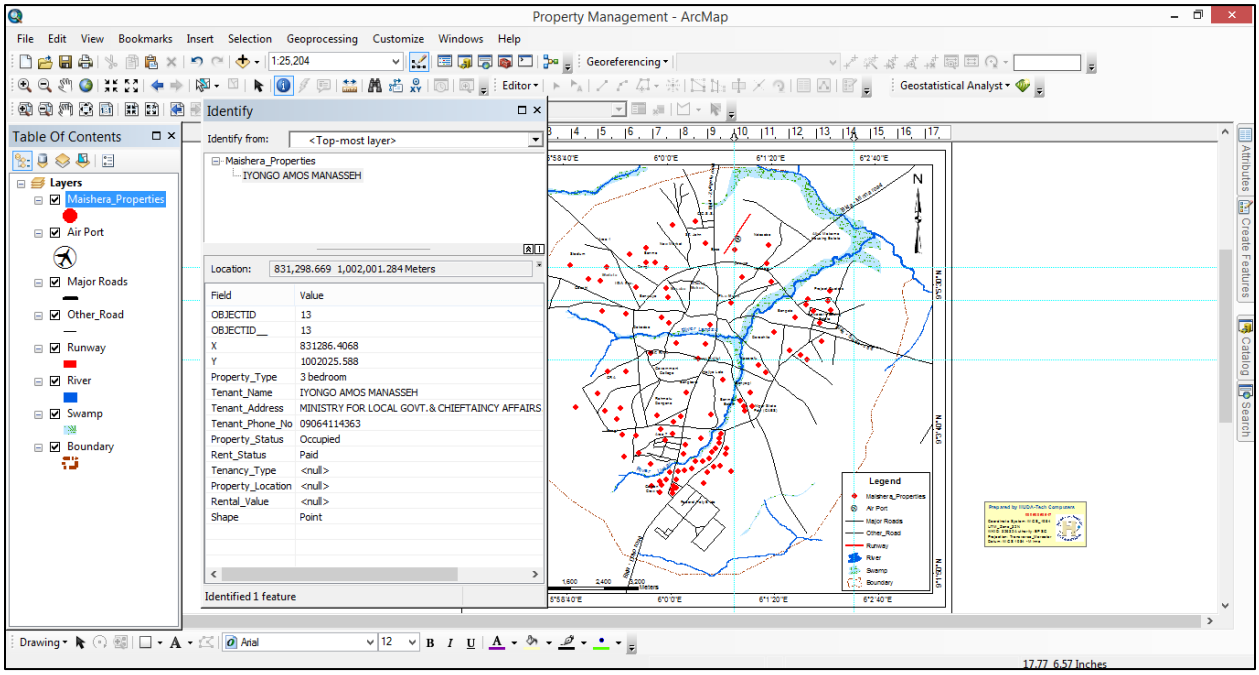

Figure 5: Using Identify Tool for Selecting Spatial Data

Source: Author's field survey, 2020

Figure 5 shows the use of 'Identify' tool on a spatial data among properties managed by Usman Maishera and Associates in Bida to display its attributes as shown in Figure 5. This is another special tool in the GIS-based property management that would allow the property managers to select properties on the map to retrieve information about the properties which is not possible using the manual system of property management. It is the quickest way of identifying property details.

CONCLUSION

The conventional geo-database is used to run spatial query, identified a property, a set of data for geo-statistics and also geospatial analysis. With this query procedure property search will be very easy for property managers or problems related to properties can easily be searched using the conventional database which cannot be easily done using manual system. Therefore the study suggests that there is a clear disparity between application of GIS in property management and manual system of property management. Identify tool will also make it easy to identify houses in spatial environment. Geo-database serve as a data bank for property managers where large number of data were linked with geographical elements in form of attribute tables for easy management of properties which cannot be carried out using manual system. Property data of estates firms can be stored and linked in term management of large portfolio which cannot be easily done using manual system. Therefore, the study recommends that property managers should be trained and oriented on the use of the GIS software for property management.

Acknowledgement

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Conflict of Interest

The authors declare that there is no conflict of interest.

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Fungi Species Associated with Invasion of Long-Term Packaged Bread Retailed Within Selected Areas in Chikun LGA of Kaduna State

***[1]Mohammed S. S. D. & [2]Kadani Z. D.**

[1]Department of Biology, Microbiology and Biotechnology, Faculty of Natural and Applied and Sciences, Nile University of Nigeria, Jabi, FCT, Abuja.
[2]Department of Microbiology, Faculty of Science, Kaduna State University, Kaduna, Nigeria

*Corresponding author: mosada78@gmail.com, sani.mohammed@nileuniversity.edu.ng, Tel; +234(0)8035861774

ABSTRACT

Bread is a confectionary food that is rich in lots of nutrients and when stored at suitable temperature for growth, permits the growth of microorganisms. Fungi species associated with invasion of long-term packaged bread retailed within selected areas in Chikun Local Government area of Kaduna State was investigated.. A total number of sixteen (16) samples were purchased from vendors at four locations (Romi, Television, Sabo and Maraba-rido) and were kept at room temperature for 14 days. Proximate analysis were carried out on selected bread samples to determine their percentage composition which included moisture, ash, protein, fat, crude fibre and cabohydrate contents. The isolation of the fungi species were carried out using standard techniques on Sabouraud dextrose agar (SDA) in duplicate. Antifungal susceptibility test was carried out using agar well diffusion technique. The proximate composition showed that sample A (SB01) had moisture content of 36.07%, 1.42% of ash, 12.78% of protein, 1.96% of fat, 0.46% of crude fibre and 47.8% of total carbohydrate. Sample B (MR02) showed 37.10% of moisture, 1.51% of ash, 12.22% of fat, 1.98% of protein, 0.42% of crude fibre and 48% of total carbohydrate. The fungi isolates identified were, *Aspergillus niger, Aspergillus flavus, Aspergillus ochraceus, Rhizopus stolonifer, Penicillium expansum, Mucor* sp and *Pichia* sp. *Aspergillus* sp had the highest occurrence with a percentage of 33% followed by *Rhizopus* sp, *Penicillium* sp, *Mucor* sp and *Pichia* sp with the percentage of 27%, 15%, 21% and 3% respectively. The antifungal susceptibility of the fungal isolates to ketoconazole, fluconazole, co-trimazole and terbinafine at different concentrations showed varying zones of inhibition against *Aspergillus niger*, *Aspergillus ochraceus*, *Penicillium expansum*, *Rhizopus stolinifer*, *Pichi*a sp and *Mucor* sp. respectively. Effort to control and prevent spoilage and quality changes during storage of bread should receive high attention so as to improve food safety.

Keywords: Bread, proximate, fungi, antifungal, inhibition

INTRODUCTION

Bread is universally accepted as a very convenient form of food that is important to all populations. Its origin dates back to the Neolithic era and is still one of the most consumed and

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acceptable staple food products in all parts of the world (Auta *et al.,* 2014). Nutritionally, bread has been categorized as a source of carbohydrates and nutrients such as magnesium, iron, selenium, B vitamins and dietary fibre (Grotts, 2011). It has significance beyond mere nutrition in many cultures because of its history and contemporary importance. Bread is also significant in Christianity as one of the Eucharistic and in other religion including paganism. Bread is used as an ingredient in other culinary preparations, such as the use of bread crumbs to provide crunchy crust or thicken sauces, sweet or savoury bread puddings, or as a binding agent in sausages and other ground meat products.

Microbial attack on stored bread (s) and the consequent waste problem causes economic losses of both the consumer and bakery industry. Food spoilage during storage has caused man significant losses (Orukotan, 2010). Bread is a major product prepared using flours. Dough is prepared from flours which undergo fermentation for which desirable microorganisms must grow. If this fermentation exceeds the required limits, it causes souring. Excessive growth of proteolytic bacteria reduces the gas holding capacity which is otherwise required for dough rising. The spoilage of bread is usually of two types viz. moldiness and ropiness. During bread making, baking is done at very high temperature, thereby there are slim chances of survival of microorganisms. Thus the contamination usually occurs when cooling is done as well as during packing, handling and from the environment. The molds which are prevalent are *Rhizopus stolonifer*(referred as bread mold), *Penicillium expansum, Aspergillus niger*. *Mucor* and *Geotrichum* also develop (Saranraj & Geetha, 2012).

Ropiness in bread is usually due to bacterial growth and is considered more prevalent in home-made breads. The chief causative organism of ropiness is *Bacillus subtilis* or *B. licheniformis.* These are spore forming bacteria with their spores surviving baking temperatures. These spores can germinate into vegetative cells, once they get suitable conditions as heat treatment activates them. In ropiness, the hydrolysis of bread flour protein (gluten) takes place by proteinases. Starch is also hydrolysed by amylases, which encourage ropiness. The manifestation of ropiness is development of yellow to brown color and soft and sticky surface. It is also accompanied by odor. Another type of spoilage of bread is chalky bread which is caused by growth of yeast like fungi *Endomycosis fibuligera* and *Trichosporon* sp. .This spoilage is characterized by development of white chalk like spots. An unusual spoilage of bread is Red or Bloody bread, which is due to the growth of bacteria *Serratia marcescens*. This organism produces a brilliant red colour on starchy food giving blood like appearance. *Neospora* and *Geotrichum* may also be involved in imparting pigmentation during spoilage of bread (Saranraj & Geetha, 2012).

A fungus (plural; fungi) is any member of the group of eukaryotic organisms that includes microorganisms such as yeasts and molds, as well as mushroom (Orukotan, 2010). These organisms are classified as a kingdom, which is separate from the other eukaryotic life kingdoms of plants and animals. A characteristic that places fungi in a different kingdom from plants, bacteria and some protists is the presence of chitin in their cell walls. Similar to animals, fungi are heterotrophs, they acquire food by absorbing dissolved molecules typically by secreting digestive enzymes into their environment. Fungi do not

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photosynthesize, growth is their means of mobility, except for spores which may travel through air or water. They are principal decomposers in ecological systems. Abundant worldwide, most fungi are inconspicuous because of the small size of their structures and their cryptic lifestyles in soil or on dead matter. They include symbionts of plants, animals or other fungi and also parasites. They play essential role in the decomposition of organic matter and have fundamental roles in nutrient cycling. They have long been used as a direct source of human food in the form of mushroom and in the fermentation of various food products, such as wine, beer and soy sauce. Since the 1940's, fungi have been used for the production of antibiotics and more recently, various enzymes produced by fungi are used industrially and in detergents. Fungi are also used as biological pesticides to control weeds, plant diseases and insects pests. Many species produce bioactive compounds called mycotoxins such as alkaloids and polyketides that are toxic to both animals and humans. Several species of fungi have been employed in various food and beverage production processes. The baker's yeast and other baked foods in cheese and sauce making and in the production of wine and soft drinks. Some species have also served as delicacies since the past ages (Orukotan, 2010).

Microbial spoilage due to molds and yeasts is of major economical importance to bakery products. Spoilage causes loss to both customers and manufacturers and these losses could be due to many individual cases such as, packaging, sanitary practice in manufacturing, storage conditions and product turnover. The mycotoxins produced by these yeasts and molds are harmful to consumers.

Several methods can be used to control microbial growth on bakery products including reformulation, freezing, and most commonly, the use of preservatives which are most commonly used to control mold growth. Reformulation involves a reduction of available water and can be achieved by dehydration, either through evaporation or freeze-drying or by high osmotically active additives e.g., sugars and salts, incorporated directly into the food. Application of these methods increases the shell life of bread. This research aimed at characterizing fungi species from long- termed packaged bread.

MATERIALS AND METHODS

Area of Study

The study area is Chikun Local Government Area is an area in Kaduna state Nigeria with its headquarters in Kujama. It has an area of 4,645km and a population of 368,200 with longitude and latitude of 10.1400°N and 7.1761°E. The samples purchased were labelled and taken to the Microbiology Laboratory of Kaduna State University for further analysis.

Collection of Bread Samples

Samples were purchased from vendors in Kaduna metropolis. Sixteen (16) loaf bread samples purchased from vendors in Romi, Television, Sabo and Maraba-rido and were stored for a period of 14 days.

Proximate Composition of Selected Bread Samples

Proximate composition of the selected bread was carried out for percentage moisture, carbohydrate, crude protein, crude fat, crude ash and crude fibre content using the methods

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described by the Association of Office Analytical Analyst (AOAC) (2005) and Mongi *et al*. (2011).

Preparation of Media

All media were prepared according to manufactures instructions.

Isolation of Fungi from Bread samples

Exactly twenty five (25) gram of each bread sample was mixed with 225ml of sterile water to make a homogenate and serial dilution at the recommended rate *i.e* 1:10 (1+9) was carried out. One (1) ml of the homogenate was taken and added to 9ml of distilled water, same procedure was repeated three more times giving dilution of up to 10^8 CFU/g. Exactly 1ml of the stock solution was taken from each diluents and dispensed into petri plates. The prepared sabouraud dextrose agar was added and the plate was swirled gently in order for the contents to mix well. It was allowed to solidify and kept at room temperature for 5 days. A sterile needle was used to pick from a distinctive colony and dropped in the middle of a freshly prepared Potato dextrose growth media. The plates was kept at room temperature for 5-7 days (Nirmala *et al.,*2016).

Characterization and Identification of Fungal Isolates

Fungi isolates were identified based on microscopic and macroscopic examination. Microscopic examination was carried by observing under the microscope for mycelial formation using lactophenol cotton blue. While the macroscopic examination was carried out observing morphological characteristics (Auta *et al*.,2014)

Antifungal Susceptibility Test (Agar well Diffusion Method)

This was performed using Mueller Hinton agar. The antifungal agents used include; Terbinafine, fluconazole, ketoconazole and cotrimazole. The inoculum used was prepared using yeasts from a 7-day subculture of potato dextrose agar, the suspension was in sterile water. The turbidity was adjusted to obtain a final concentration to match that of a 0.5 McFarland standard. A sterile wire loop was used to pick from the inoculum and streaked all over the surface of the agar. A sterile wire loop was used to pick from 7-day moulds and dropped in the middle of agar plate. 200mg of each antifungal agent was dispensed into 10ml of sterile water. A sterile cork borer was used to cut out four wells, each 4mm in diameter and o.1ml of each of antifungal agent's suspension was into the wells. The plates were kept at room temperature for 4- 6 days after which zones of inhibition were measured (Colella *et al*., 2008).

RESULTS

Table 1 shows the average proximate composition two selected bread samples 'A' (SB01) and' B' (MR02). the composites were measured in percentage (%). For sample A, moisture (36.07%), Ash (1.42%), Protein (12.78%), Fat (1.98%), Crudefibre (0.46%) and a total of 47.31%. for sample B, Moisture (37.10%), Ash (1.82%), Protein (12.78%), Fat (1.96%), Crude fibre (0.46%) and a total of 46.72% as shown in the table below. Table 2 shows the characteristics of organisms isolated from the bread samples. *Aspergillus niger, Aspergillus flavus, Aspergillus ochraceus, Mucor* sp*, Rhizopus stolonifer, Penicillium expansum* and *Pichia* sp were the fungi isolated as shown in the table below. Table 3 shows the occurrence of fungi

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species in bread samples at different locations. *Aspergillus* sp and *Rhizopus* sp occurred in all locations, *Penicillium* sp in two locations while *Pichia* sp and *Mucor* sp in one and three locations respectively as shown in the table below. Table 4 shows the percentage of occurrence of fungi species in the bread samples. *Aspergillus* sp was 33%, *Rhizopus* sp 27%, *Penicillium* sp 15%, *Mucor* sp and *Pichia* sp 3% as shown in the table below. Table 5 shows the inhibitory effects antifungal agents against the probable organisms. Zones of inhibition were observed and measured in millimetres (mm) no zone of inhibition was observed with Ketoconazole against *Pichia* sp and Fluconazole against *Aspergillus ochraceus* as shown in the table below.

Table 1 Average Proximate Composition of Selected Bread Samples

Parameters (%)	Samples	
	A (SB01)	B (MR02)
Moisture	36.07	37.10
Ash	1.42	1.51
Protein	12.78	12.22
Fat	1.96	1.98
Crude fibre	0.46	0.42
CHO	47.31	48

A(SB01): Bread sample 01 from location 01 of Sabo, **B (MR02):** Bread sample 02 from location 02 of Maraba-rido.

Table 2: Characteristics of Fungi Isolated from the Bread Samples

Colonial Appearance	**Microscopic Appearance**	**Identified Isolates**
Velvet dark brown to black colour	Large, globose, dark brown conidial heads.	*Aspergillus niger*
Greenish yellow surface with white boarder	Septate with long conidiophores	*Aspergillus flavus*
Pale yellow coloured conidiophores with powdery mass	Pebble-like sclerotia	*Aspergillus ochraceus*
Large white colonies which later turn into black	Hyphae with rhizoids columella. Sporangia with shaped tip.	*Mucor* sp.
Blue-green mold, smooth conidia and rough stries around the apex	Septate hypha, hyaline with branched conidiophores	*Penicillium expamsum*
White cotton-like colony initially becoming brownish to blackish.	Sporangiospores, smooth walled, non septate**.**	*Rhizopus stolonifer*
White/creamy colonies.	**S**pherical acuminate cells	*Pichia* sp.

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Table 3 Occurrence of Fungi Species in Bread samples

Fungal Isolates	Sample locations			
	Romi	Television	Sabo	Maraba-rido
Aspergillus sp	+	+	+	+
Rhizopus sp	+	+	+	+
Penicillium sp	_	+	_	+
Pichia sp	_	_	+	_
Mucor sp	+	+	+	_

Key;**+ (present), _ (Absent**)

Table 4 Percentage Occurrence of Fungi in Bread Samples

Fungi species	**Percentage (%)**
Aspergillus sp	33
Rhizopus sp	27
Penicillium sp	15
Mucor sp	21
Pichia sp	3

Table 5 Inhibitory Effect of Antifungal Agents against Fungi Isolates

Fungal Isolates	**Antifungal Agents**			
	FLU	**COT**	**KET**	**TER**
	Zones of Inhibition (mm)			
Aspergillus niger	19	13	22	24
Aspergillus flavus	12	11	16	19
Aspergillus ochraceus	NZ	15	14	25
Penicillium expansum	13	16	11	22
Rhizopus stolonifera	15	13	9	21
Pichia sp	14	21	NZ	20
Mucor sp	17	15	12	18

Key; FLU (Fluconazole), COT (co-trimazole), KET (ketoconazole), TER (Terbinafine)
NZ= No zone of inhinition, mm= Millimeter

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DISCUSSION

The main purpose of carrying out proximate analysis is to determine how much major composites (moisture, ash, protein, fat and crude fibre) exist in food. The composites of the bread samples analysed met with the Nigerian Industrial Standard (NIS) limit which means that they were nutritious and 100% acceptable for consumption. Researchers such as Mongi *et al.* (2011) carried out proximate analysis using coco-yam wheat bread, he also proved that the composites were 100% acceptable. The fungi isolated from the bread samples were *Aspergillus niger, Aspergillus flavus, Aspergillus ochraceus, Penicillium expansum, Rhizopus stolonifer, Mucor* sp and *Pichia* sp. During spoilage, *Rhizopus* and *Mucor* (mostly found in old bread) are the first to occur, followed by *Aspergillus* and *Penicillium* although, *Aspergillus* has greater significance in tropical areas (Banwart, 2004). *Pichia* sp, which is a yeast-like fungi causes an uncommon type of bread spoilage known as chalky bread which is characterized by chalky spots all over the surface of the bread. The findings of this research is similar to that of Nwakanma & Unachukwu (2015) who isolated and identified *Rhizopus* sp, *Fusarium* sp, *Peniciilium* sp, *Mucor* sp and *Aspergillus* sp as organisms that cause the spoilage of bread. Mold spoilage occur after baking as they cannot survive baking conditions, contamination sources include packaging and poor handling environment. While yeasts contamination sources include slicing machines and conveyor belts (Saranraj & Geetha, 2012).

The susceptibility of the fungal isolates to four selected antifungal agents (Ketoconazole, Fluconazole, Co-trimazole and Terbinafine) was carried out. Lesser zones of inhibition was observed with azoles (Ketoconazole, Fluconazole, Co-trimazole) while greater zones of inhibition were observed with Terbinafine. No zone of inhibition was observed for Fluconazole and ketoconazole against *Aspergillus ochraceus* and *Pichia* sp respectively, this could be that the drugs are not active against them. From the findings of the research, it was discovered that Terbinafine had greater antifungal activity than azoles. This is similar to the research of Bagyalakshmi *et al.* (2006) who used ketoconazole, fluconazole and Amphotericin B against ocular fungal isolates using three methods of *in vitro* antifungal susceptible test to the determine the Minimum Inhibitory Concentration (MIC).

CONCLUSION

Proximate analysis carried out on the bread samples proved that they were nutritious and safe for consumption as the bread were line with Nigerian Industrial Standard (NIS) limit. This research confirms that fungi (molds and yeasts) were associated with the spoilage of bread and among the organisms isolated, *Aspergillus* sp was discovered to have higher occurrence. Anti-fungal activity of the selected antifungal agents (Ketoconazole, Cotrimazole, Fluconazole and Terbinafine) against the probable isolates were effective. Terbinafine had higher activity and inactivity of some of the agents maybe due to resistance of the organisms to the antifungal agents.

RECOMMENDATIONS

Preventive measures and urgent attention is needed to minimize occurrence of fungi so as to reduce economic loss and avoid diseases as a result of consuming deteriorated or spoilt bread. Proper hygiene should be taken seriously while handling, packaging and storage. Materials (cream mixers, savor bags, roots, etc) which

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come in contact with bread must be washed hot or disinfected. Cleaning plans should be made to guarantee that the risk of foreign bodies in the bakery is drastically reduced (no glass in production rooms, no other small items that could fall in the dough). The use of bio-preservatives also should be employed in order to increase the shelf life and quality of bread.

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Water Tank Level Indicator Leakage Detection and Automatic Pump Controlling System

***[1]Adamu A., [2]Badeggi Y. A., [1]Ibrahim A., [1]Gana U. M., [1]Maipan-uku J. Y., [2]Abubakar A. & [1]Yusuf M.**

[1]Department of Computer Science, Ibrahim Badamasi Babangida University, Lapai, Niger State, Nigeria
[2]Department of Mathematical Sciences, Ibrahim Badamasi Babangida University, Lapai, Niger State, Nigeria

*Corresponding Author Email: aadam@ibbu.edu.ng

ABSTRACT

An electric sensors regulator suitable for sensing water level and leakage detection was realised using Arduno MEGA 2560 Microcontroller. Theses enable the entire system functionality to be tested as threshold indicator for water level and detection of leakages, thus displaying in Liquid Crystal Display (LCD) output. The developed water level sensor and leakage indicator was tested in real time by using plastic storage containers as tanks, pump and sensors. Experimental results indicated that the system is capable of performing the required functionalities of sensing and reporting leakages, and also indicating minimum and maximum water level threshold from reservoir or overhead tank by maintaining sets of minimum and maximum levels of one litres and ten litres respectively thereby switching the pump ON in the case of minimum threshold and OFF in the case of maximum threshold.

Keywords: *Water Level Indicator, Leakage Detector, Sensor, Microcontroller*

INTRODUCTION

The daily routine of both human and animals begins with water. Water is one of the basic need of life and means to survive. Hostels, hotel, companies and farms depend on overhead tank for their daily usage. The water level indicator circuits are used in factories, chemical plants, and electrical substations and other liquid storage systems to indicate level of liquid. There are many other possible uses of this simple system, examples include monitoring a pump pit (to control pump activation), rainfall detection, and leakage detection. Electronic water level circuits have the capability of alerting if there is a water leak somewhere in the factory. When the water level is too high or too low or exceed the higher limit, it can detect the water level easily by triggering an alarm sound or displaying different colors of light bulb. We can also measure the fuel level in motor vehicles and the liquid level containers which are huge in the companies. An automatic water level control detects the water level in the tank and also ensures continuous water flow round the timer. This overhead tank are mostly made of opaque material to prevent dust and mosquito infestation, many time we switch and forget to turn off the overhead tank because most water level are unknown which lead to water scarcity and wastage. Water level measurement can be achieved by determining the distance from the

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upper surface of a liquid in a reservoir or vessel or any arbitrarily chosen mark located above or below this surface by itself. The water level measurement is not an independent physical quantities describing the state of a substance through direct and indirect level; some examples of direct level measurement are dipstick, capacitor type, liquid level radiation type and liquid level measurement. The dipstick measurement is very simple OFF and ON system that trigger the pumping machine when the water level in the storage tank has reach its maximum level.

Electronics circuit has undergone tremendous changes since the invention of a triode by LEE DE FOREST in 1907; they are one of the active component likes resistors, inductors and capacitors in the 1970's. The circuits were separated at distinct unit, connected by soldered lead. With the invention of a transistor in 1984 by W.H Brattain, the electronic circuit became considerably reduced in size. It was due to the fact that transistors were not only cheaper, more reliable and less power consumption but was much smaller in size than an electronic tube. To take advantage of small transistors size, the passive component too was reduced in size there by making the entire circuit very small. The components were further reduced in size by eliminating bulky wiring and tie point. In the early 1960 new field of micro-electronics was born primarily to meet the requirement of the military which was to reduce the size of it electronics equipment to approximately one tenth of it than existing volume. This drive extreme reduction in the size of electronic circuit has led to the development of micro-electronics circuit called Integrated Circuits (ICs) which are so small than their actual construction is done by technicians using high powered microscopes.

Most water head tank at Ibrahim Badamasi Babangida (IBB) university Lapai depend on human intervention/supervision in case of leakage or minimum/ maximum threshold indicator of water level. Water level indicator can simply be defined as a system of getting information of water within a reservoir (Oyndrilla, Aranyak and Debasis, 2016). Water indicator and leakage detector are significantly important in order to reduce wastage of water from storage tank. The system has an attached automatic pumping machine to do the refilling, once a command is sent to initiate at lower threshold, its switch OFF the pump once the liquid gets to the maximum threshold. It also detects leakages, indicate and switch ON the pump when the tank or reservoir is empty. Nigeria is said to be suffering from economic water scarcity due to the inability of the stake holders to properly manage and protect these resources for socioeconomic development and environmental sustainability. Therefore, the significance of monitoring and management of water storage tanks in IBB University and Nigeria as a whole cannot be over emphasized, as it is of utmost importance to preserve water for human beings.

This paper developed water tank level indicator, leakage detection and pump controlling system in order to reduce wastage of water in storage tanks at IBB University. The system detects, indicates, monitor, controls and maintain the water level in the overhead tank and ensures the continuous flows of water round the clock without the stress of human intervention and monitoring of going to switch the pump ON or OFF thereby, saving time, energy, water, and prevent the pump from overworking. The remaining sections of the paper discuses the literature review, the methodology, the results

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and the discussion and recommendation for future work.

Review of Related Works

An integrated circuit is a complete circuit in which both the active and passive component are fabricated on a tiny single chip of silicon, examples are transistors and Field Effect Transistors (FET). Ebere *et al*., (2013) used copper sensors positioned at a particular level in tank as level sensor and considers electrical conductivity of water and immersed metal contacts (copper) to compare the voltage corresponding to the water level with reference to voltage. Debasis, Oyndrilla and Aranyak, (2016) developed automatic water level indicator using transistor, water sensor, power supply and sets of regulators. Anyanwu, Mbajiorgu and Anoliefo, (2012) developed water level controller using MC140066 integrated circuit to control water level in real time their by switching OFF/ON the regulator when the water level attained minimum/maximum threshold. Sourove *et al*., (2016) developed water level detector and automatic pump controlling system using microcontroller and sets of sensors. According to Pandey *et al.* (2011), agricultural land management practices are compatible with the preservation of water resources; hydrological diagnoses are needed in order to choose the alternative land uses, cultivation practices and their spatial arrangements. Kon *et.al.* (2009) explained the use of image sensor for ensuring water level as the most recent approach different from other types of sensors, it can provide the surrounding information around the sensor as well as the water level so that the measured data can be confirmed. It also has an advantage that it is unaffected by weather. Jerry (2010) deduced that Ultrasound echo ranging transducers can be used in either wetted(contact) or non-wetted(non-contact) configuration for continuous measurements of liquid level. An interesting application of wetted transducers is as depth finders and fish finders for ships and boats. On-wetted traducers can also be used with bilk materials such as grains and powders. In another related research, Sakharov *et al*. (2003) proposed a liquid level sensor by using ultrasonic lamb wave where the characteristics of the acoustic lamb wave propagating in a steel plate was observed experimenting, but the non linearity of the system renders it impracticable. Kaphungkui, (2014) utilizes the switching effects of a transistor which will be either in cut off mode or saturation mode depending upon the signal level applied to its base and a 555 timer control the state of output. A relay is used to switch the pump ON and only when the high output is produce from both dirt detection and when water level is low otherwise the pump switches OFF. Rasyid, Lee and Sudarsono, (2015) proposed an e-health sensor system that can be used to read vital health signals and store in the database server by using body temperature sensor and a blood oxygen sensor. These systems required used of vital signals collected from sensors and sensors data reading to a desktop application as well as stored data in the database and display the data via a website in the form of report that can be accessed remotely. Salomi, Amar and Anurag (2016) developed sensor that digitally sense body temperature and heart rate using arduino. Adriano was mainly used because it can sense the environment by receiving input from variety of sensors and can affect its surroundings by controlling lights, motors, and other actuators. The microcontroller on the board is programmed using the Arduino programming language. LM35 is used for the sense body temperature. Body

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temperature is a basic parameter for monitoring and diagnosing human health. Heart beat sensor was used for sensing heart rate. The device allows one to measure their Mean Arterial Pressure (MAP) in about one minute and the accurate body temperature will be displayed. The system can also be used to measure physiological parameters, such as Heart rate (Systolic and Diastolic) and Pulse rate.

METHODOLOGY

An efficient, and non intrusive means of indicating water level, detecting leakages of water, controls, monitor and managing water tank was achieved by using MC 14066 Integrated circuit and Arduno MEGA 2560 Microcontroller. The Arduno MEGA 2560 Microcontroller is designed based on ATmega 2560 microprocessor which runs at speed o 16MHz. it contains 54 digital output/input pins; 16 analog inputs, 4 hardware serial port and 15 digital output that can be used for Pulse Width Modulation (PWM) specifically for getting analog results with digital means output as shown in **Figure 1**.

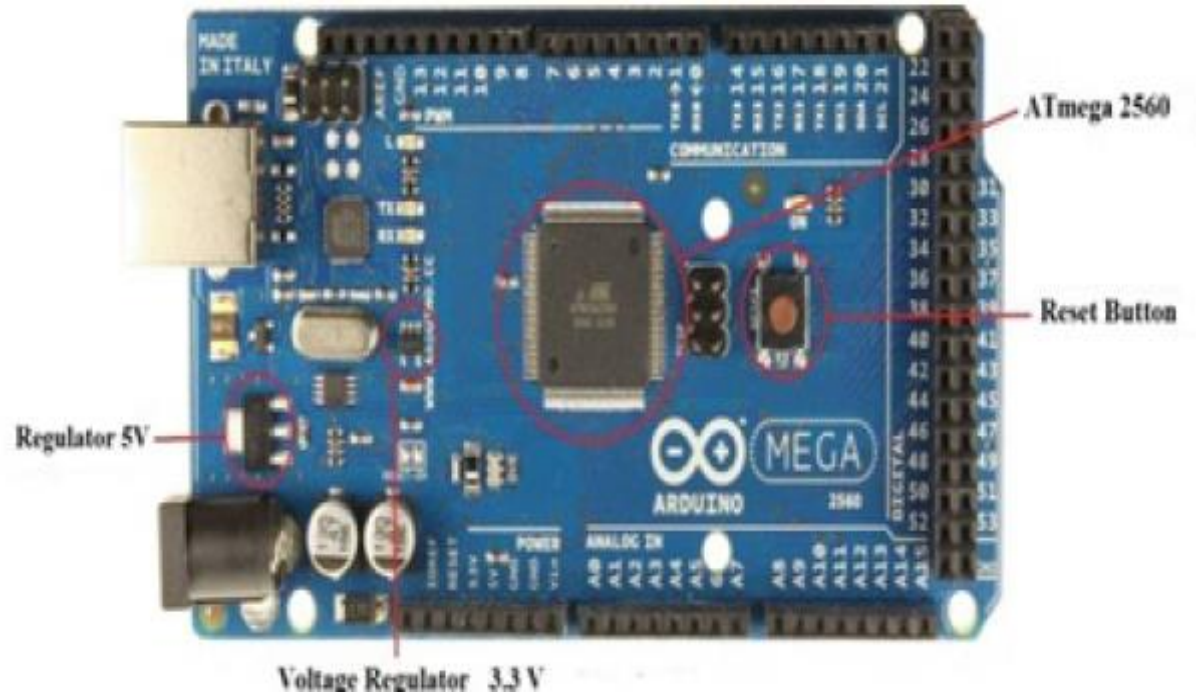

Figure 1: Arduno MEGA 2560 Microcontroller

The microcontroller is basically used to interpret the received signal. The programming of microcontroller is responsible for the display of welcome message, leakage detector, level control and sending of error messages and testing the functionality of the system (hardware, the software, the integration) and troubleshooting incase of system malfunctions or failure. Other hardware used are water sensors, transistors, storage tank, environment were tank is located, control switch or relay, pipe, tape, gum and power supply. The sensors are used for measuring water level and leakages. The architecture of the system is displayed in **Figure 2.**

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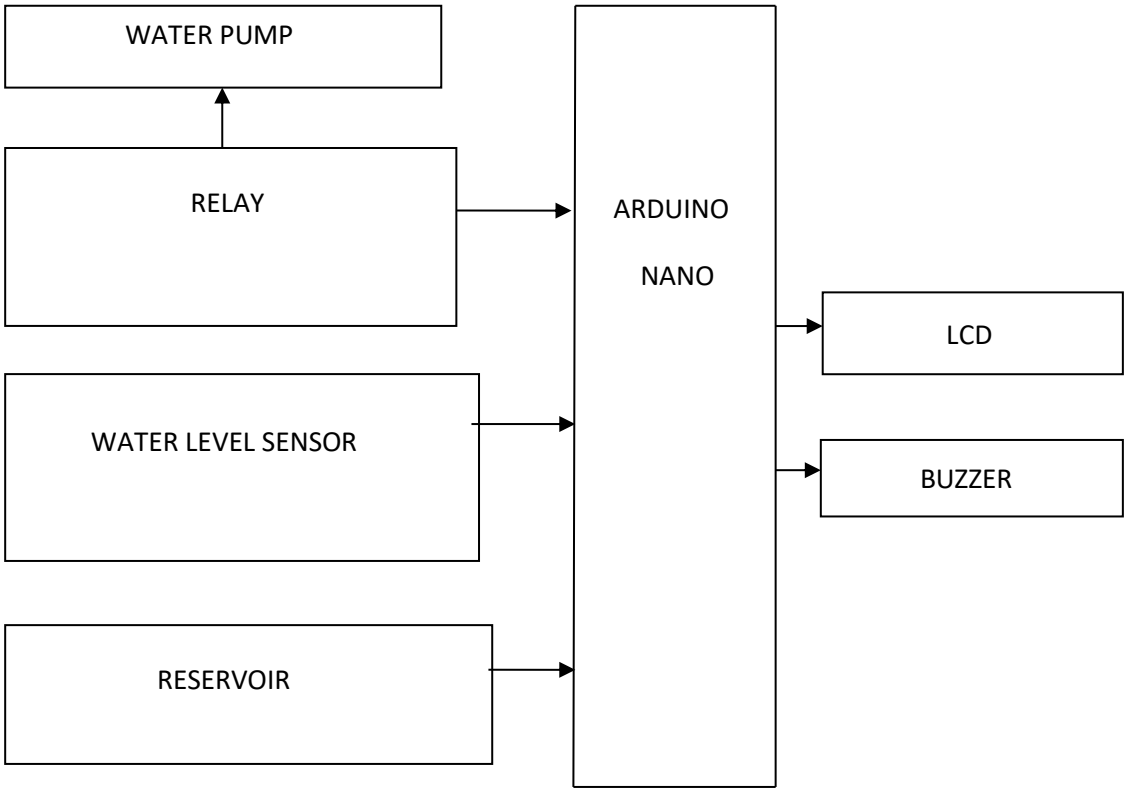

Figure 2: System Architecture

The architecture consist of Arduino microcontroller, water pump sensor, sets of relay, water pump, buzzer and water tank (reservoir). These components are activated by DC voltage with fixed value of 12 volt. The water pump supply water to the overhead tank (reservoir) at a very high pressure when turned ON. The water sensors detect leakage by collecting information. This information are controlled by the Arduino Mega 2560 microcontroller which decides the actions to be carried out; trigger alarm in case of malfunction or turn ON the water pump if the water threshold is low and turn OFF the water pump when the threshold is high using the electrical relays to mainly activate the water pump and valves based on the data received from the sensors.

Table 1: Arduino Maga 2560 Specification

Specification	Arduino Mega 2560
Serial Port	4
PWM Port	15
Analog Output	16
Digital Input/output Pins	54
Clock Speed	16 MHz
EEPROM	4KB
Data Memory	8KB
Flash Memory	256KB
Processor	ATMega 2560

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Based on the design specification and requirement of the system, the specific functions perform by the system is to control water level and detect leakages which occur at the surrounding or distribution units. Thus, the design is experimentally true when the sensors detect the required signal and transmit acceptable results to the hardware component specification as shown in the driven circuit diagram in **Figure 3.**

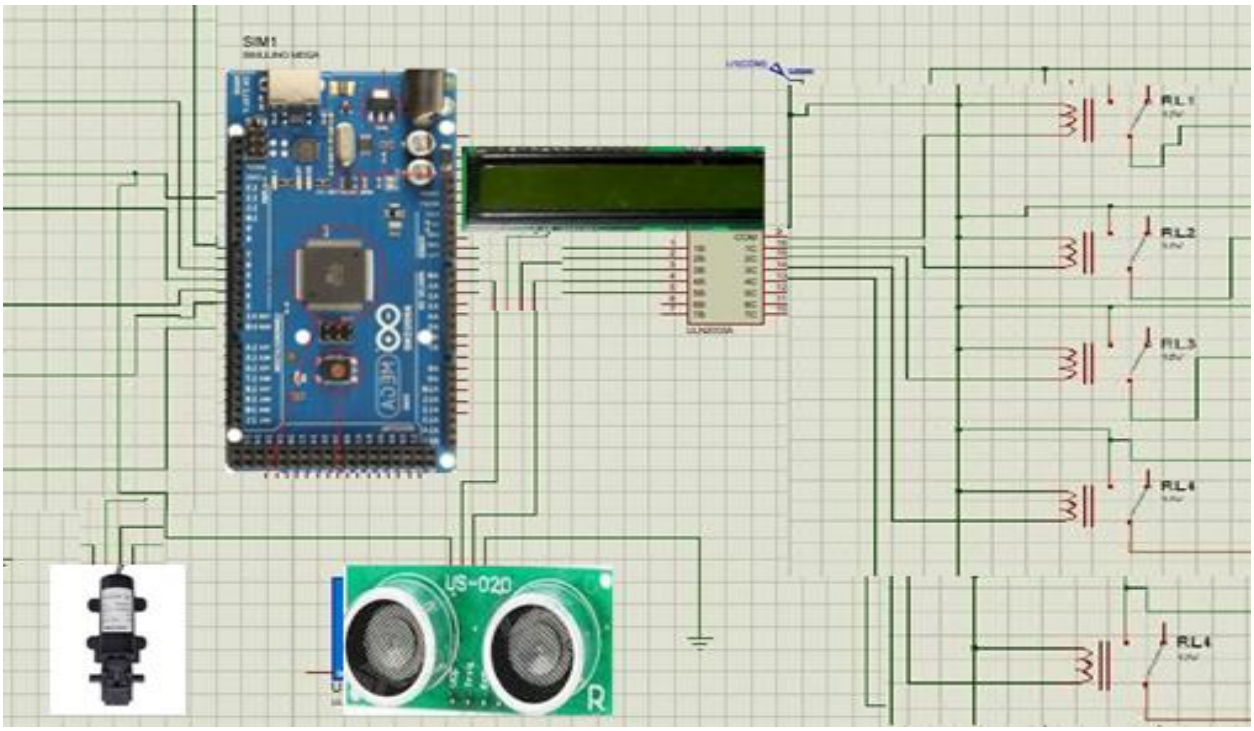

Figure 3: Complete Circuit Driving Diagram

The following codes are used to determine minimum and maximum threshold of the water level; the program work by initializing Pin to 1 and LCD to 12. The water level variable is set at 30 liters for maximum threshold and 5 liters for minimum threshold to read and transmit by the sensors:

```
Int Water_level;
Const intSensorPin=1              // Sensor Pin Connected to Pin 1
Const intLCDPin=12               // LCD Connected to Pin 12
Void Setup () {
Serial.begin (2560);
PinMode(SensorPin.INPUT);     //Set the input rate to transfer data
PinMode(LCDPin.OUTPUT);     //Display the LCD output
```

.

```
}
Void loop ()
{
Water_level= analogRead (SensorPin); // Arduino reads the value of water level from sensor
Serial.Prinln(water_level);      //Send water level reading from sensor for debuggin
delay (3000);         //delay for 3s
}
If(waterlevel>=30)
digitalWrite(LCDPin, Waterlevel);
Serial.Println("Water Level is High, Pump Goes OFF");
}
else
{
digitalWrite(LCDPin, Waterlevel);
}
}
```

The power cable is used to power the system. It consist of three main components; conductors, dielectric and sheath. LCD (liquid crystal display) is a type of flat panel display which uses liquid crystals in its primary operation. The Jumper wire is an electrical cable wire, or group of cable with a connector or pin at each end as shown in **Figure 4.**

Figure 4: Jumper Wire

The buzzer gives alarm or sound in the water system, it is an electrical device that makes a sound and is used for signaling. The buzzer is depicted in **Figure 5.**

Figure 5: Buzzer

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RESULTS AND DISCUSSION

The application module of system includes water level indicator, water leakage detector and reporting module. The water level indicator module sense and display the water level in the tank to ensure that there is no shortage or wastage of water by switching ON or OFF the pumping machine respectively. Other Error messages in case of malfunctions and reports are displayed on the Liquid Crystal Display (LCD). Alarm is triggered by the use of buzzer device in case of malfunctions from pumping machine or when water level and leakage sensors are faulty. The electronic circuits and LCD are shown in **Figure 6.**

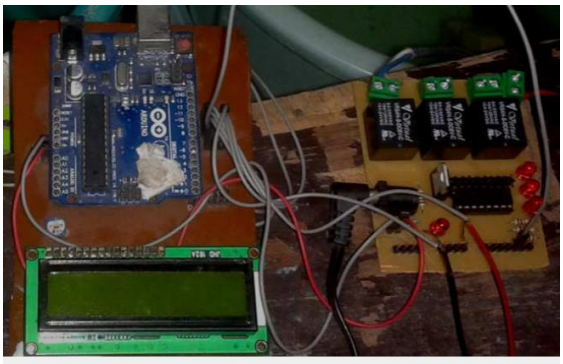

Figure 6: LCD and other Circuits

Experiment I: Maximum (High) Level Water Control

To test the maximum threshold control, we set the maximum water threshold at 30 liters level with a sensor attach at that level to detect and send signal as the water level reach the 30 liters level, a DC (12V) water pump was used to supply water into the storage tank at high pressure. As the water attain the maximum level, the sensor detect and send an output signal to the Arduino microcontroller which trigger the relay to power OFF the water pump, the buzzer also trigger alarm and the LCD screen display the message "Water Level High, Pump OFF".

Experiment II: Minimum (Low) Level Water Control

To test the minimum threshold control, we set the minimum water threshold at 5 liters level with a sensor attach at that level to detect and send signal as the water level reaches the 5 liters level, water was release from the storage tank for distribution to users in order to reduce the water level, the process continues until when the water level reduced to 5 liters and the sensor send signal to the Arduino which trigger the relay to power ON the water pump to supply water into the tank, the buzzer trigger an alarm and the LCD screen display the message " Water Level Low, Pump ON". The system maintains these processes by ascertaining the water level limits to control the threshold as long as there is power supply.

Experiment III: Leakage Detection

To test the functionality of leakage detection, a 5 mm hole was punch at the distribution unit of the pipe, water was released from the distribution valve, a leakage could be observed at the distribution unit, within 2-5 seconds, the buzzer trigger alarm due to the signal received from the leakage detection sensor and the LCD display the message "Leakage at distributing unit".

The summary of the three experiments are depicted in **Table 2**.

Table 2: Summary of Experiments

S/N	WATER LEVEL/Leakage	PUMP STATE	BUZZER
1	Full/High	OFF	ON
2	Low	ON	ON
3	Sense Leakage	OFF	ON

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CONCLUSION AND RECOMMENDATIONS

Based on the results collected and analysis of those results from the study of this project, it can be deduced that, implementation of water storage level detection monitoring and reporting system will go a long way in improving water efficiency in IBB university and environment which will reduce or eliminate the problems of shortages of water. The design and implementation of water storage level detection monitoring and reporting system, consist of Arduino microcontroller, water pump, sensor, liquid crystal display, relay and buzzer. The developed system has proven that it is possible to integrate and deploy an automated water level indicator, leakage detector, monitoring and reporting system in IBB University storage tanks and Nigeria as a whole. Further research can be conducted using similar technology in oil and gas industries.

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Design and Implementation of Invigilator(s)/Supervisor(s) Roster for Semester Examination Using USSD Code

[*1]Adamu A., [1]Ibrahim A., [1]Adamu A. I., [1]Maipan-uku J. Y., [1]Gana U. M., [1]Awal A., [2]Badeggi Y. A, [1]Kawu A. A. & [1]Lawal O. F.

[1]Department of Computer Science, Ibrahim Badamasi Babangida University, Lapai, Niger State, Nigeria

[2]Department of Mathematical Sciences, Ibrahim Badamasi Babangida University, Lapai, Niger State, Nigeria

*Corresponding Author: aadam@ibbu.edu.ng

ABSTRACT

Unstructured Supplementary Service Data (USSD) also referred to as feature code or quick code has become monumental part of services and products offered by Telecommunication Operators, and its application to various areas of telecommunication and real world scenarios. It is a protocol of communication utilized by Global System for Mobile communications (GSM) cell phones to communicate with mobile telecommunication network operators and applicable to various areas of trend in modern information technology. This research presents a system that will deliver examination roster/timetable using Unstructured Supplementary Service Data (USSD code) to invigilator's phones with USSD code capability. The research also explore the usability, convenience and speed of the USSD technology in an education context where invigilator who have to check their examination roster only need to dial the required USSD code instead of using the departmental notice board. This technology can, therefore, be highly efficient, effective and easily accessible in a timely manner.

Keywords: *USSD, Examination Roaster, Invigilator, Telecommunication Operators, GSM*

INTRODUCTION

We are now in the era of information evolution where computers and mobile phones have gone a long way making communication among people easier and faster. Telephone system has been the major means of communication which has been invented several years ago. This communication system had undergone a great improvement that it now supports many technologies and provide many services to subscribers such as Short Messaging Service (SMS), Interactive-Voice-Response Technology (IVR) and Unstructured Supplementary Service Data (USSD) which are most available in all mobile phones being produced today. Due to its speed and ease of use; customized Unstructured Supplementary Service Data (USSD) has become a technology that is widely used as a means of communication for sending and receiving text to and from a mobile phone and the codes can be used by anyone regardless of whether they are

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computer expert or not. The use of USSD in most higher education has recently gain advance applications in developing countries especially in semester result checking and examination time table as discussed by Kawu et. al, (2020). Another area in which USSD can be applied in higher educational institution includes staff welfare, attendance and leave management system (Adamu *et al*., 2021). Most departments in higher educational institution are information driven, and the administrative and academic staff drives and carry out day to day activities. Some of these activities are manually carried out while others are automated, it is the responsibility of departmental and faculty examination officers to design and keep record of these activities such as invigilators roaster and general examination time table which are manually carried out. The manual system of accessing and scheduling of Invigilators Roster/Timetable in Higher Institutions is always very tedious. The scheduling is done by the school's exam and record department. The number of lecturers in each department and their various capacities must be put into consideration, for each of the sections; levels and departments, there will be a time slot for each of the supervising day. Manually scheduling of this time is confusing and takes a long period of time to produce and excellent and perfect roster that is free from clashes.

The proposed system of invigilators roster for semester's exam using USSD code aside from allowing supervisors to request for invigilating roaster and other exam information, it will also notify the invigilator once their roster is out. Unlike Short Messaging Service (SMS), USSD does not require internet; its creates real time connection during session, allows up to 182 alphanumeric character in length, allows two way exchange of data in sequence and can be accessed by most kind of mobile cell phone. Communication is establish by dialling the USSD code beginning with asterisk (*) symbol followed by the digits that comprise data/commands or password and terminated by (#) symbol. Group of digits may be separated by asterisk symbol. Communication between the user, mobile cell phones and other devices are typically in real-time through a network or server. After successfully establishing connection between the user and backend system, users directly interact from their cell phone by following the prompt and making various selections from the menus. In higher institutions of developing countries, invigilators roaster are paper based, the request and management are carried out using conventional method. Conventional method of requesting and managing invigilators roaster for semester examination entails examination officers to design, print and placed invigilators roaster in departmental or faculty notice board. This method is prone to error, require more paper work and time consuming. Hence the need for automated technique that is free from error, faster and easy to manage with no or less paper work. USSD technique for semester invigilation/supervision and management system employs Global System Mobile (GSM) technology to automate and ease allocation, tracking and general management of examination monitoring. Hence, the system can be used for effective and efficient management of exam monitoring.

The remaining sections of the paper are divided into four. Section two discuses literature review and section three describes the methodology used in development of the system. Section four described the result obtained and section five

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concludes and gives recommendations for future works.

Review of Related Works

Various techniques have been proposed and adopted by many researchers for USSD application. USSD has been applicable to different areas of telecommunication such as mobile commerce, mobile chatting, call back services, prepaid balance inquiries, mobile banking and software upgrade (Gupta, 2010; Sanganagouda, 2011). These services are available as pull based resources and services such as currency updates, stock market report, sport updates, polling/voting emergency service, advertising and promotion of new product and services. Kawu *et al*. (2020) developed a mobile USSD-SMS Interactive Result Checking System for Resource Constrained Settings. Moses *et al.* (2013) proposed model for location based mobile advertising system using USSD and SMS symbolic to display menu for user registration and information access. Rob *et al.* (2017) developed sensors Empowerment and Accountability (SEMA) platform in Tanzanian for monitoring the functionality status of rural water service maintenance using USSD technique. Ojo and Ogundare (2018) developed an offline USSD system for accessing agricultural E-Commerce product and services by farmers. This was achieved by using offline module to manage crop inventory and other basic details by farmers with the aid of mobile cell phone. Baraka *et al*. (2013) suggests method of security that is more secure while using SMS and USSD technologies in mobile banking. Hridita (2018) documents an internship report on leave management system but due to time factor, confidentiality of organizational data and resources limitation only few details of the system were described. Mishal *et al.* (2017) developed an intranet based leave management system that can be accessed within an organization. The system can be used to request, approve and generates reports of leave but only on works on intranet. Manish *et al*. (2015) developed leave and pay roll management system for requesting, viewing of history and granting/rejecting of leave request. Rushitha *et al*. (2019) developed Android based leave management system for effective and efficient management of staff leave request and approval/rejection. Although the system can manage staff leave efficiently, it can only work on Android Operating System platform as such cannot be used on non Android phones. Isaac *et al*. (2018) developed prototyped of cloud based employee management information system for African small and medium enterprises consisting of four modules; leave management, payroll management, staff appraisal and record management. Though the system is designed to cover all aspect of human resources in African small and medium enterprises, only prototype of the proposed system was developed at the time of carrying out this research. Vikrant *et al*. (2017) developed an intranet based student leave management system that automates the work flow of leave approval and rejection. Stephen *et al*. (2018) developed algorithm for scheduling leave in Nigeria university system for academic staff. The system processes service delivery by determining staff mix by rank and lecturer-to-student ratio.

METHODOLOGY

This section gives an in-depth description of the architectural design and development of USSD system for monitoring and management of invocator's roaster in semester examination at Ibrahim Badamasi Babangida University, Lapai, Niger State, Nigeria. The architecture of USSD system is shown in Figure 1. The developed USSD

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system was designed around Adamu *et al*., *a.l* (2021). The system is designed in such a way that a supervisors and invigilators can search, track, locate examination venue at their convenient time in timely manner by dialing the required USSD code using a mobile phone. The system was design by integrating the USSD channel into standard GSM architecture. The architecture consists of three parts; the front end, the middle end and the back end.

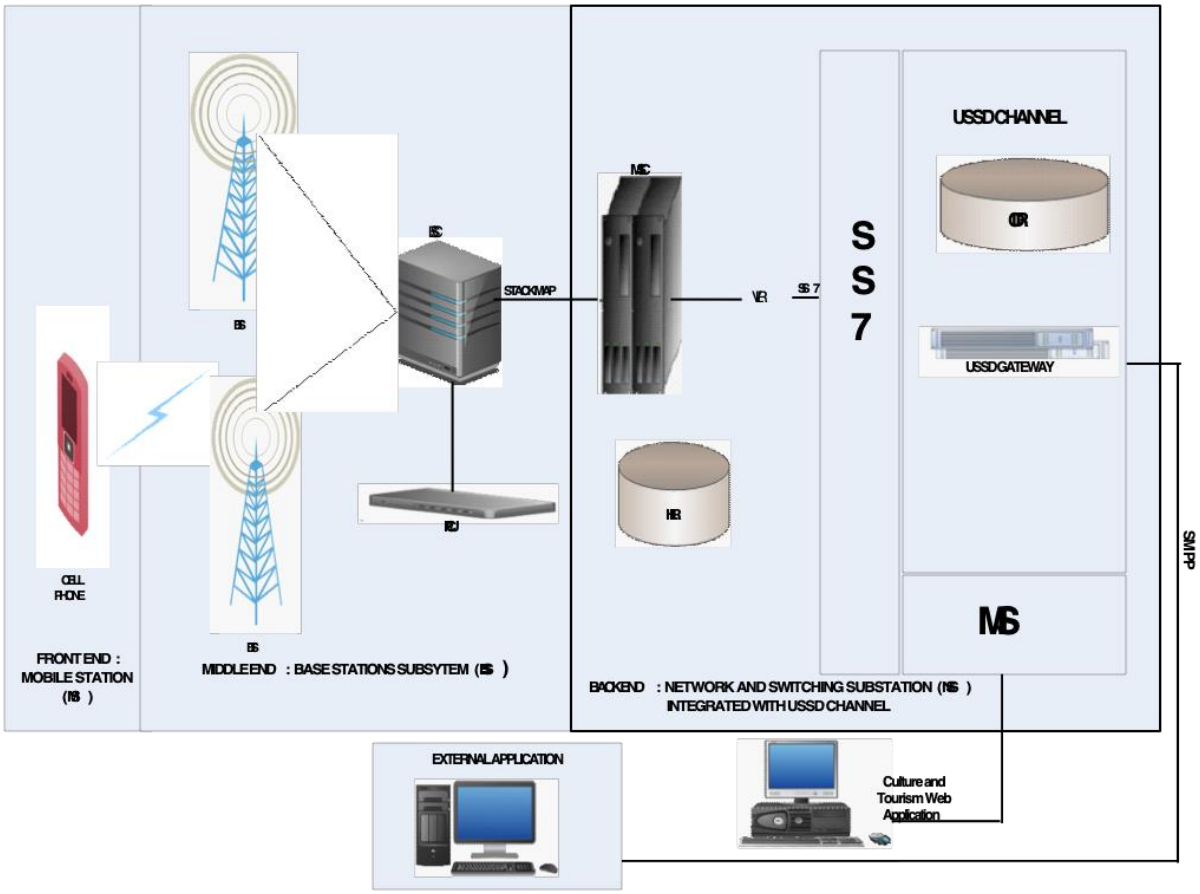

Figure 1: Architecture of the USSD System (Adamu *et al*., 2021)

The front end is also known as Mobile Station (MS) unit, the MS unit is the unit which the invigilator sees and operates with the aid of mobile cell phone configured to enable USSD interface connection between the front end and middle end, connection between these end is establish when the user dials the required string. The middle end also known as Base Service Substation (BSS) consists of the Base Transceiver Station (BTS) and Base Station controller (BSC) elements. The fundamental functions of these elements are associated with establishing communication between the invigilators mobile device and the network. The BTS network consist of antennas with associated radio transmitter and receiver for directly transmitting and receiving signals with the invigilators mobile cell phone and also equipment for encrypting/decrypting communication. The function of the BTS is controlled by parent BSC through Base Station Control Function (BCF). The BSC controls group of base transceiver stations by managing radio resources, allocating channels and controlling hand over within the group of

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BTS. This enables several users of the USSD system to concurrently access the network. Further connection between the user mobile cell phone, the middle end and back end (network and switching substation) is enabled via the BTS and BSC using stack Mobile Application Part (MAP) protocol. The essential function of the MAP protocol is enrooting messages using USSD; MAP also contains layers which are present on both MS and network server handler in the network nodes. The back end generally known as Network and Switching Substation (NSS) consists of Mobile Service Switching Centre (MSC), the Home Location Register (HLR) and the Visitor Location Register (VLR) elements integrated with the culture and tourism USSD channel. The culture and tourism management USSD channel consists of database called Call Data Record (CDR) where all staff data are stored. A USSD gateway; Classic USSD was collaborated into the system. The overall interaction is depicted using Data Flow Diagram (DFD) and UML diagram. The DFD and UML diagrams are shown in Figure 2 and 3 respectively. The diagrams show interactions within system and relationships with each other. The system has users which include administrators, head of departments, departmental and faculty examiners, and the invigilator. The invigilator has the privilege to view their available semester roster/timetable.

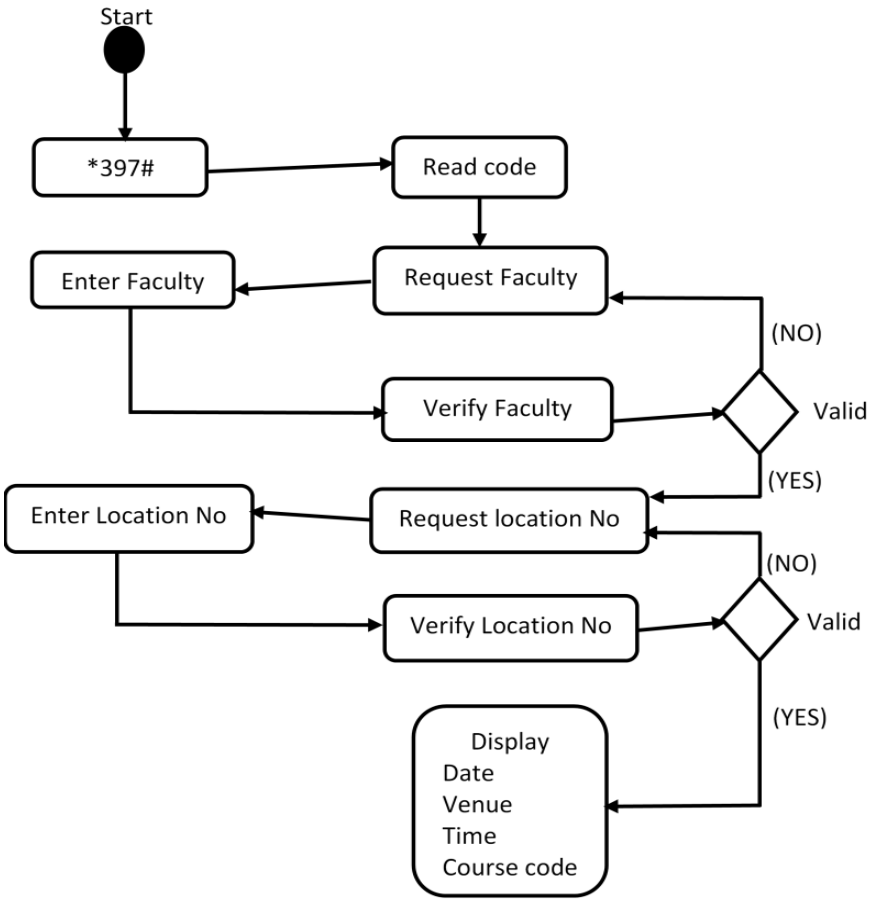

Figure 2: DFD Diagram Depicting Interactions within the System

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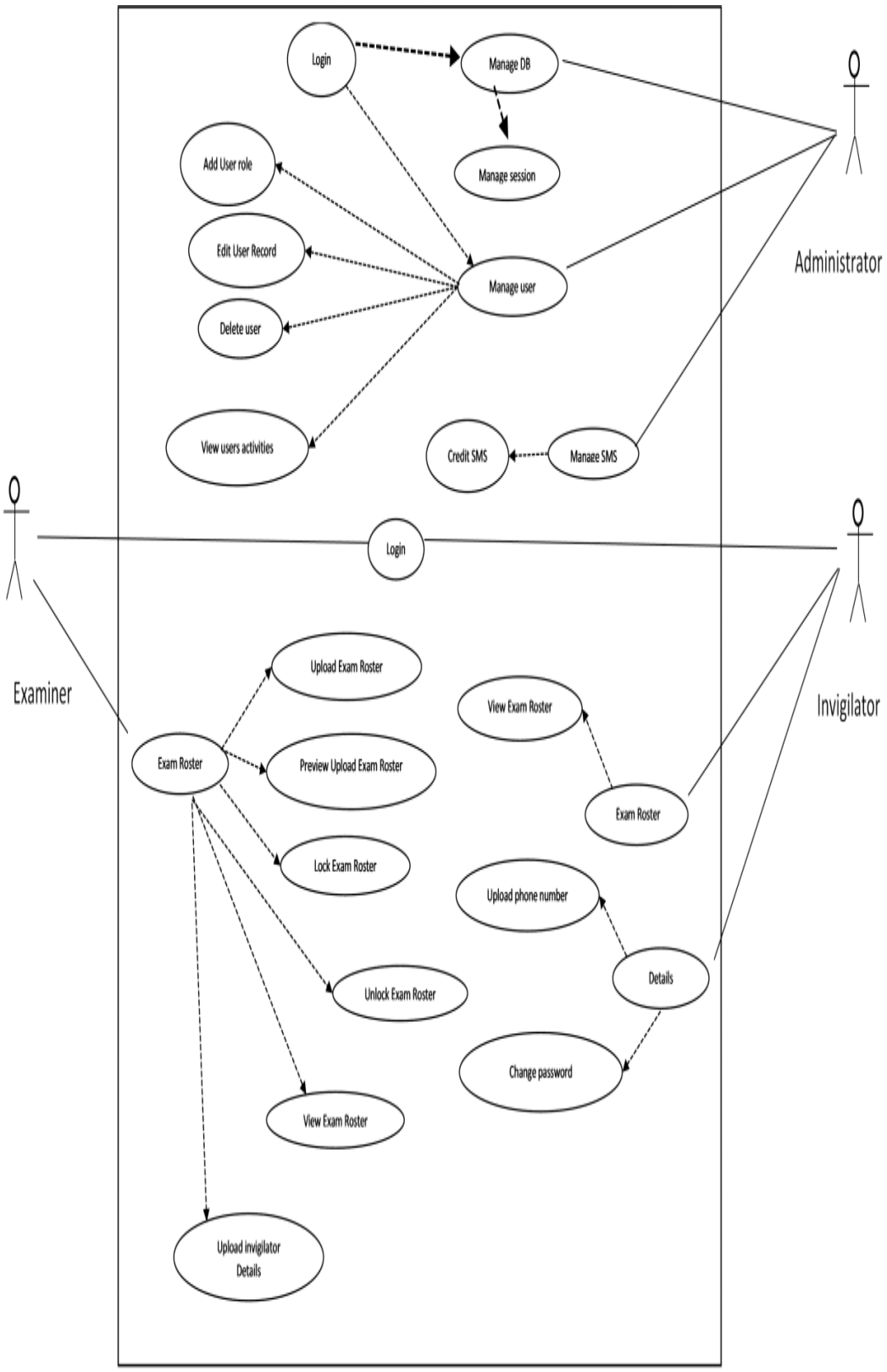

Figure 3: UML Diagram Depicting Interactions of the System

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The system is implemented using PHP for the overall programming, MySQL for the database and Windows 7 operating system as development environment with Adobe dream weaver CS3 IDE. Apache TOMCAT web server was used to host the system locally. Two interfaces were developed; one side with the mobile operators which requires setting up of SS7 stack and the USSD application over the stack. On the other side, HTTP based API's were used for the USSD application. The minimum hardware requirement for the development of the system include network of Intel P4 computers with 100 GB hard disks capacity, 4GB RAM, mobile cell phone and 2x1.6 Hz CPU processor with 3.5 GB. An 80 GB hard disk is also required for the virtual machine configuration of the web server.

INFERENCES

The increasing sophistication of the educational sector has brought about the need for up-to-date knowledge of data and a fast access to them.

Having identified the series of calamities caused by the manual method of generation of Invigilator's Timetable, there is the challenged to analyze these problems and proffer possible solution through the use of computer technology.

The work, therefore extols the use of micro-computers in developing an interactive, easy invigilator's timetable for computer science department institute of Basic and Applied Sciences, Kwara State Polytechnic Ilorin for the security of data and data integrity among other specialized measures and for a more vibrant and efficient operation.

RESULT AND DISCUSSION

Invigilator initiates the USSD service of the semester examination roaster by dialing *397# for registration and *384*29999# to commence various operations as shown in Figure 4 and 5 respectively; in defined format **"[Dial USSD code no.] [Select the faculty] [The supervising location No.] [Date request for] [Select period]"** Once the invigilator request is received by the system, it processes the request and relay the response back to the invigilator within a shortest period of time.

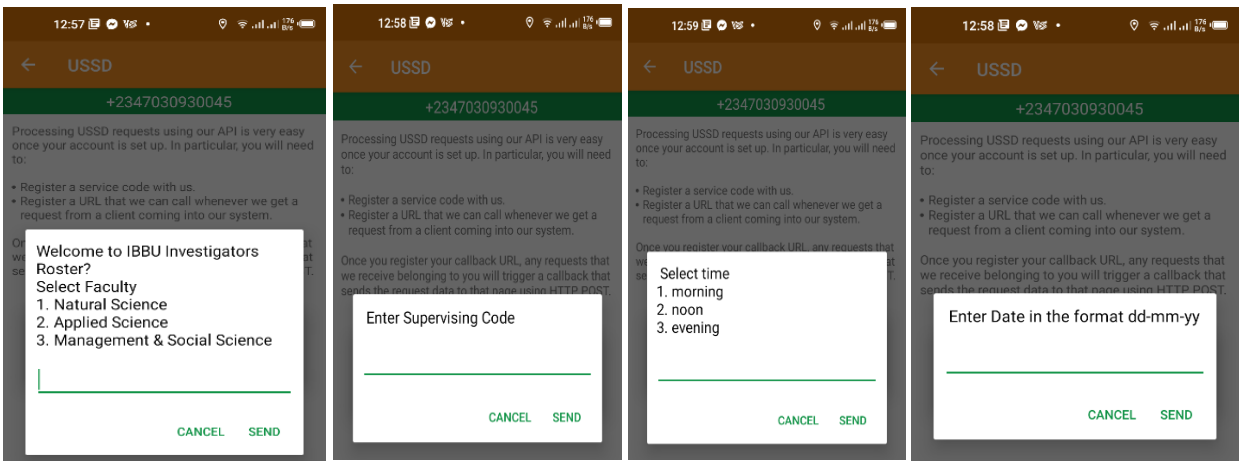

Figure 4: Various Prompt from the USSD Operations

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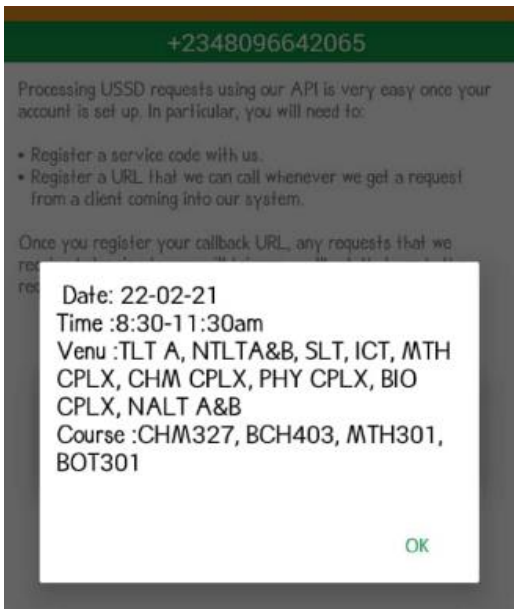

Figure 5: Final Output of the USSD Operation

SYSTEM PERFORMANCE EVALUATION

The system was tested and evaluated at Ibrahim Badamasi Babangida University Lapai, staff were selected to test the developed system and compare it with the conventional method used by invigilators and supervisors. Usability evaluation Method is used to evaluate the efficiency, accuracy and flexibility of the system. The usability study, evaluates the system's response time in delivering message to invigilator's phone and the suitability of the interface in terms of how easy it is to perform basic functions (such as, Roster/Timetable upload and Invigilators details upload). Flow sequence of using USSD is illustrated using a particulars invigilators from computer science department as shown in Figure 6.

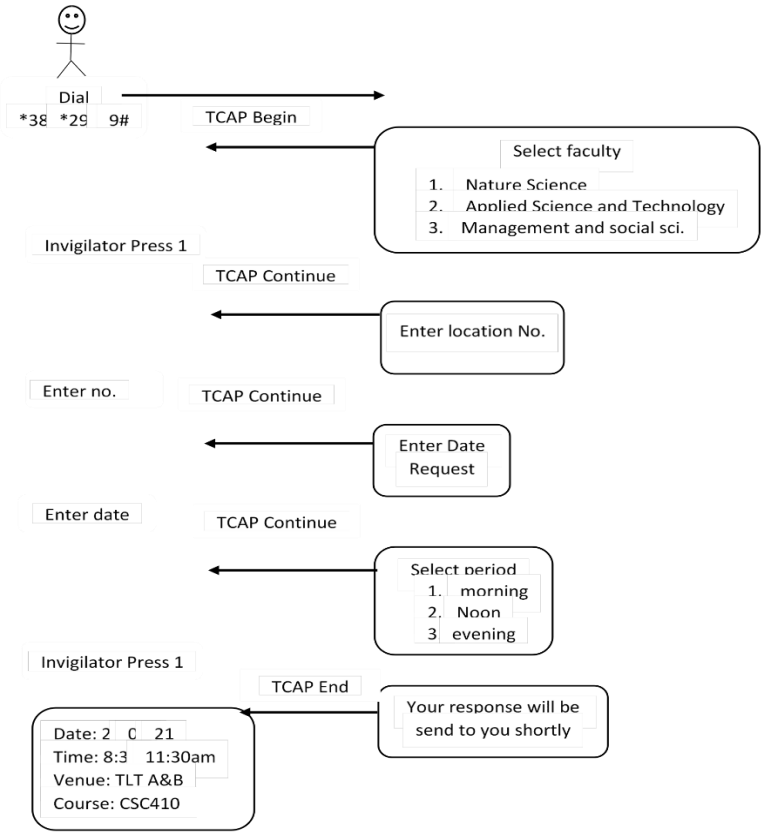

Figure 6: MAP/TCAP message sequence required to check the roster via the USSD code.

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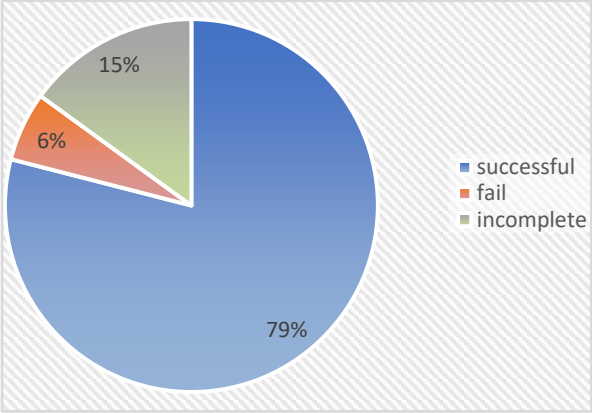
At the end of the tests, a total of 25 USSD sessions were recorded as shown I Table 1; indicating an 89% reliability of access using USSD with four telecommunication network operators. This does not compare with access through SMS short code which should expectedly be lower given the number of telcos supported or the limitations in view of the DND policy.

Table 1: USSD Sessions

m	Success	Failed	Incomplete
=> 25	17	3	5



USSD SEMESTER CHART

Figure 7: USSD Success and Failure

CONCLUSSION AND RECOMMENDATION

Invigilators Roster for semester's examination using USSD code is an innovative approach for requesting roster for exam monitoring and management. It should be widely used by universities in addition to existing means like website, email, SMS and IVR. The system utilized the versatility and portability of the mobile USSD code; it is designed as to allow invigilators get semester roster efficiently at their own convenience. Invigilators can check and receive their venue and time of invigilation instantly, without queuing at the notice board for a manual way of displaying roster. Though this system is in its nascent stage and more design refinement, exploration and evaluation is request. It will be great to extend the current design using the IVR as the user access route to the system rather than the USSD given the cost implications. The cost of USSD services is relatively high for HEI in developing countries, but IVR may lower this cost and could provide more interesting discourse.

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Effects of *Craseonysteris thonglongyai* (Bumblebee Bat) Droppings and Chemical Fertilizer on the Concentration of Some Antioxidants in the Leaf of C*nidoscolus chayamansa* (Tree Spinach)

[*1,2]Danazumi N. & [1]Musa A.

[1]Department of Biochemistry, Faculty of Natural Sciences Ibrahim Badamasi University Lapai, Niger State, Nigeria.
[2]Department of Science Laboratory Technology Federal Polytechnic Bida, Niger State, Nigeria

*Email: faithdestiny12@gmail.com, 09036594007, 08052858308

ABSTRACT

Cnidoscolus chayamansa is a highly valued plant because it provides valuable nutrients and antioxidants for maintenance of good health and prevention of some diseases like coronary heart diseases, diabetes, high blood pressure, cataracts, obesity among others. The concentrations of these nutrients in the plant are influenced by the soil nutrient compositions. The experiment was conducted to investigate the effects of *Craseonysteris thonglongyai* droppings and chemical fertilizer on some antioxidants (β-carotene, lycopenes, carotenoid, chlorophyll, tocopherol and ascorbic acid) content in the leaf of *C. chayamansa.* The concentrations of β-carotene, lycopene, total carotenoid, chlorophyll and tocopherol were determined by spectrophotometric method while ascorbic acid was assayed by titrimetric method. The result showed that the application of chemical fertilizer has no significant effects (P>0.05) on the concentration of β-carotene and chlorophyll in *C. chayamansa,* while their concentrations increases significantly with the application of *C. thonglongyai* droppings. Similarly, both chemical fertilizer and *C. thonglongyai* droppings significantly increase (P<0.05) the concentration of total carotenoid and lycopene, while the carotenoid and lycopene contents of those treated with *C. thonglongyai* droppings was significantly higher (P<0.05) compared to that of chemical fertilizer. Tocopherol and ascorbic acid contents in *C. chayamansa* were not significantly affected (P>0.05) with both chemical and *C. thonglongyai* droppings. The results of this study concludes that the concentrations of some antioxidant increase with chemical fertilizer and *C. thonglongyai*, while the concentrations of these antioxidants were higher in the vegetable treated with *C.thonglongyai* droppings.

Keywords: *Cnidoscolus chayamansa, Craseonysteis thonglongyai,* spectrophotometric, antioxidants, fertilizer.

INTRODUCTION

There is an increasing need to provide adequate and quality food for the entire people in the whole world today. Sequel to this is the acute need for increasing awareness about varieties and quality of food in relation to health which is significantly influencing our modern agricultural practice and food industries (Hornick, and Weiss, 2011). Approximately 80% of the world

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population depends on natural products particularly plant and vegetables for their health and healing (Aliyu, 2003).

Vegetables are good sources of antioxidants and nutrients for good health and are potential sources of mineral elements, vitamins, and antioxidants required for the maintenance of good health (Musa *et al*., 2015). Adequate intake of vegetables has been linked with a lowered prevalence of degenerative diseases in humans (Musa *et al*., 2015). Nigerian families select vegetables as an integral part of their daily meal because these vegetables are cheap and easy to cook. They can be cooked together with staple food, with the addition of condiments or they can be eaten raw (Antia *et al*., 2006).

Tree spinach (*C. chayamansa*) is cultivated in home gardens as such can be used throughout the year (Adeniran *et al*., 2013; Lucky and Festus, 2014). This vegetable is among the most important vegetables eaten in Nigeria. It is called "IyanaIpaja" by 'Yoruba' in south western region and in south eastern region it is called "Hospital too far", in the Northern part of the country it is called "Asibiti kusa" by 'Hausas' (Zarmai *et al.,* 2017; Lennox *et al*., 2018) because of its blood boosting effects. According to Fasuyi and Kehinde (2009). The vegetables widely consumed because of its health and nutritional benefits as it contains essential micronutrients required for the maintenance of good health and prevention of degenerative diseases. However, the antioxidant contents in *C. chayamansa* like any other vegetables are influenced by nutrient content and soil compositions. This research is undertaken to evaluate the influence of chemical and organic manures on the concentrations of antioxidants, in *C. chayamansa*, because farmers use these chemical and organic fertilizers to augment the poor soil nutrient.

MATERIALS AND METHODS

Study area: Pot experiment was conducted in the Department of Biochemistry, Faculty of Natural Sciences, Ibrahim Badamasi Babangida University, Lapai, Niger State.

Study duration: The experiment was conducted between 6th July to 23rd October 2019. The raining season occurs between April to October with mean annual rainfall of 1334 mm, with August and September having the highest rainfall of 300-330 mm (Musa *at al*., 2016).

Source of seedlings: Cuttings of *Cnidosculus chayamansa* were obtained from a home garden in Shanu village near Police Secondary School, Minna, Niger State.

Source of manures: Chicken droppings were obtained from poultry House along Tagwai Dam road after federal Government Secondary school, Minna Niger State while *Craseonycteris thonglongyai* (Bumblebee bat) droppings were obtained from a cave in Edati Local Government Area of Niger State, Nigeria. Chicken droppings and chemical fertilizer was obtained from Ndagi farm, Tagwai dam raod and Minna Gwadabe market respectively

Chemicals: All the chemicals used in this work are of analytical grade and were produced by British Drug House (BDH) and Sigma Chemical Company, England

Analysis of soil samples and manures: The soil sample used in this study was collected at 0-20 cm depth from Ibrahim Badamasi Babangida University, Lapai. This was bulked dried, ground and sieved through 2 mm sieve to remove debris. The sub-sample was then analyzed for particle size, pH, organic carbon, exchangeable acidity,

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exchangeable Ca, Mg, K and Na and total Nitrogen (Musa *at al*., 2016).

Experimental design, layout and nursery management: The experiment was laid out in a complete randomize design (CRD) and replicated three times. There are four treatments in this experiment; control, application of 100g each of *C. thonglongyai* and chicken droppings, application of recommended rate of chemical fertilizer. The experimental area and the surrounding were kept clean to prevent harboring of pest. The pots were lifted from time to time to prevent the roots of the plants from growing out of the polythene bags.

Manure treatment: The Chicken droppings and Bat droppings were dried and ground into powder, application was carried out three weeks after sprouting at the recommended rate of the four different treatments, namely:

Control (no Fertilizer application):	0g, 20 kg soil
*Craseonycteristhonglongyai*droppings:	100g/20 kg soil
Chicken droppings:	100g/20 kg soil
Chemical Fertilizer:	60 mg Nitrogen, 60 mg Phosphorous, 44 mg Morish of potash (K) / 20kg soil, (NIHORT, 1983; Musa *et al*., 2017).

Leaves of *C. chayamansa* cultivated under different soil fertility condition were harvested at vegetative phase of plant development (8–12 months) and used for determination of antioxidants).

Analysis of antioxidant in *Cnidoscolus chayamansa*

Estimation of beta-carotene: β-carotene was determined using the methods described by Musa *et al*., (2010)

Estimation of total carotenoids and lycopene: Estimation of total carotenoid and lycopenewere estimated using the methods described by Zakaria *et.al.*, (1979).

Estimation of chlorophyll: Chlorophyll content was estimated according to the method of Withman *et. al.,* (1971).

Estimation of tocopherol: Tocopherol was estimated by the Emmeric-Engel reaction as reported by Rosenberg (1992).

Estimation of ascorbic acid: The amount ascorbic acid in *C. chayamansa* leaves was estimated using titrimetric method described by Jone and Hughes, (1983)

Results and discussion

The result of the determination of Physical and chemical properties of the soil used for pot experiment is shown in Table 1.

Table1: Physical and chemical properties of the soil used for experiment

Parameter	Values
pH(H_2O)	6.2
pH($CaCl_2$)	6.25
CEC (cmol/kg)	2.88
Organic carbon %	8.67
Organic matter %	2.05
p (mg/kg)	13.96
Total nitrogen %	1.00

The result showed that the soil is slightly acidic in $CaCl_2$ and in water. The soil texture is loamy-sandy, which suggests that the soil has a moderate water retention capacity. The nitrogen and phosphorous contents of the soil are

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moderate. The concentrations of calcium and magnesium are moderate, whereas sodium is low, the potassium content is very low in the soil. The result also showed that the soil has a low concentration of organic carbon (Musa *et al*., 2017).

The result of the analysis of *C. thonglongyai* and chicken droppings used for the experiment is presented in Table 2.

Table 2: Physical and chemical properties of *C. thonglongyai* and chicken droppings used for the experiment.

Parameter	Chicken Droppings	C. *thonglongyai* Droppings
pH(H_2O)	6.82	6.38
pH($CaCl_2$)	5.72	5.56
CEC (cmol/kg)	18.7	23.7
Organic carbon (%)	18.7	16.1
Organic matter (%)	30.33	27.83
Acidity (cmol/kg)	0.03	0.03
Total nitrogen (%)	14.0	30.4

Values are MEAN±SEM of triplicate determinations.

The organic carbon, organic matter, total nitrogen and available phosphorous were very high in *C. thonglongyai* droppings while its pH (6.38) is slightly acidic. Similarly, the chicken droppings are very high in nitrogen, organic carbon, organic matter, and available phosphorous. The pH of the manure is slightly acidic (6.82) while conductivity or cation exchange capacity is moderate (Musa *et al*., 2017).

The result of the **e**ffect of organic manure and chemical fertilizer on the concentrations of antioxidants in *C. chayamansa* is presented in table 3.

Table 3 Effect of organic manure and chemical fertilizer on the concentrations of some antioxidants in C. *chayamansa*

Antioxidants	Control (no application)	Chicken Droppings	C. *thonglongyai* Droppings	Chemical Fertilizer
β-carotene (µg/100g)	2623.67± 93.42^a	3340.00± 26.20^b	4003.33±42.10^c	2416.33±164.05^a
Carotinoid (mg/g)	933.33± 26.67^a	1857.87± 32.91^c	2213.07± 77.73^d	1370.13± 65.07^b
Lycopenes (mg/g)	156.00± 18.01^a	426.81± 5.61^c	567.63± 27.22^d	384.38± 19.39^b
Chlorophyll (mg/100g)	0.02± 0.00^a	0.03± 0.00^b	0.04± 0.00^c	0.02± 0.00^a
Tocopherol (µg/100g)	0.01± 0.00^a	0.03± 0.01^a	0.02± 0.00^a	0.01± 0.00^a
Ascobic acid (mg/100g)	44.33± 2.85^a	46.67± 2.91^a	49.17± 0.88^a	42.67± 1.67^a
I.C_{50} Values µg/cm³	2.79 ± 3.96^a	2.93 ± 0.64^a	2.98 ± 2.16^a	2.85 ± 1.21^a

Values are MEAN±SEM of triplicate determinations. Mean values on the same row with different superscripts are significantly different ($p \leq 0.05$). IC_{50} (Half maximal inhibitory concentration)

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The results presented in table 3 shows a significant increase in β-carotene, chlorophyll, lycopene and carotenoid concentrations in *C. chayamansa* treated with *C. thonglongyai* and chicken droppings when compared to control. This might be due to the increase amount and activity of chlorophyll which is a light absorbing pigment and lycopene which is an important precursor for the biosynthesis of many carotenoids including β-carotene, carotenoid and lycopene (Havling, 2006). These results agree with the findings of Nithiya *et al.*, 2015 and Musa *et al*, 2016 who all reported that organic manures improve the nutritional quality and antioxidant contents in plants along with the soil health. The increase in the concentration of β-carotene, carotenoid and lycopene in the vegetable treated with organic manures may be due to increased content and activity of chlorophyll and associated with light absorbing pigment following the application of manures (Musa *et al*., 2017). The β-carotene and chlorophyll content of the vegetable treated with *C. thonglongyai* droppings were significantly higher than the vegetable treated with chicken droppings whereas lycopene and carotenoid concentrations in *C. chayamansa* treated with chicken droppings were significantly higher than the vegetable treated with *C. thonglongyai* droppings. Arisha *et al.,* (2003) explained that organic manures stimulate many genus of microorganisms, enhance the microbial activity of soil and encourage the plant growth and nutrient uptake. The activity of these microorganisms with high nutrient content of the manures may probably augment the organic matter and soil biological interactions that may be capable of mending the soil for nutrients uptake and promote plant health (Musa *et al*., 2016, Musa *et al*., 2017).

The result of this research correlates with the observations of several previous researchers who have reported increase of antioxidants content of vegetables treated with organic fertilizers (Nithiya *et al.*, 2015 and Musa *et al*, 2016). Nutrients in organic manures are more readily available since the materials have to decompose and organic nutrients mineralized before absorption (Makinde *et al*., 2010), organic manure activates many different species of micro-organisms involved in the release of phytohormones and may stimulate the plant growth and nutrients content (Arisha *et al*., 2013)

CONCLUSION

This experiment found more β-carotene, more carotenoids, more lycopene, more chlorophyll, more Tocopherols and more vitamin C in *C. chayamansa* fertilized with *C. thonglongyai*, chicken dropping respectively when compared with the plants fertilized with chemical fertilizer.

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Geoelectrical Exploration for Groundwater at Day Secondary School Maikunkele Town, Niger State, Nigeria

[*1]Muhammad A., [2]Suleiman I. K., [3]Tsepav M. T., [4]Umar S.,[5]Adetona A. Abbass & [6]Aminu A

[1,2,3,4]Department of Physics, Ibrahim Badamasi Babangida University, Lapai, Nigeria
[5]Department of Geophysics, Federal University of Technology, Minna, Nigeria
[6]Department of Physics, Nasarawa State University, Keffi, Nigeria

*Corresponding Author: kaleabdullahi75@gmail.com

ABSTRACT

Vertical Electrical Sounding (VES) was used to review the groundwater potential for effective development and management in Maikunkele, Niger State, Nigeria. Thirty (30) VES points were examined using Schlumberger configuration with current electrode spacing ranging from 2.0 to 200 m. The VES data obtained were interpreted using WinResist computer software. The VES results revealed heterogeneous subsurface geologic sequence probe up to 20.20 m and beyond. The variation in lithology consists of sand/clayey/sandy-clay topsoil with resistivity varying from 115 to 1237 $\Omega m$and which wereunderlay bylayers of clay with resistivities varying from 41-461 Ωm. The third layer indicateda continuation of clay deposits of resistivitiesranging from 15.62-100 Ωm while the fourth layer in some VES points showeda combination of gravely sand with resistivity varying from 2674-4271 Ωm with a highest resistivity at the basement bedrock of 7920-16541 down to infinity. The weathered basement which corresponds to the viable points that can be dug for groundwater has it lowest resistivity at VES A2 with resistivity of 5.2 Ωm, followed by VES A6 with average layered resistivity of 76.9 Ωm, VES E4 with resistivity of 32.70 Ωm and VES E5 with resistivity of 169.20 Ωm, respectively.

Keywords: Lithology, Aquifer Potential, Geoelectric section, Basement and Vertical Electrical Sounding (VES)

INTRODUCTION

The search for groundwater has become paramount across the entire universe. This is because groundwater is considered one of the safest sources of potable water to both man and animals which could be for drinking, industrial and agricultural use (Horque *et al.,* 2009). Groundwater is the water that is embedded beneath the ground filling the pore spaces between tiny grains in sediment and classic sedimentary rock and occupying every little crevices in rocks. Basically, rain and snow are known to be the major sources of groundwater when they settle on or flow into the ground to form groundwater. However, the amount of water that finally percolates into the ground is strongly affected by climate change, soil type, the nature of rock present, topography and type of vegetation.

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According to Plummer *et al.,* (1999) and Water for Africa (2010), approximately 15% of rainwater ends up as groundwater with a slight variation of about 1 to 20 % for both local and regional. It is surprising to note that about 0.61 % groundwater of the global water distribution is about 60 times the availability of water in rivers and lakes on the surface of the earth (Water for Africa, 2010).

Electrical resistivity investigation is important in determining the groundwater potential of a particular area (Olasehinde, 1999; Ariyo*et al.,*2009; Anudu *et al.,* 2011 and Okoye *et al.,* 2004). The resistivity of any rock is largely influenced by the presence of trapped groundwater and this serves as an electrolyte. The mineral particles that form the shape of a rock are considered better resistors than groundwater, so the greater the amount of water contained by a rock/sediment, the lower its resistivity, provided the water is not salty. This deduction strongly depends on the part of the rock with more pore spaces and the fraction of this pore spaces filled with water (Lowrie, 1997).

2-D resistivity survey is usually employed in research involving relatively shallow depth anomalies, for example, geotechnical and environmental studies (Cosenza *et al.*,2006; Chamber *et al.,* 2006 and Auken, 2006). A major achievement in recent times in geophysics is the introduction of 2-D electrical imaging/tomography profiling to map out regions with moderately complex geology (Griffiths and Barker, 1993). However, VES technique probes deeper beneath the surface especially in regions with rugged terrain (Hamza *et al.*, 2006). Schlumberger electrode array gives better information about the subsurface as this method utilises expanding the current electrodes to attain deeper depth. The purpose of this research is to characterize the subsurface lithology of the region under investigation using VES employing the Schlumberger arrangement.

Site Description

The study area, Maikunkele is located between latitudes 09^{o} 40’37.17’’ to 09 41’37.15’’ N and longitudes 06^{o}29’51.66’’ to 06^{o} 30’51.55’’ E (Figure 1). The area is easily accessible by road via Minna-Kontagora road. The area falls within the south western part of Minna. The geology of the study area is part of sheet 164 of Minna and lies within the basement complex region of Nigeria. The area has distinct wet and dry seasons.

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Figure 1: Location Map of the Study Area (Abdullahi and Adetona, 2017)

Theory of Electrical Resistivity

The process of electrical resistivity investigation involves sending electrical current, I, via current electrodes and the returning electrical signal (voltage) is measured across the two ends of potential electrodes which turns out to be the potential difference, V. The most applicable array types include Wenner, Schlumberger, Pole-pole, Dipole-dipole and pole-dipole arrays. The fundamental equation for resistivity can be derived from Ohm's law (Grant and West, 1965; Dobrin and Savit, 1988; and Nwankwo, 2010).

$$\rho = \frac{RA}{L} \tag{1}$$

whereρ is the resistivity, R is resistance, L is the length of a homogenous conducting cylinder with cross-sectional area, A. Since the earth is anisotropic in nature, equation (1) can be further transformed as:

$$\rho_a = \frac{\Delta V}{I} 2\pi r \tag{2}$$

where $2\pi r$ represents the geometric factor, K which is constant for every electrode configuration. Schlumberger array was employed in this research with a geometrical factor given as:

$$G = \pi \frac{\left[\left(\frac{AB}{2}\right)^2\right] - \left[\left(\frac{MN}{2}\right)^2\right]}{2\left(\frac{MN}{2}\right)} \tag{3}$$

Apparent resistivity, ρ_a is the bulk average of all rocks and soil influence on the motion of the applied current. In a 2-D electrical resistivity survey, both the current and potential electrodes are placed in a linear array. ERT is a technique used to picture the bulk electrical resistivity pattern and distribution beneath the earth subsurface. Hence, resistivity is an intrinsic property of every material used to study the characteristics of the subsurface features. On the other hand, VES uses collinear configuration to generate a 1-D vertical apparent resistivity plotted against depth model of the subsurface at a particular sounding point. This method

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involves acquiring several potential differences at successively greater electrode spacing while maintaining a fixed central reference point. The induced current travels farther and deeper layers at greater electrode spacing. The measured potential difference is directly proportional to the variation in lithology. The variation of apparent resistivity estimated from the potential difference can give information about overburden thickness, depth to aquifer and depth and thickness of subsurface strata. A comprehensive review of electrical properties can be found in (SchOn, 2004) whereas a complete explanation of electrical methods can be found in Telford *et al.,* (1990 and Ward(1990).

Table 1: Resistivity of some common rocks and minerals (Telford and Sheriff, 1984).

Rocks/Minerals	**RESISTIVITY (Ohm's-m)**
Clay and Marl	1-67
Topsoil	67 – 100
Clayey soil	100 – 133
Sandy soil	670 – 1330
Limestone	67 – 1000
Sandstone	33 – 6700
Sand and gravels	100 – 180
Schist	10 – 1000
Granite	25 – 1500
Surface water (in igneous rock)	30 – 500
Groundwater (in igneous rock)	30-150
Weathered laterite	200 – 500
Fresh laterite	500 – 600
Weathered/Fractured basement	100-500
Fresh basement	>1000

Materials and Method

In this study, Vertical Electrical Sounding (VES) using Schlumberger configuration was applied in order to examine the variations in resistivity of materials with depth. Resistivity meter with model number G41 manufactured by Geotron was used to acquire field resistivity data. The entire survey was carried out with the help of Schlumberger array since it is usually characterized to delineate electrical resistivities associated with the change in lithology and/or hydrological characteristics (Hodlur, 2006). Furthermore, the Schlumberger electrode array has greater probing ability and is less affected by local heterogeneity.

Five profiles each having six (6) VES points were gridded making a total of thirty (30) DC sounding points as shown in Figure 2. The maximum spacing of half current electrode AB/2 (in the case of Schlumberger array) is equals the potential electrode spacing and it varied between 1 m to 100 m which is wide enough to give the required objectives of the research. The target parameter (apparent resistivity) was finally calculated at each sounding point using equation (2). The earth subsurface resistivity value is equal to the true earth resistivity if and only if the earth is isotropic (uniform).

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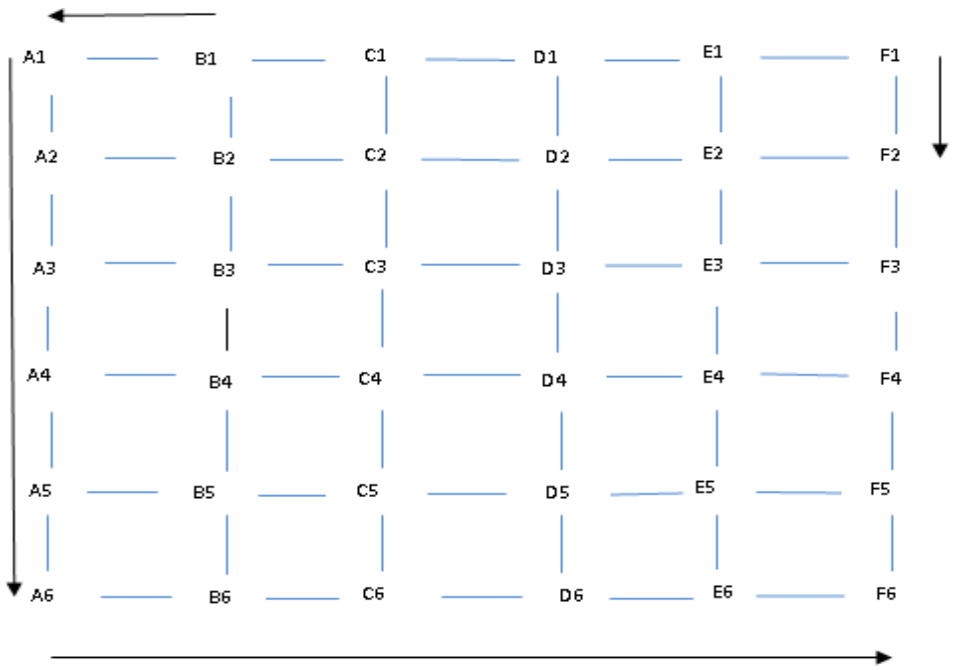

Figure 2: Profile layout and location of the VES points

RESULTS AND DISCUSSION

Methods of geophysics seek to reveal hidden information down beneath the earth's crust. Therefore, a geophysical investigation is one of the major tools used in studying the characteristics of the earth's subsurface (Nordiana *et al.,* 2012). Tables 2a, 2b, 2c and 2d shows the results of the VES curves at each sounding points and their corresponding lithology. After proper interpretation of the generated geologic sections and their correlations with nearby geologic sections of borehole log samples, the area under study is underlain with four geologic layers corresponding to topsoil, sand/clayey/sandy-clay, progression of clay soil, combination of sand/gravel (fracture basement) and fresh basement with resistivity values ranging between 113-1322 Ωm, 42-462 Ωm, 16.1-92 Ωm and 7760-15342 Ωm, respectively. The dominant curve types obtained in the area are AK, H, HK, KH and QH (Figure a, b, c, d and e). Figure 2shows the frequency of the different curve types that dominate the area of study. Qualitative hydrological deduction can be made from the different curve types to ascertain the possibility of groundwater (Singh, 1984). Furthermore, Satpathy and Kanugo, (1976) reported that the H and KH are often associated with the presence of groundwater.

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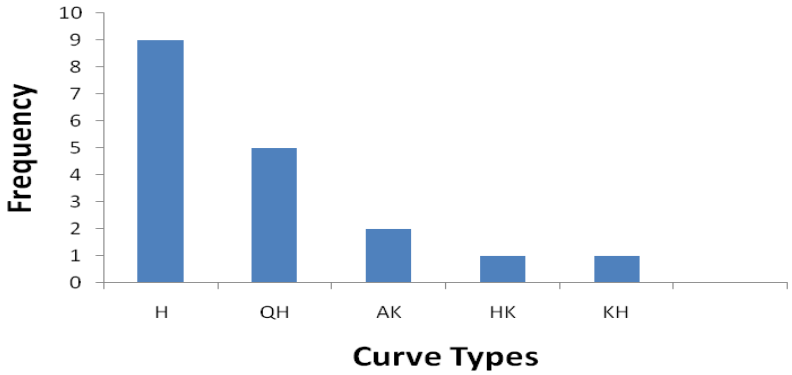
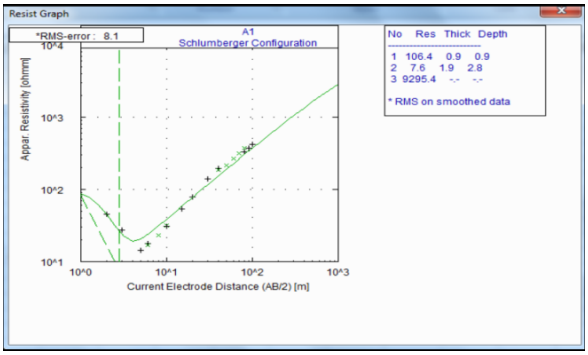
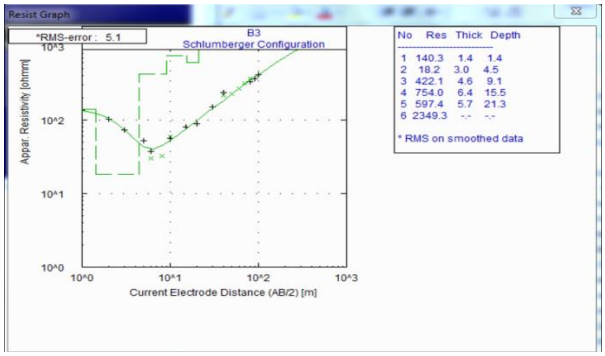


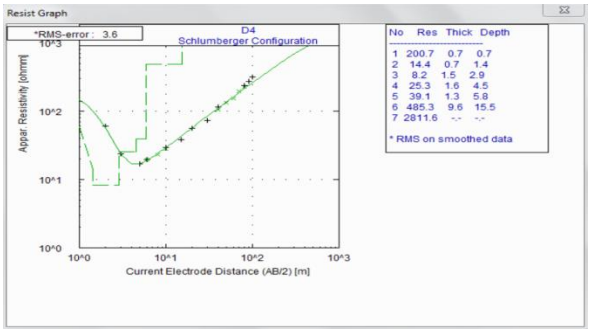
Figure 3: Frequency of predominant curve types obtained in the area of study.



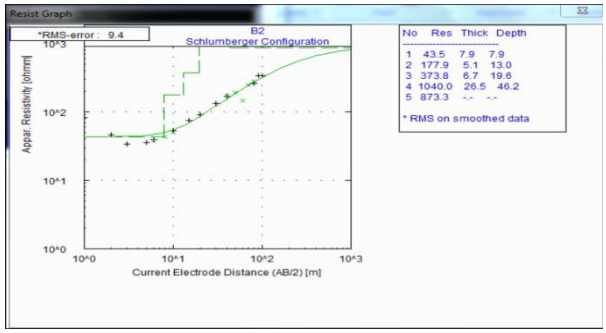
HK curve type (VES B3)



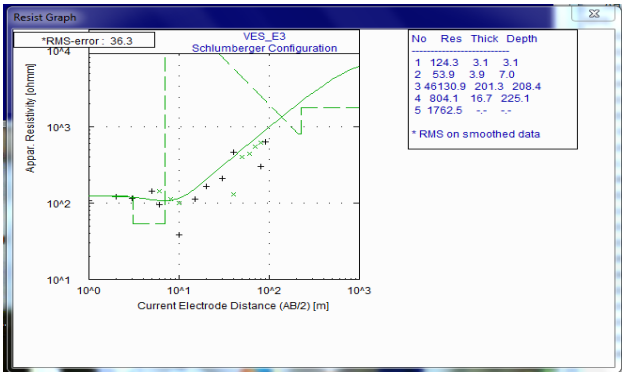
AK curve type (VES D2)



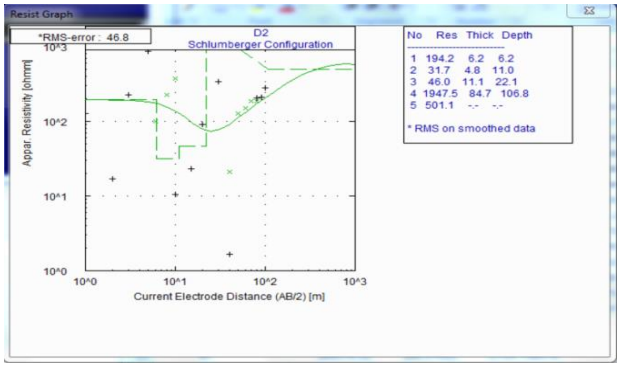
HK curve type (VES D4)



QH curve type (VES B2)



HK curve type (VES C3)



AK curve type (VES C2)

Figure 3: Samples of predominant curve types obtained in the area of study

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Table 2a: Summary of VES results along profile A in the study area

VES POINT	NO OF LAYER	LAYER AVERAGE RESISTIVITY (Ωm)	THICKNESS (m)	DEPTH (m)	TYPE OF CURVE	Infer Lithology
A_1	1	38.3	20.0	20.0	H	Topsoil (clayey sand)
	2	1065.1	20.0	40.0		Clay
	3	2915.3	∞	∞		Sand
A_2	1	3.4	0.3	0.3	KH	Topsoil (clayey sand)
	2	889.05	11.65	11.95		Sandy clay
	3	5.2	∞	∞		Bedrock
A_3	1	171.0	0.3	0.3	K	Topsoil
	2	253.05	5.06	5.09		Sandy clay
	3	122.9	∞	∞		Topsoil (clayey sand)
A_4	1	1.4	7.0	7.0	A	Clay
	2	132.55	20.65	27.65		Sand
	3	2263.6	∞	∞		Topsoil (clayey sand)
A_5	1	3.8	0.3	0.3	A	Sandy clay
	2	237.4	4.7	5.00		Bedrock
	3	1794.8	∞	∞		Top soil
A_6	1	365.9	3.0	3.0	H	Sandy clay
	2	81.0	37.5	40.50		Topsoil (clayey sand)
	3	76.9	∞	∞		Clay

Table 2b: Summary of VES results along profile B in the study area

VES STATION	NO OF LAYER	LAYER AVERAGE RESISTIVITY (Ωm)	THICKNESS (m)	DEPTH (m)	TYPE OF CURVE	Infer Lithology
B_1	1	179.70	5.00	5.00	A	Topsoil (clayey sand)
	2	457.00	15.00	20.00		Clay
	3	38937.60	∞	20.00		Sand
B_2	1	45.00	5.00	5.00	A	Topsoil (clayey sand)
	2	344.15	5.00	10.00		Sandy clay
	3	1233.6	∞	10.00		Bedrock
B_3	1	281.30	5.00	5.00	AK	Topsoil
	2	41057.85	5.00	10.00		Sandy clay
	3	884.40	∞	10.00		Topsoil (clayey sand)
B_4	1	161.20	20.00	20.00	A	Clay
	2	2001.40	10.00	30.00		Sand
	3	9949.50	∞	30.00		Topsoil (clayey sand)
B_5	1	94.80	0.80	0.80	A	Sandy clay
	2	247.50	2.40	3.20		Bedrock
	3	3369.00	∞	3.20		Topsoil
B_6	1	127.70	1.10	1.10	H	Sandy clay
	2	54.32	5.10	8.30		Clay
	3	8887	∞	∞		Bedrock

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Table 2c: Summary of VES results along profile C in the study area

VES STATION	NO OF LAYER	LAYER AVERAGE RESISTIVITY (Ωm)	THICKNESS (m)	DEPTH (m)	TYPE OF CURVE	Infer Lithology
C_1	1	54.90	0.50	0.50	K	Topsoil (clayey sand)
	2	50017.25	13.90	3.70		Clay
	3	10000.00	∞	14.4		Sand
C_2	1	92.50	8.00	8.00	QH	Topsoil (clayey sand)
	2	645.20	18.00	10. 00		Sandy clay
	3	48071.90	∞	26.00		Bedrock
C_3	1	47.20	1.00	1.00	A	Topsoil
	2	4655.45	10.00	10.00		Sandy clay
	3	22015.2	∞	11.00		Top soil (clayey sand)
C_4	1	10.00	0.700	0.70	K	Clay
	2	4956.35	0.30	16.10		Sand
	3	972.1	∞	1.00		Topsoil (clayey sand)
C_5	1	91.10	6.00	6.00	K	Sandy clay
	2	1141.15	6.00	20.00		Bedrock
	3	829.00	∞	12.00		Topsoil
C_6	1	10.30	0.30	0.30	A	Sandy clay
	2	1127.35	0.10	7.20		Sandy clay
	3	1530.40	∞	0.40		Bedrock

Table 2d: Summary of VES results along profile D in the study area

VES STATION	NO OF LAYER	LAYER AVERAGE RESISTIVITY (Ωm)	THICKNESS (m)	DEPTH (m)	TYPE OF CURVE	Infer Lithology
D_1	1	216.40	0.70	0.70	A	Topsoil (clayey sand)
	2	938.75	0.20	0.90		Clay
	3	78723.4	∞	0.90		Sand
D_2	1	101.80	5.20	5.20	A	Topsoil (clayey sand)
	2	5993.95	8.00	13.20		Sandy clay
	3	100000.00	∞	13.20		Bedrock
D_3	1	230.1	0.60	0.60	A	Topsoil
	2	35304.50	3.50	4.10		Sandy clay
	3	100000.00	∞	4.10		Topsoil (clayey sand)
D_4	1	2.10	0.30	0.30	HK	Clay
	2	150.40	0.90	1.20		Sand
	3	26918.50	∞	1.20		Topsoil (clayey sand)
D_5	1	457.2	0.5	0.50	H	Sandy clay
	2	34.45	0.5	1.00		Bedrock
	3	2159.7	∞	1.00		Topsoil
D_6	1	18.60	5.4	5.40	A	Sandy clay
	2	295.70	0.4	5.80		Sandy clay
	3	27586.00	∞	5.80		Bedrock

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Table 2e: Summary of VES results along profile E in the study area

VES STATION	NO OF LAYER	LAYER AVERAGE RESISTIVITY (Ωm)	THICKNESS (m)	DEPTH (m)	TYPE OF CURVE	Infer Lithology
E_1	1	393.10	5.00	5.00	H	Topsoil (clayey sand)
	2	61.70	5.00	15.00		Clay
	3	22968.80	∞	10.00		Sand
E_2	1	147.20	6.00	6.00	H	Topsoil (clayey sand)
	2	115.35	4.00	10.00		Sandy clay
	3	3143.50	∞	10.00		Bedrock
E_3	1	124.3	3.10	3.10	AK	Topsoil
	2	23092.40	16.10	70.00		Sandy clay
	3	804.10	∞	19.20		Topsoil (clayey sand)
E_4	1	38.50	15.00	15.00	Q	Clay
	2	32.20	5.00	20.00		Sand
	3	32.70	∞	20.00		Topsoil (clayey sand)
E_5	1	3.30	5.00	5.00	A	Sandy clay
	2	111.65	10.00	10.00		Bedrock
	3	169.20	∞	15.00		Topsoil
E_6	1	157.60	2.00	2.00	K	Sandy clay
	2	793.50	2.00	15.00		Sandy clay
	3	170.70	∞	4.00		Bedrock

Interpretation of Geo-electric Vertical Section along Profile A

The vertical geo-electric section of profile A is as shown in figure (4.8a) while its corresponding geologic section is shown in figure (4.8b). The map is contoured at an interval of 70 Ωm. This map can be divided into three layers.

Profile A has top soil with resistivity ranging from 140 Ωm to about 900 Ωm. This high resistivity is due to human influence. It is follow by a layer of clay saturated with ground water with resistivity ranging from 600 Ωm to 900 Ωm on VES points A1-A3. The low resistivity values observed on A4-A6 which is as low as 70 Ωm is a clear indication of weathered basement up to a depth of 30 metres.

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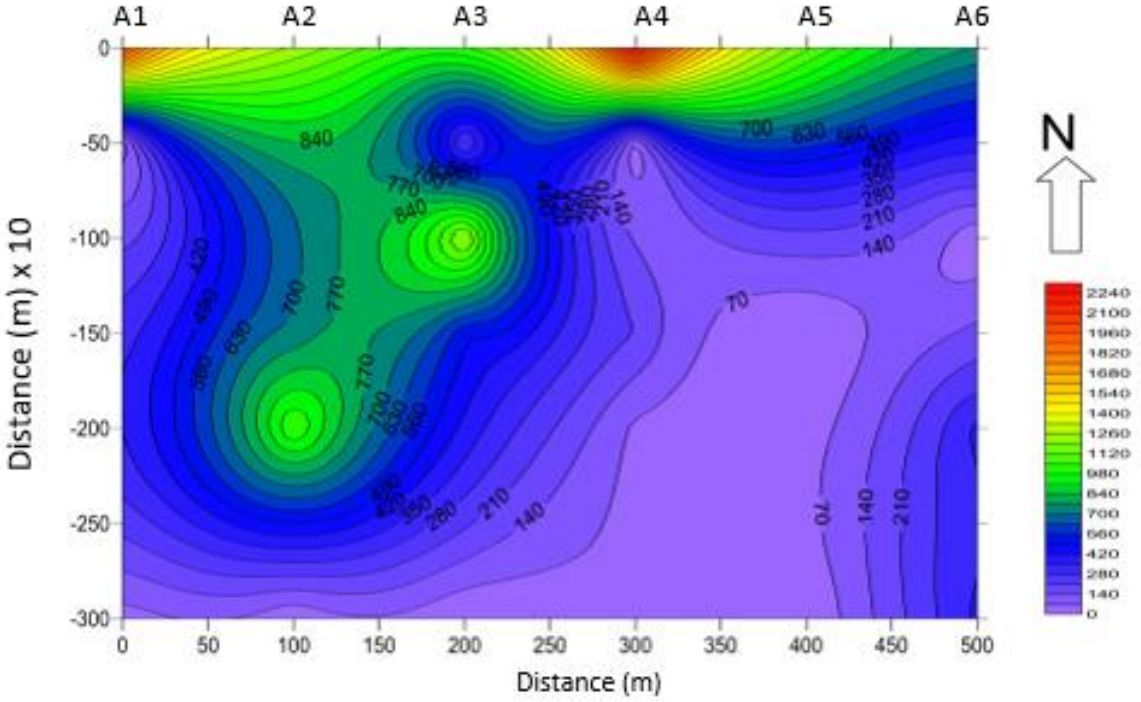

Figure 4a: Geoelectric Vertical section along Profile A (Contour Interval is 70 Ωm)

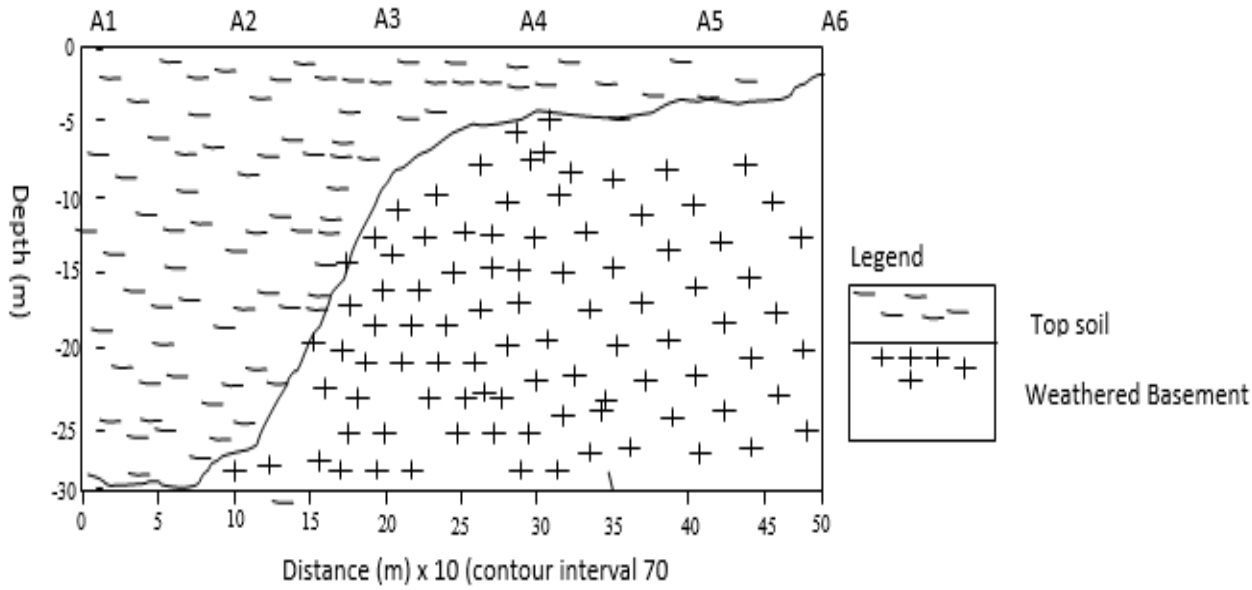

Figure 4b: Vertical Section through Profile A

Interpretation of Geo-electric Vertical Section along Profile B

The vertical geo-electric section of profile B is as shown in figure (4.9a) while its corresponding geologic section is shown in figure (4.9b). The map is contoured at an interval of 300 Ωm. This map can be divided into three layers. The top soil with resistivity range from 300 Ωm-600 Ωm isolated point B3 with high resistivity is a

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concrete from construction of school building. The second layer of clay saturated with ground water with low resistivity ranging from 300 Ωm downward dominate the area to about 30 metres

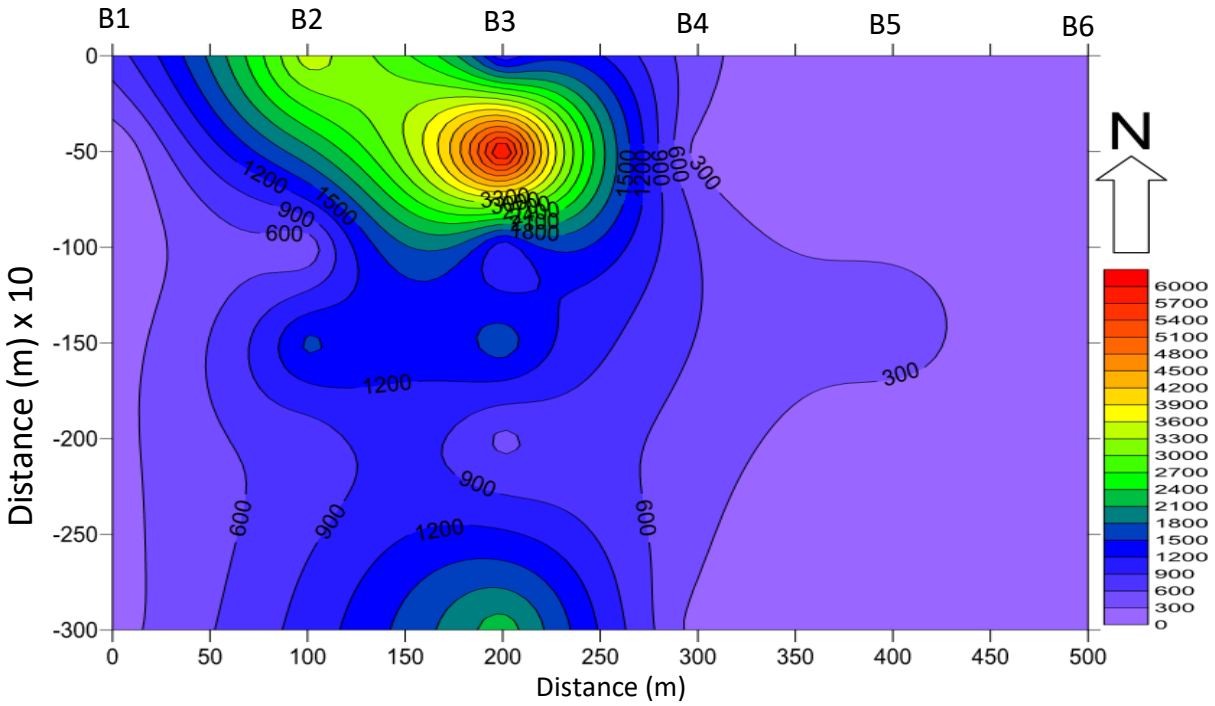

Figure 5a: Geoelectric Vertical section along Profile B (Contour Interval is 300 Ωm)

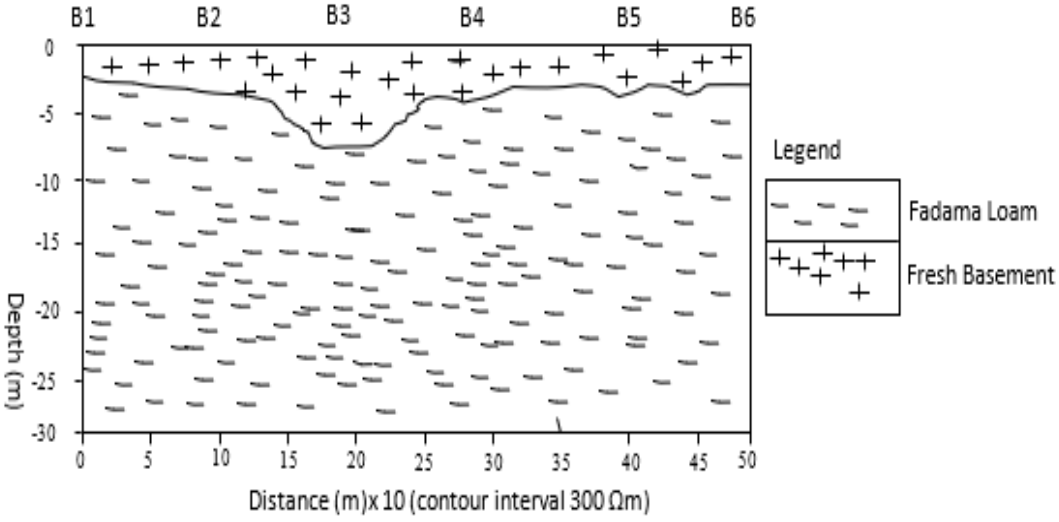

Figure 5b: Vertical Section through Profile B

Interpretation of Geo-electric Vertical Section along Profile C

The vertical geo-electric section of profile C is as shown in figure (4.10a) while its corresponding geologic section is shown in figure (4.10b). The map is contoured at an interval of 200 Ωm. This map can be divided into three layers.

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The first layer has resistivity values ranging between 10.00 Ωm to 92.00 Ωm as shown in table 4.3. This spread through the entire profile and at different depths. The highest resistivity value of (92.00 Ωm is found at VES C_2 and the lowest value of 10.00 Ωm is at VES C_4), which is an outcrop due to erosion. The thickness of this layer ranges between 0.3 m to 8.0 m. The highest thickness of about 20.0 m occurs at VES B4, while the least thickness of about 0.8 m occurs at VES B5. The lithology around this area suggests that this layer could be sand and gravels. The second layer could be fresh basement with very negligible aquifer potential. The layer has resistivity values ranging between 200.00 Ωm to 1800.00 Ωm. The layer covers the entire profile.

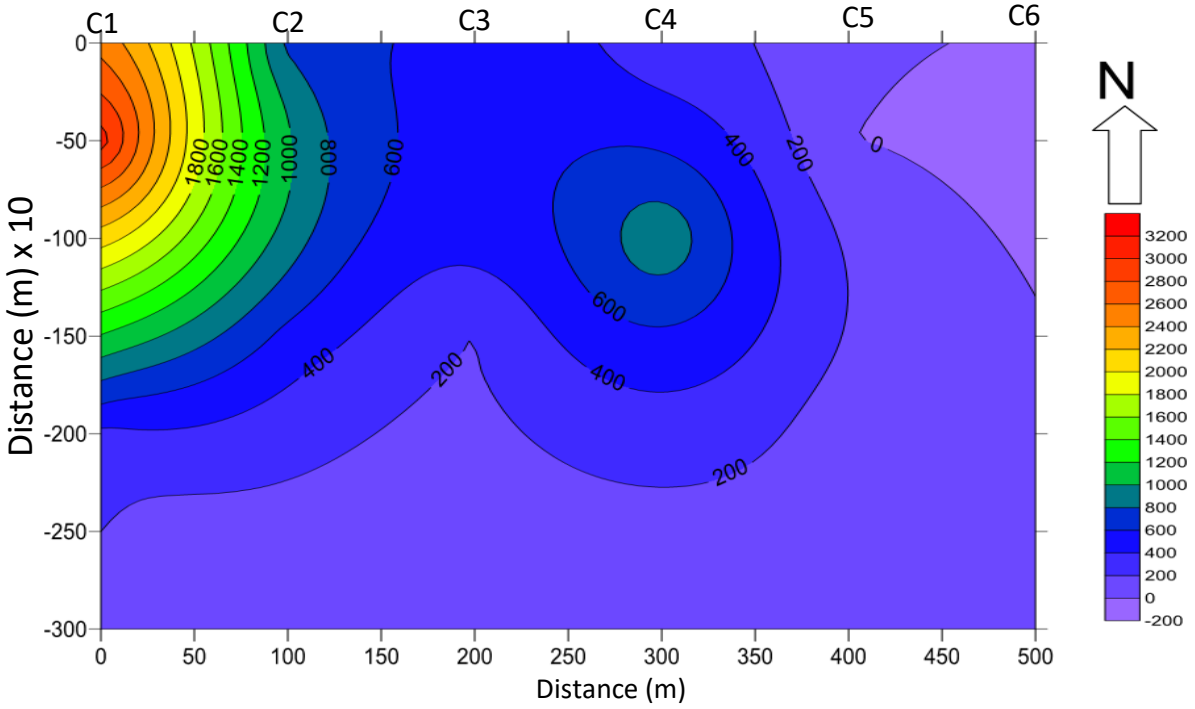

Figure 6a: Geoelectric Vertical section along Profile C (Contour Interv al is 200 Ωm)

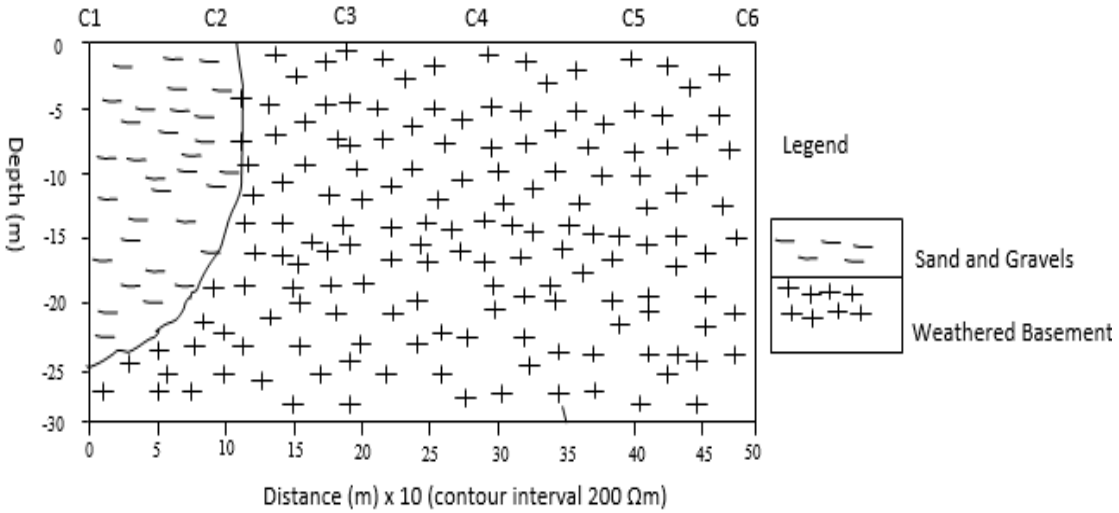

Figure 6b: Vertical Section through Profile C

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Interpretation of Geo-electric Vertical Section along Profile D

The vertical geo-electric section of profile D is as shown in figure (4.11a) while its corresponding geologic section is shown in figure (4.11b). The map is contoured at an interval of 300 Ωm. This map can be divided into two (2) layers.

Profile D has lateritic top soil observed at D1, D2, D5 and D6 with resistivity ranging from 40 Ωm to 120 Ωm. There is an outcrop of granite at D3 which correspond to high resistivity greater than 700 Ωm outward to about 30 metres. The weathered basement stretches up to 30 metres across the profile.

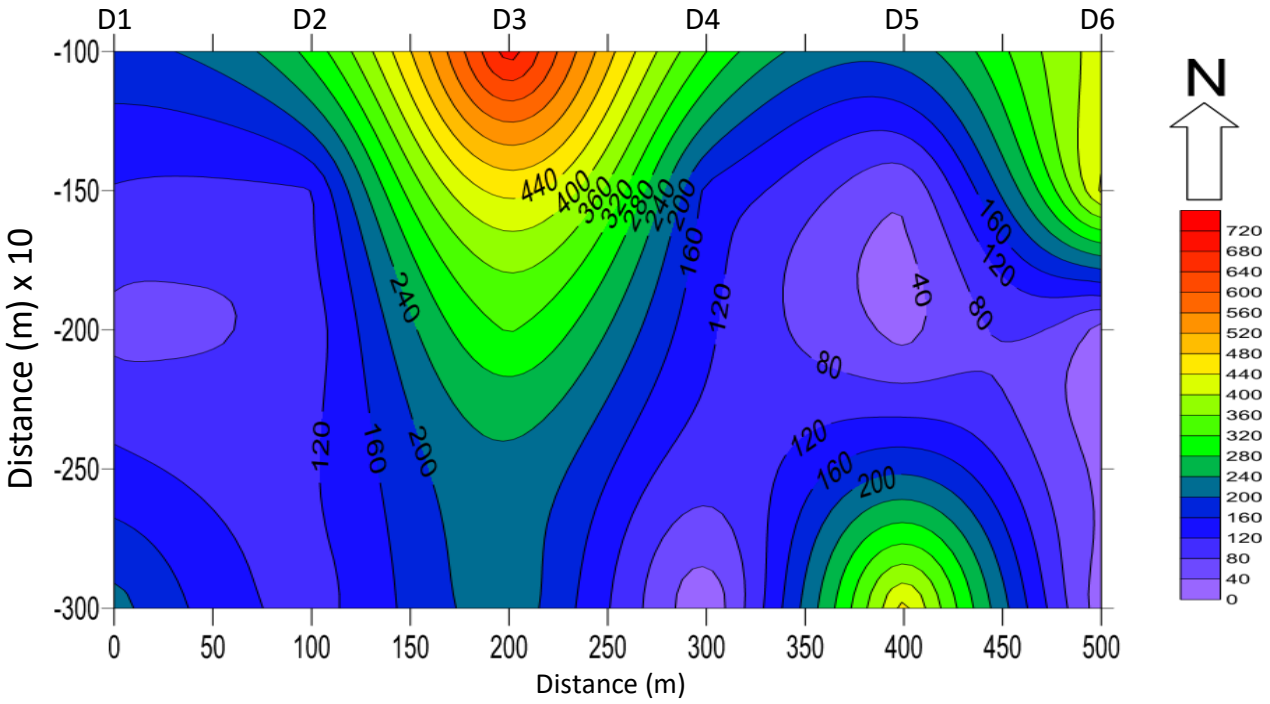

Figure 7a: Geoelectric Vertical section along Profile D (Contour Interval is 300 Ωm)

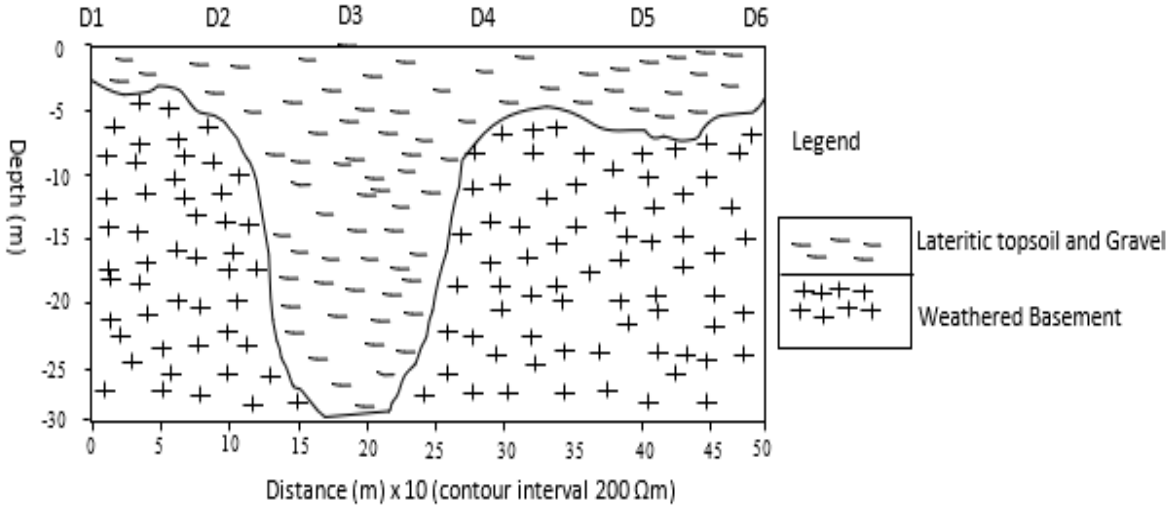

Figure 7b: Vertical Section through Profile D

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Interpretation of Geo-electric Vertical Section along Profile E

The vertical geo-electric section of profile E is as shown in figure (4.12a) while its corresponding geologic section is shown in figure (4.12b). The map is contoured at an interval of 120 Ωm. This map can be divided into two layers.

Profile E has top soil from E2-E6 with low resistivity ranging from 40 Ωm to about 760 Ωm. A high resistivity outcrop of weathered basement is found at E1 which extends 30 metres from E2-E6.

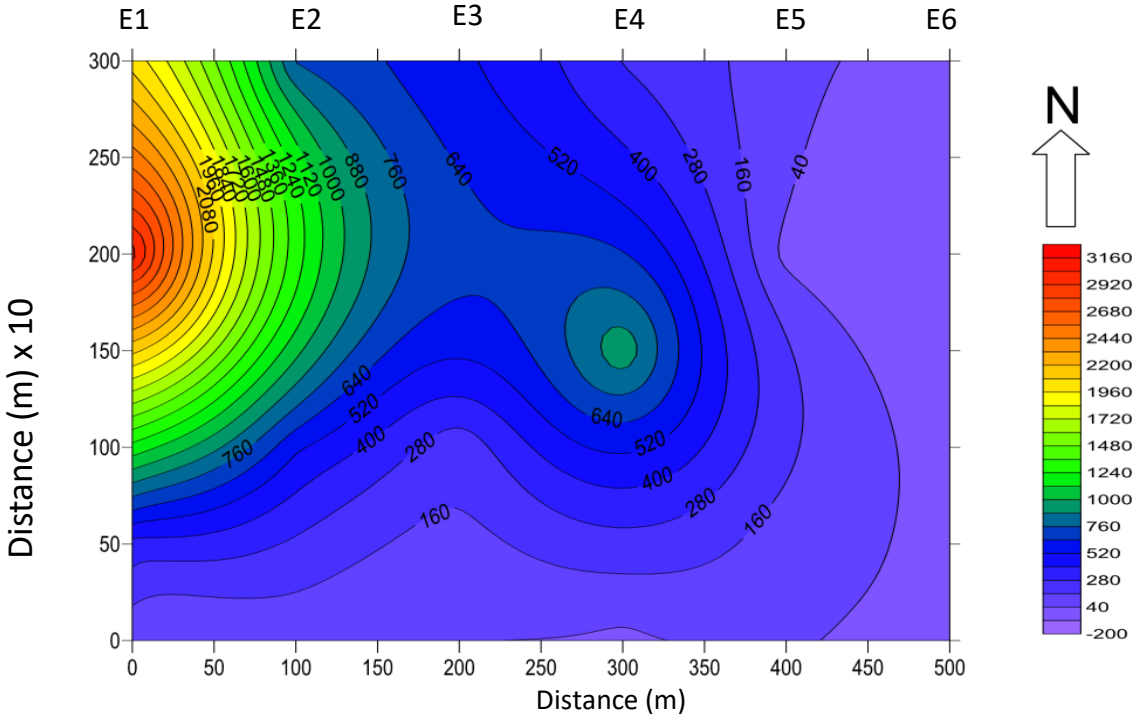

Figure 8a: Geoelectric Vertical section along Profile E (Contour Interval is 120 Ωm)

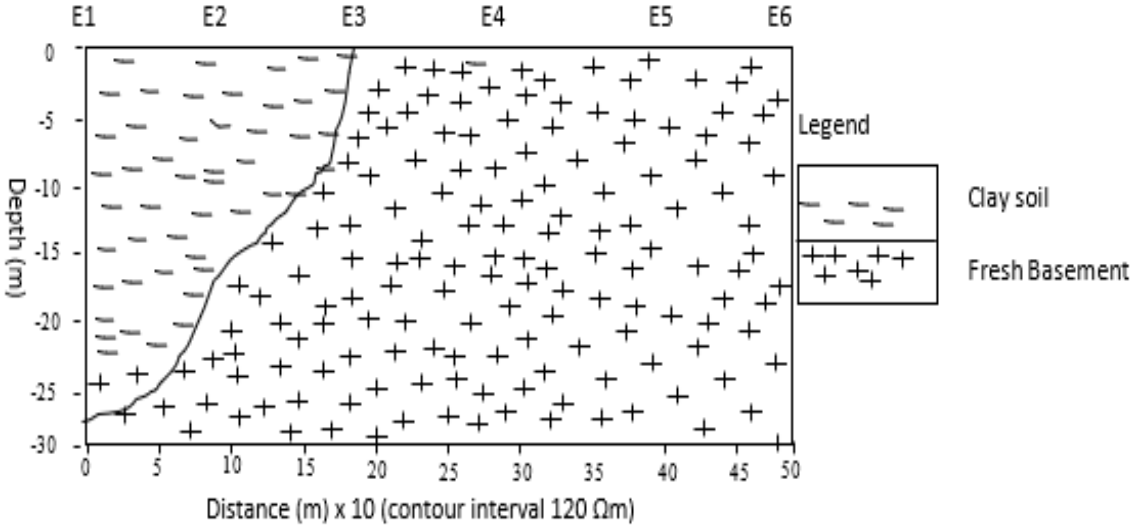

Figure 8b: Vertical Section through Profile E

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CONCLUSION

From the discussion so far, electrical resistivity method was employed to characterize the subsurface for general lithology of rock formations at Maikunkele, Niger State. Electrical resistivity results indicate the electrical properties of rocks soil, matric and grain size; which varied within the fractured bedrock. Both the horizontal profiling and the vertical electrical sounding (VES) were carried out and 30 VES points were sounded.

The study area is mostly two-layer formations. The top soil and weathered basement. The top layer resistivity ranges from 100 to 190, the weathered basement ranges from 100 to 475 and the highest thickness of about 20.00 m. The weathered basement which corresponds to the viable points that can be dug for groundwater has it lowest resistivity at VES A2 with resistivity of 5.2Ωm, followed by VES A6 with average layered resistivity of 76.9Ωm, VES E4 with resistivity of 32.70Ωm and VES E5 with resistivity of 169.20Ωm, respectively. The weathered basement rock is deepest at north western part of the survey area. The fresh basement (solid bed rock) has a characteristic high resistivity value. The resistivity value is maximum at about 100000 in the area investigated and much lower in other sections especially where the materials rock matrix is not so compacted, and less resistive and therefore less competent.

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Assessment of Tamarind, *Tamarindus indica* Pulp as Immune Booster in the Blood of Broiler Chickens

***Sode M. A. & Mohammed A.**

Department of Biochemistry & Biotechnology, IBB University Lapai.

**Email:* abusideek20@gmail.com; *Phone number*: +2347034598075.

ABSTRACT

Evidence in the literature suggests that the use of antibiotics in broilers as Growth Promoter Agents (GPA) leads to increased antibiotic resistance, presence of antibiotics residues in feeds and environment that compromises human and animal health. Restriction on use of Anti-microbial Growth Promoters has stimulated interest in plant residues with immune boosting properties such as *Tamarindus indica.* This present study was undertaken to investigate the full blood count of broiler chickens due to tamarind pulp and tetracycline administration *in vivo* and compared to a control. Broiler chickens in 3 groups of 40 chickens each were administered either drinking water (Control), drinking water containing dissolved tamarind pulp (25g/L; Tamarind treatment) or drinking water with dissolved tetracycline (0.2g/L; Tetracycline treatment) daily for 6 weeks. At the end of the trial, blood samples were collected from the brachial vein and assessed for full blood count. The lymphocyte count in the tamarind and tetracycline groups were significantly ($p<0.05$) higher than the control group. All other blood parameters evaluated did not significantly ($p<0.05$) differ across all treatment groups. From the results of this trial, it may be concluded that Tamarind pulp has potential as a Growth Promoter Agent (GPA) substitute, as lymphocytes participate in immune activities in animals.

Key words: Broiler, Tamarind pulp, Antibiotic, Immunity, Blood parameter

INTRODUCTION

Tamarind (*Tamarindus indica*) is a leguminous tree (family Fabaceae) bearing edible fruit that is indigenous to tropical Africa (Rivers and Mark, 2017). It grows wild in Africa in locales as diverse as Sudan, Cameroon, Nigeria, Zambia and Tanzania (Mohammed, 2019). The genus Tamarindus is monotypic, meaning that it contains only this species. The tamarind tree produces brown, pod-like fruits that contain sweet, tangy pulp, which is used in cuisines around the world (Borah, 2018; Manalo, 2021). The pulp is also used in traditional medicine and as a metal polish. The proximate analysis of tamarind pulp revealed moisture content 16.82%, ash content 4.63%, crude fiber 18.75%, protein content 18.84%, fat content 5.4% and carbohydrate content 35.56% (Mahima and Sharma, 2018). Both root and stem bark ethanolic extracts of the tamarind possess anti-inflammatory and antioxidant activities. The stem bark extract showed better activity in reducing carrageenan-induced edema and much better scavenging potential in chicks compared to the root extract. Both root and stem extract,

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however, have similar total antioxidant capacities and total phenolic contents (Lawrence, Michel and Samuel 2020).

Plant Feed Additives (PFA) as dietary ingredients in animal nutrition is a growing area of interest owing to the ban of in-feed antibiotic additives (Christaki *et al*., 2019, Franz *et al*., 2010) The PFA (phenolic acids, phenolic di-terpenes, flavonoids, and volatile oils) are considered natural antioxidants, because they are able to donate hydrogens and therefore to interrupt the oxidative chain in tissues. Generally, PFA can trap free radicals and chelate metals (Brewer, 2011, Villano *et al*., 2008). The PFA also prevents peroxide formation by modifying the activities of antioxidant enzymes (Angeloni and Hrelia, 2020)

Poultry farming is the domestication of birds for meat, egg and some industrial materials like feather and faecal droppings (Famuwagun, 2020). Over 60 billion chickens are slaughtered for consumption annually (Faunalytics, 2019; Compassion in World Farming, CIWF, 2018). As at 2018, the annual poultry meat consumption of four important African economies including Nigeria was 176,287.266MT (Nigeria), 1.1 millionMT (Egypt), 10,922.46MT (Ethiopia), and 1.95 millionMT (South Africa) (Organisation for Economic Cooperation and Development, OECD, 2019; World Bank, WB, 2020). It has been estimated that by 2030 the demand for poultry products across the African continent will increase by 60%. Currently, consumption across the continent is almost 100 million tonnes for both poultry meat and egg products whereas in Nigeria, 192.69 MT is consumed annually (World Economic Forum, WEF, 2019). There is a gap between consumption and availability of chicken. The lagging increase in domestic poultry production may be explained by numerous problems that the farmers encounter, including opportunistic infections. (Ahmed and Mohammed, 2015).

To increase production, feed additives in form of Antimicrobial Growth Promoters (AGPs) are used for growth promotion and disease control at lower therapeutic doses on the basis of the observations made since 1946 (Elagib *et al*., 2015). Unfortunately, due to the risk posed by AGPs to create cross-resistance to antibiotics used in human medicine and their presence in animal products has led to their use to drop significantly. This is as they are banned in some cases in the formulation of fodders and for use in general animal husbandry (Gaucher *et al.*, 2015). Additives used in animal feeds are diverse and heterogeneous. As a substitute for the synthetic growth promoters, agents like probiotics, prebiotics, phytobiotics, and enzymes have been utilized as feed additives in broilers (Elgedawwy *et al.,* 2020; El-Hindawy *et al.,* 2019; Reda *et al.,* 2019).

The restriction on AGPs has stimulated interest in medicinal plants, which was revived in recent times because of their efficacy in providing cost effective therapy to several diseases due to secondary metabolites abundant in plants (Liu *et al*., 2020, Aljumaah *et al*., 2020). These compounds known as phytochemicals, have been found to be responsible for the antioxidant properties of plants (Martel *et al*., 2020). This study was thus designed to investigate the potential effects of dissolving *Tamarindus indica* pulp in the drinking water of broiler chickens on the immune system of broiler chickens.

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METHODOLOGY

Experimental Set up and Animal Grouping.

The tamarind pulp was all purchased from a single seller in Paiko, Niger State, Nigeria. The tamarind pulps were aggregated into an average of 50g each. 120 week-old broiler starter birds were purchased from Zartech Farms limited in Ibadan, Oyo State Nigeria. Chicks were reared in cages of dimension 1.5m x 1.5m x 2.0m; length x breadth x height, that were previously cleaned and disinfected. The acclimatized 120 broiler chicks were weighed with an electronic digital scale. The birds were randomly divided into three groups of 40 birds each. Each group was assigned into four replicates of 10 birds each. The birds were fed commercial Ultima starter feed and the tamarind pulp dissolved in the drinking water of the tamarind group and tetracycline in the drinking water of the tetracycline group for the six weeks starter period. Feed and water were offered to the birds in all the different treatments *ad-libitum*. All birds were reared without vaccination. Chicks were reared under approximately natural photoperiod of 12/12 hours of light/dark cycles for period of six weeks and cages were cleaned at the end of every week.

Group 1; Control group (C), this is the negative treatment group with drinking water absent of any treatment.

Group 2; Tetracycline group (AT), this is the positive treatment group administered with tetracycline 0.2g/L of water.

Group 3; Tamarind group (TD), this is the experimental treatment group administered with tamarind 25g/L of water.

Collection of blood from experimental birds

The experimental sampling procedure involved the collection of blood from the test broiler chickens. At the end of the sixth (6th) week, nine birds were randomly selected from each treatment. The blood sample was obtained by veni-puncturing the brachial veins (wing veins) of the experimental birds (broilers), according to method described by Lisa and Leanne, (2013). This was done by plucking a few feathers from the ventral surface of the humeral region of the right wing to aid visualization. The skin was then sterilized with methylated spirit, and was afterwards punctured with 2mm syringe to obtain 2ml of blood sample. The blood sample was transferred into appropriate sample bottles; some with anticoagulants and some plain. The serum was obtained by centrifugation using an electric desktop lab centrifuge (EU Plug 220V, Jersey, UK) for 10 minutes at 3,000 rpm. The serum was collected by the use of micro pipette and transferred into anticoagulant free test-tube and stored in refrigerator (4°C) for subsequent analyses.

Determination of haematological parameters

Haematological components including red blood cells (RBC), haemoglobin (Hb), Mean cell volume (MCV), hematocrit (HCT%), Platelet number (PLT), white blood cells (WBC), lyphocytes (LYM), Mean cell haemoglobin, (MCH), Mean cell haemoglobin concentration (MCHC), Mean platelet volume (MPV), were determined using the automated haematologic analyzer (ABACUS 3CT, Diatron, USA), employing the methods described by Dacie and Lewis, (1991).

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RESULTS AND DISCUSSION

Table 1: Haematology parameters of broiler chickens fed tamarind pulp dissolved in their drinking water for 6 weeks starter period.

	AT	TD	C
HB	11.53±0.62 [a]	11.60±0.38 [a]	10.07±0.29 [a]
PCV	34.67±1.86 [a]	35.00±1.15 [a]	30.33±0.88 [a]
RBC	5.73±0.37 [a]	5.80±0.17 [a]	5.03±0.15 [a]
WBC	3.73±0.37 [a]	4.03±0.03 [a]	3.13±0.24 [a]
NEUTR	17.00±1.53 [a]	16.00±1.15 [a]	13.67±1.20 [a]
LYMPH	53.67±3.38 [b]	54.67±1.76 [b]	45.67±1.20 [a]
MONOCYT	2.33±0.33 [a]	2.67±0.33 [a]	2.00±00 [a]
EOSIN	1.00±00	1.00±00	1.00±00
BASOPH	1.00±00	1.00±00	1.00±00
MCV	60.43±0.22 [a]	60.30±0.17 [a]	60.23±0.12 [a]
MCH	20.07±0.03 [a]	19.97±0.09a	20.00±00a
MCHC	33.23±0.03 [a]	33.10±0.15 [a]	33.17±0.07 [a]

Data are expressed as mean ± SEM (n = 3).
Mean ± SEM followed by different letter within a row are significantly different (P <0.05).
SEM=Standard Error of Mean.
AT = Tetracycline group
TD = Tamarind group
C = Control group
HB, Haemoglobin; PCV, Packed Cell Volume; RBC, Red blood cell number; WBC, White blood cell number; MCV, Mean cell volume; LYMPH, Lymphocytes; MCH, Mean cell haemoglobin; MCHC, Mean cell haemoglobin concentration.

From the Table, all the haematological parameters except lymphocytes did not significantly (P > 0.05) differ across all the treatments. The neutrophils count in the tetracycline and tamarind groups were numerically higher than the control group but not significantly (P > 0.05) different. Lymphocytes count did not significantly (P > 0.05) vary between the tetracycline and tamarind groups, however they are both significantly (P > 0.05) higher than the control group.

CONCLUSION

This study shows lymphocyte count in the tamarind and tetracycline groups were significantly (p<0.05) higher than the control group. Also, the performance of the tamarind and tetracycline were statistically similar. From the results of this trial, it may be concluded that Tamarind pulp has potential as a Growth Promoter Agent (GPA) substitute, as lymphocytes participate in immune activities in animals.

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CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

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Analytical Solution to Heat Conduction of Polystyrene Silver Nanoparticle (PS/AgNPs) Composite in Spherical Coordinate

[1]Imrana M. H., [*1]Yabagi J. A., [2]Chukwude A. E., [1]Ndanusa B., [1]Bello M. L. & [1]Yusuf T. U.

[1]Department of Physics, Ibrahim Badamasi Babangida University Lapai, Niger State, Nigeria
[2]Department of Physics University of Nigeria Nsuka

*Corresponding Email: jibrinbida@yahoo.com, mimrana507@gmail.com +2348065654037

ABSTRACT

The analytical solution to temperature field for a spherical nanocomposite subjected to heat transfer was derived. The nanocomposite is considered to be spherical, with time-dependent thermal properties. Advancement of thermal-insulation of polymer nanocomposite needs a theoretical model in order to predict the overall thermal conductivity. The heat transfer equation was solved, using separation of variable methods and analytical solution obtained for the heat conduction in the multilayered sphere of polystyrene silver nanoparticles (PS/AgNPs) composite. The two layers of the sphere in their radial direction depend on time and have spatially non uniform internal heat sources. Heat is transferred within the sphere of PS by conduction and to the outer surface of the sphere to the AgNPs by convection. The computer code was developed for numerical analysis using MATLAB environment to investigate the selection of time step in the simulations of the temperature distribution. The results obtained showed that the surface temperature of AgNPs increases from 26 ℃ at 10 sec to 423 ℃ at 30 sec, due to conductive heat transfer, the heat is transferred to inner sphere of the nanoparticle; therefore the temperature also increased within the nanocomposite with increases in time. The results obtained are compared with experimental values and are in good agreement. The results are used to approximate the problems and the time consuming complex numerical calculations attributed to polymer nanocomposite. A thermal property of polystyrene silver nanoparticle (PS/AgNPs) composite plays a vital role in the development, performance, and reliability of various electronic devices.

Keywords: *Polystyrene; Silver nanoparticles; Temperature; Heat conduction; Spherical composite.*

INTRODUCTION

Nanoparticles dispersed with polymer matrix known as nanocomposite are promising for heat transfer enhancement due to their high thermal conductivity (Ravisankar & Tara, 2013). Nanocomposite sphere covered with a spherical shell of different materials has received some attention due to their unique properties and potential applications in remote sensing and electronic appliances. Nanoshells (NSHs) are spherical or cylindrical particles with diameters typically ranging in nanoscale, which are a new class of nanoparticles (NPs) with highly tunable thermal properties and suited to nanoscale polymer engineering. In general, two layer NSHs

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are the core-shell NPs, which represent an example of nanostructures with a tunable plasmon resonance and may be considered by combining various types of dielectric materials, metals and semiconductors (Prodan & Nordlande, 2003). Such systems exhibit a strong thermal absorption peak, which is absent in bulk counterparts. The important feature of this type of nanostructures is the possibility of controlling their thermal properties by the variation of their geometry and shell material because the electronic and thermal properties of NSHs are determined by both their size and shape (Hirsch *et al.*, 2006). Furthermore, the temperature and absorption of the heat by NSHs (core-shell NPs) are related to the physical and geometrical parameters of particles such as size, shape, structure, orientation, and surrounding medium (Mishchenko *et al,* 2004). This dependency on the size and composition of each nanoshell layer can be designed to either absorb or transfer heat over much of the visible part of the composite (Pena-Rodrıguez & Pal, 2010).

Many researchers have solved the heat conduction problem in a polymer nanocomposite medium. For instance, Jain & Singh (2010) proposed an analytical series solution for the transient boundary-value problem of multilayer heat conduction in spherical coordinates by using the separation of variable method. Nourazar & Dalir (2014) used the eigenfunction expansion method to solve the problem of three-dimensional transient heat conduction in a multilayer cylinder. Haji & Beck (2002) used Green's function method to obtain temperature distribution in a three-dimensional two-layer orthotropic slab. Lu *et al.* (2006) combined separation of variables and Laplace transforms to solve the transient conduction in the two-dimensional cylindrical and spherical media. Monte (2004) used the eigenfunction method to studied the temperature distribution for the heat conduction in a two-dimensional - layer isotropic slab with homogenous boundary condition. Chiang *et al.* (2008) studied the thermal properties of composite materials as a function of temperature by using the Maxwell-Garnett and Bruggeman models for temperatures up to the melting points of materials. Link & El-Sayed (2004) studied the size and temperature dependence of the plasmon absorption of colloidal gold NPs and the plasmon band width was found to follow the predicted behavior as it increases with decreasing size in the intrinsic size region (mean diameter smaller than 25 nm), and also increases with increasing size in the extrinsic size region (mean diameter larger than 25 nm). Also, Chen *et al.* (2015) theoretically investigated the temperature dependence of the optical/spectroscopic properties of metallic nanoparticles (NPs) using the Gersten-Nitzan model. It was observed that the resonance frequencies of this type of nanostructures can be tuned by varying the dimensions of nanoparticles. The temperature dependence of the surface Plasmon resonance and optical absorption leads to a broadening and spectral shift. Bouillard *et al.* (2012) investigated the effect of temperature on the optical properties of two different types of plasmonic nanostructures. It was showed that the extinction of a plasmonic nanorod material can be efficiently controlled with temperature with transmission changes by nearly a factor of 10 between room and liquid nitrogen temperatures, while temperature effects in plasmonic crystals are relatively weak (transmission changes only up to 20%). Recently, temperature dependence of the resonance in spherical silver nanoparticles (AgNPs) embedded in silica host matrix in the temperature range of

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17–170 ℃ has been investigated by Yeshchenko *et al*. (2012). The authors found that as the size of the nanoparticles decreases, the temperature of the nanoparticles NPs also increases. The temperature distribution of nanocomposite and, thus, the thermal properties of such nanostructures at elevated temperatures are of interest to study and some researchers were dedicated to study the influence of temperature on the thermal conductivity of NPs. In this research work, the effect of temperature on the nanocomposite absorption due to heat transfer in core-shell nanoparticles composite NPs is explores. This was achieved by obtaining an analytical solution to heat conduction of polymer nanocomposite structure having spherical geometry, using a separation of variable method.

METHODOLOGY

THEORY AND PROCEDURE

As a starting point for the calculation of the thermal conductivity of NPs, a core-shell NPs embedded in a host medium with polymer matrix is consider in which heat transfer from the inner sphere of the composite to the outer sphere of the composite. Because the origin of thermal phenomena is the interaction of temperature distribution within the material, and the strongest thermal interactions are usually through their size, shape and volume fraction interaction. Core-shell NPs consist of an inner core of radius r and an outer shell of radius R surrounded by acontinuum environment as shown in Figure 1a and b.

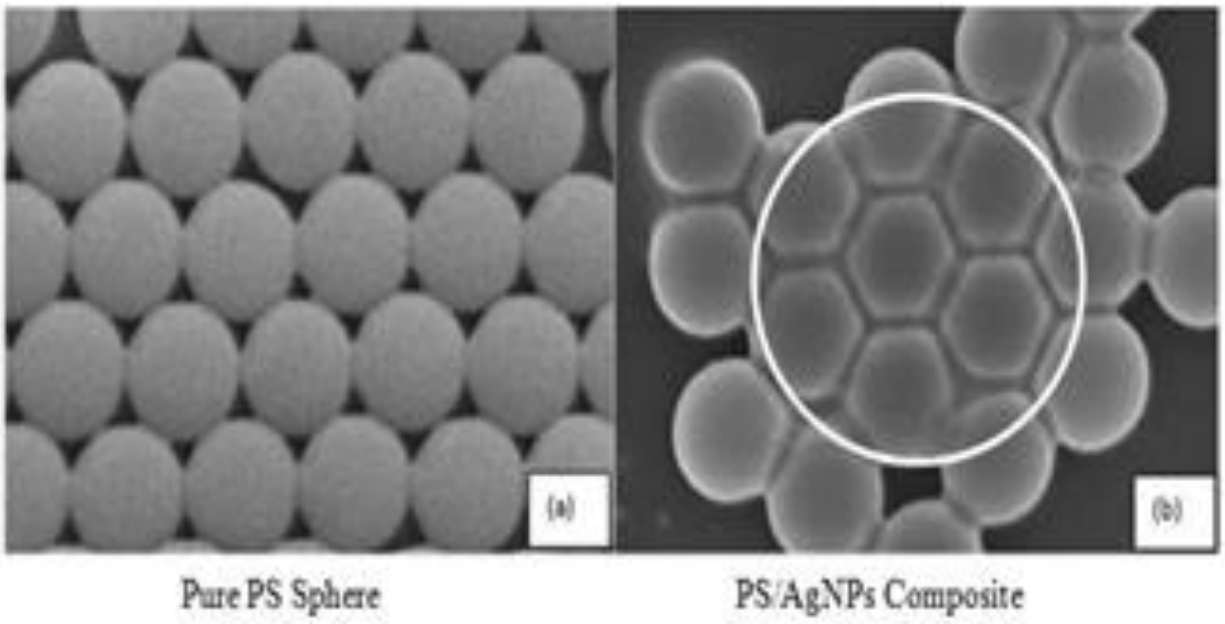

Figure 1a: SEM Image of a Pure PS and PS/AgNPs Composite adopted from (Yabagi *et al.,* 2017)

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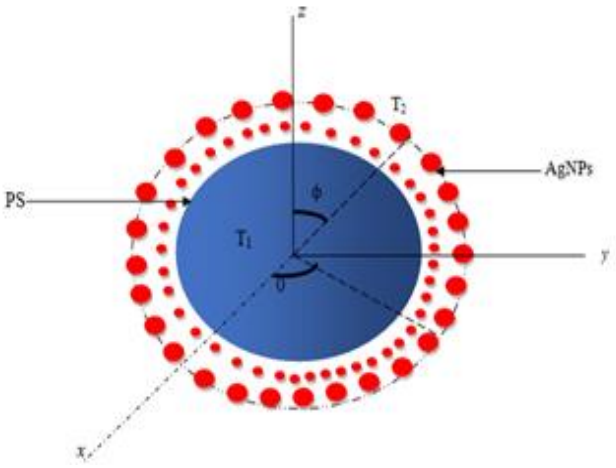

Figure 1b: Geometrical disposition of Polymer nanocomposite structure in spherical coordinate System

Furthermore, the dependence of the temperature on nanoparticle dimensions can be found in the thermal diffusivity α . Formulation is based on the quasi-static approximation for the absorption and scattering cross section of nanocomposite because this approximation neglects the retardation effects and it is valid for systems, whose dimensions are much smaller. Thus, by solving of Laplace's equation for the temperature distribution in each region, the heat conduction in different regions of spherical core-shell of nanocomposite can be obtained. However, the thermal respond of the composite depends on the temperature distribution, when nanoparticles (NPs) are immersed in the polymer matrix.

MATHEMATICAL FORMULATION

The Governing Heat Equation in Spherical Geometry

Several assumptions have been considered in this paper. Material inside the sphere is considered to start at a non uniform temperature where the material depends on radius r and time t covered with insulation on the outer surface to reduce heat loses. The coordinate r is taken to measure the position radial out from the centre of the sphere with outer surface given with initial value $T(r,0)=T_1$, $T(R,t)=T_2$ and $T(0,t)=T_0$ where *R* is the radius of sphere from the origin (Figure1b). The heat conduction equation in spherical coordinates, assuming constant physical properties and no heat generation within the sphere is given by Dada *et al.* (2009).

$$\frac{1}{\alpha}\frac{dT_{PNC}}{dt}=\frac{1}{r^2}\frac{d}{dr}\left(r^2\frac{dT_{PNC}}{dr}\right)+\frac{1}{r^2\sin\theta}\frac{d}{d\theta}\left(\sin\theta\frac{dT_{PNC}}{d\theta}\right)+\frac{1}{r^2\sin^2\theta}\frac{d^2T_{PNC}}{d\phi^2} \qquad (1)$$

where, T_{PNC} is the temperature of the polymer nanocomposite (PNC), that depends on position and time, (r,θ,ϕ) is off-axis coordinate systems of polymer composite structure, α is the Thermal diffusivity

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express as $\alpha = \frac{\lambda}{\rho C_p}$, where λ is the thermal conductivity ρ is the density and C_p is specific heat capacity of the composite structure respectively.

If $T_{PNC} = T_{PNC}(r,\theta,\phi,t)$, by using separation of variable

$$T_{PNC} = F(r,\theta,\phi)U(t) \tag{2}$$

We have

$$\frac{1}{\alpha}F\frac{dU(t)}{dt} = \left\{\frac{d}{dr}\left(r^2U(t)\frac{dF}{dr}\right) + \frac{1}{r^2\sin\theta}\frac{d}{d\theta}\left(\sin\theta U(t)\frac{dF}{dr}\right) + \frac{1}{r^2\sin^2\theta}U(t)\frac{d^2F}{d\phi^2}\right\} \tag{3}$$

By factoring out U(t*)* from right hand side of Equation (3), and dividing through by $FU(t)$

$$\frac{1}{U\alpha}\frac{dU(t)}{dt} = \frac{1}{F}\left\{\frac{d}{dr}\left(r^2\frac{dF}{dr}\right) + \frac{1}{r^2\sin\theta}\frac{d}{d\theta}\left(\sin\theta\frac{dF}{dr}\right) + \frac{1}{r^2\sin^2\theta}\frac{d^2F}{d\phi^2}\right\} \tag{4}$$

Equating each side of equation (4) to a constant $-\beta^2$ since, both sides are independent of each other.

$$\frac{1}{U\alpha}\frac{dU(t)}{dt} = \frac{1}{F}\left\{\frac{d}{dr}\left(r^2\frac{dF}{dr}\right) + \frac{1}{r^2\sin\theta}\frac{d}{d\theta}\left(\sin\theta\frac{dF}{dr}\right) + \frac{1}{r^2\sin^2\theta}\frac{d^2F}{d\phi^2}\right\} = -\beta^2 \tag{5}$$

$$\frac{dU}{U\alpha} = -\beta^2 dt \; U(t) = Ae^{-\beta^2\alpha t} \tag{6}$$

Equation (6) is a partial solution to equation (5) where A is a constant

From equation (5) it can be express as,

$$\frac{1}{F}\left\{\frac{d}{dr}\left(r^2\frac{dF}{dr}\right) + \frac{1}{r^2\sin\theta}\frac{d}{d\theta}\left(\sin\theta\frac{dF}{dr}\right) + \frac{1}{r^2\sin^2\theta}\frac{d^2F}{d\phi^2}\right\} = -\beta^2 \tag{7}$$

Equation (7) depends on three parameters r,θ and ϕ which can be solve, using the standard approach of separation of variables, i.e., by writing

$$F(r,\theta,\phi) = R(r)\Theta(\theta)\Phi(\phi) \tag{8}$$

Putting equation (8) into equation (7) gives

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$$\frac{1}{R\Theta\Phi}\left\{\frac{d}{dr}\left(r^2\frac{dR\Theta\Phi}{dr}\right)+\frac{1}{r^2\sin\theta}\frac{d}{d\theta}\left(\sin\theta\frac{dR\Theta\Phi}{dr}\right)+\frac{1}{r^2\sin^2\theta}\frac{d^2R\Theta\Phi}{d\phi^2}\right\}=-\beta^2 \quad (9)$$

$$\frac{1}{R\Theta\Phi}\left\{\Theta\Phi\frac{d}{dr}\left(r^2\frac{dR}{dr}\right)+R\Phi\frac{1}{r^2\sin\theta}\frac{d}{d\theta}\left(\sin\theta\frac{d\Theta}{dr}\right)+R\Theta\frac{1}{r^2\sin^2\theta}\frac{d^2\Phi}{d\phi^2}\right\}+\beta^2=0 \quad (10)$$

$$\frac{1}{R}\frac{d}{dr}\left(r^2\frac{dR}{dr}\right)+\frac{1}{\Theta}\frac{1}{r^2\sin\theta}\frac{d}{d\theta}\left(\sin\theta\frac{d\Theta}{dr}\right)+\frac{1}{\Phi}\frac{1}{r^2\sin^2\theta}\frac{d^2\Phi}{d\phi^2}+\beta^2=0 \quad (11)$$

Multiplying the second, third and fourth term of equation (11) by r^2 to make each term depends on its parameter.

$$\frac{1}{R}\frac{d}{dr}\left(r^2\frac{dR}{dr}\right)+\frac{1}{\Theta}\frac{1}{\sin\theta}\frac{d}{d\theta}\left(\sin\theta\frac{d\Theta}{dr}\right)+\frac{1}{\Phi}\frac{1}{\sin^2\theta}\frac{d^2\Phi}{d\phi^2}+\beta^2r^2=0 \quad (12)$$

$$\frac{1}{R}\frac{d}{dr}\left(r^2\frac{dR}{\partial r}\right)+\beta^2r^2+\frac{1}{\Theta\sin\theta}\frac{d}{d\theta}\left(\sin\theta\frac{d\Theta}{d\theta}\right)+\frac{1}{\Phi\sin^2\theta}\frac{d^2\Phi}{d\phi^2}=0 \quad (13)$$

Multiplying the last term of equation (13) by $\sin^2\theta$, so that the last term involves ϕ, only and must be equal to a constant $-m^2$, where as the first two terms depends only on r,θ

$$\frac{1}{\Phi}\frac{d^2\Phi}{d\phi^2}=-m^2 \quad (14)$$

Equation (14) has a solution as

$$\Phi(\phi)=e^{im\phi} \quad (15)$$

Substitute equation (14) into equation (13), gives

$$\frac{1}{R}\frac{d}{dr}\left(r^2\frac{dR}{\partial r}\right)+\beta^2r^2+\frac{1}{\Theta\sin\theta}\frac{d}{d\theta}\left(\sin\theta\frac{d\Theta}{d\theta}\right)-\frac{m^2}{\sin^2\theta}=0 \quad (16)$$

The third and fourth terms of equation (16), are only a function of θ (whereas the first two term only depend on r), and must therefore be equated to a constant $l(l+1)$, i.e.

$$\frac{1}{\Theta\sin\theta}\frac{d}{d\theta}\left(\sin\theta\frac{d\Theta}{d\theta}\right)-\frac{m^2}{\sin^2\theta}=-l(l+1) \quad (17)$$

with substitution $x=\cos\theta$, for spherical coordinate, Equation (17) becomes,

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$$\frac{d}{dx}\left[\left(1-x^2\right)\frac{d\Theta(x)}{dx}\right]+\left(l(l+1)-\frac{m^2}{1-x^2}\right)\Theta(x)=0 \tag{18}$$

Equation (18) is called the Associated Legendre equation, so the solution is given as

$$\Theta(x)=P_l^m(x)\text{, when } x=\cos\theta, \tag{19}$$

where $P_l^m(\cos\theta)$ are Associated Legendre Polynomials, and $l=0,1,2$, and m runs over integer values from $-l$ to l. If l is not an integer, we can show that the solution of Equation (18) diverges for $\cos\theta=1$ or -1 ($\theta=0$ or 1).

The functions Θ and Φ are often combined into a spherical harmonic, $\mathrm{Y}_l^m(\theta,\phi)$, where

$$\mathrm{Y}_l^m(\theta,\phi)=\beta\mathrm{P}_l^m(\cos\theta)e^{im\phi}, \tag{20}$$

where β is the normalization constant which can be determined by normalizing equation (20).
By taking the radial part of the equation (16) to get the solution as Bessel function

$$\frac{d}{dr}\left(r^2\frac{dR}{dr}\right)+\left[\beta^2r^2-l(l+1)\right]R=0 \tag{21}$$

Or equivalently

$$r^2\frac{d^2R}{dr^2}+2r\frac{dR}{dr}+\left[\beta^2r^2-l(l+1)\right]R=0 \tag{22}$$

$$r^2\frac{d^2Z}{dr^2}+r\frac{dZ}{dr}+\left[\beta^2r^2-\left(l+1/2\right)^2\right]Z=0 \tag{23}$$

Equation (23) is Bessel's Equation and their solutions are Bessel functions of half-integral order $l+1/2$.
The normalize solutions are $J_{n+1/2}(\beta.r)$ and $N_{n+1/2}(\beta.r)$

$$R(r)=\mathrm{A}\frac{J_{n+1/2}(\beta.r)}{\sqrt{\beta.r}}+\mathrm{B}\frac{\mathrm{N}_{n+1/2}(\beta.r)}{\sqrt{\beta.r}} \tag{24}$$

If $\sqrt{\beta.r}=\sqrt{\varepsilon}$, $J_{n+1/2}=J_n$, and $\mathrm{N}_{n+1/2}=n_n$ then equation (24) becomes

$$R(r)=\mathrm{A}J_n\frac{(\beta.r)}{\sqrt{\varepsilon}}+\mathrm{B}n_n\frac{(\beta.r)}{\sqrt{\varepsilon}} \tag{25}$$

Equations (25) are known as spherical Bessel functions and are denoted $J_n(kr)$ as first kind and $n_n(kr)$ as second kind defined as

$$J_n(x)=\sqrt{\frac{\pi}{2x}}J_{l+1/2}(x)\text{, and } n_n(x)=\sqrt{\frac{\pi}{2x}}\mathrm{N}_{l+1/2}(x) \tag{26}$$

The solution from equation (25) reduces to

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$$R(r) = \mathrm{A}J_n(\beta.r) + \mathrm{B}n_n(\beta.r) \tag{27}$$

If the solution is assumed to be finite at the origin, then the second kind of Bessel function $n_n(\beta.r) = 0$ and equation (27) reduce to

$$R(r) = \mathrm{A}J_n(\beta.r) \tag{28}$$

Hence, the general solution to heat conduction equation in spherical coordinate system which is regular at the origin is given as

$$T_{PNC}(r,\theta,\phi,t) = \sum_{l=0}^{\infty}\sum_{m=-l}^{l}\sum_{t=0}^{\infty} a_{lm}J_l(\beta\cdot r)\mathrm{Y}_l^m(\theta,\phi)e^{-\beta^2\alpha\, t} \tag{29}$$

Initial and Boundary Conditions

Consider two concentric spheres of radius r_1 and r_2 as illustrated in Figure 2. The two concentric spheres are sufficiently large in the radial direction and their radius r_1 and r_2 are very large to their thickness, in order to ensure only the temperature gradient in the radial direction r. Therefore taking $r_1 = r_2 + \delta r_1$ for inner sphere containing polystyrene (PS), and $r_2 = R + \delta r_2$ for outer sphere containing silver nanoparticle (AgNPs). δr_1 and δr_2 are thickness of (PS) and (AgNPs) respectively.

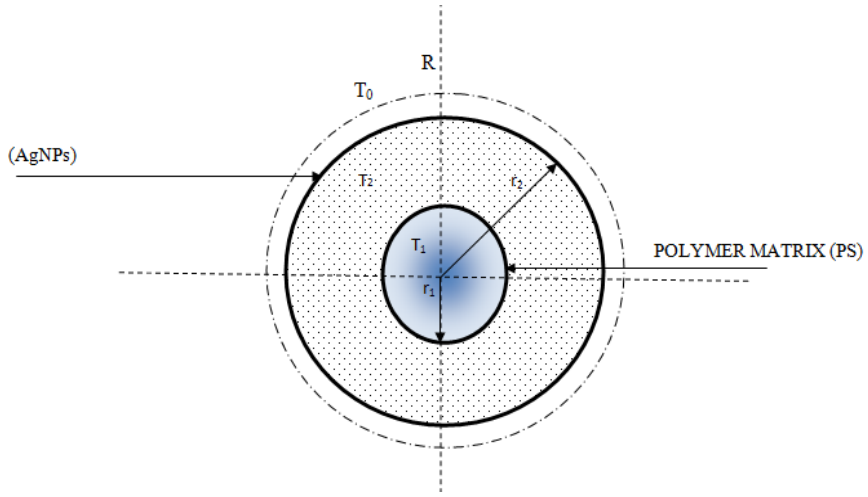

Figure 2: Cross-section of polystyrene silver nanocomposite (PS/AgNPs), the first layer is situated between r_1 and r_2 and the second layer is between r_2 and R

Subject to temperature boundary conditions for t>0:

$$\frac{\partial T_1}{\partial r} = 0 \qquad \text{at } r = r_1 \tag{30}$$

$$\lambda_1 \frac{\partial T_1}{\partial r} = \lambda_2 \frac{\partial T_2}{\partial r} \qquad \text{at } r = r_2 \tag{31}$$

.

$$\lambda_2 \frac{\partial T_2}{\partial r} + T_2 = 0 \qquad \text{at } r = R, \tag{32}$$

where λ_1 and λ_2 are the thermal conductivity of the composite material.

And the initial conditions, at $t = 0$:

$$T_1 = T_2 = T_0 \qquad \text{at } r_1 < r < r_2 \tag{33}$$

$$T_1 = T_0 = \frac{Q}{\rho \cdot C_\upsilon} \qquad \text{at } r_1 < r < R \tag{34}$$

In order to obtain the analytical solution for the heat equation in spherical nanocomposite structure, the temperature distribution $T_1(r,t)$ and $T_2(r,t)$ in the inner sphere and outer sphere respectively is express by considering equation (24) and (25)

$$T_1(r,t) = e^{-\beta_n^2 \alpha\, t}\left[\mathrm{A}_{1n} J_n\left(\frac{\beta_n}{\sqrt{\varepsilon_1}} \cdot r \right) + \mathrm{B}_{1n} n_n\left(\frac{\beta_n}{\sqrt{\varepsilon_1}} \cdot r \right) \right] \tag{35}$$

$$T_2(r,t) = e^{-\beta_n^2 \alpha\, t}\left[\mathrm{A}_{2n} J_n\left(\frac{\beta_n}{\sqrt{\varepsilon_2}} \cdot r \right) + \mathrm{B}_{2n} n_n\left(\frac{\beta_n}{\sqrt{\varepsilon_2}} \cdot r \right) \right], \tag{36}$$

where J_n and n_n are the Bessel functions of the first and second kind, The coefficients A_{1n}, B_{1n}, A_{2n}, and B_{2n} are determined from the temperature boundary conditions by defining the following quantities (Tamene *et al,* 2007).

Temperatures Prediction of Polymer Nanocomposite Structure (PS/AgNPs)

The temperatures distribution in the first sphere of Polystyrene (PS) is given by (35), which must satisfy the initial conditions.

Let consider that

$$\mathrm{T}(r,t) = e^{-\beta_n^2 \alpha t} f_i(r), \tag{37}$$

where, $f_i(r)$ is a function that depends on temperatures position of nanoparticles given by (Youcef & Bougriou, 2006)

$$f_i(r) = \sum_n C_n \varphi_{in}(r) \tag{38}$$

Therefore, the initial conditions at t = 0 are

$$f_i(r) = \mathrm{T}_0' \qquad r_1 \le r \le r_2 \tag{39}$$

$$f_i(r) = \mathrm{T}_0 \qquad r_2 \le r \le R \tag{40}$$

By applying the operator $\frac{\lambda_i}{\varepsilon_i} \cdot \int_{r_i}^{r_{i+1}} \varphi_{ir} r dr$ to both sides of equation (38), gives

$$\sum_{i=1}^{\mathrm{M}} \frac{\lambda_i}{\varepsilon_i} \cdot \int_{r_i}^{r_{i+1}} \varphi_{in}(r) \cdot f_i(r)\ r dr = \sum_n C_n \cdot \left[\sum_{i=1}^{\mathrm{M}} \frac{\lambda_i}{\varepsilon_i} \cdot \int_{r_i}^{r_{i+1}} \varphi_{in} \cdot \varphi_{in} r dr \right] \tag{41}$$

.

where M is the number of concentric spheres which is equal to 2, and r_i is the radial position of the concentric sphere in the selected point.

The orthogonality and normalization N_n expression is defined respectively, as follow

$$\sum_{i=1}^{\mathrm{M}} \frac{\lambda_i}{\varepsilon_i} \cdot \int_{r_i}^{r_{i+1}} \varphi_{in} \cdot \varphi_{in} r dr = \begin{cases} 0 & if \quad n \neq r \\ \mathrm{N}_n & if \quad n = r \end{cases} \tag{42}$$

and

$$\mathrm{N}_n = \sum_{i=1}^{\mathrm{M}} \frac{\lambda_i}{\varepsilon_i} \cdot \int_{r_i}^{r_{i+1}} \varphi_{in}^2 \cdot r dr \tag{43}$$

By substituting equation (43) into equation (41), gives

$$\sum_{i=1}^{\mathrm{M}} \frac{\lambda_i}{\varepsilon_i} \cdot \int_{r_i}^{r_{i+1}} \varphi_{in}(r) \cdot f_i(r) \ r dr = \sum_n C_n \cdot \mathrm{N}_n \tag{44}$$

Divide equation (44) through by C_n

$$C_n = \frac{1}{\mathrm{N}_n} \cdot \sum_{i=1}^{\mathrm{M}} \frac{\lambda_i}{\varepsilon_i} \cdot \int_{r_i}^{r_{i+1}} \varphi_{in}(r) \cdot f_i(r) \ r dr \tag{45}$$

Substitute equation (45) into equation (38), gives

$$f_i(r) = \sum_n \varphi_{in}(r) \cdot \left\{ \frac{1}{\mathrm{N}_n} \cdot \sum_{i=1}^{\mathrm{M}} \frac{\lambda_i}{\varepsilon_i} \cdot \int_{r_i}^{r_{i+1}} \varphi_{in}(r) \cdot f_i(r) \ r dr \cdot \right\} \tag{46}$$

Therefore, putting equation (46) into equation (37) gives the expression of temperature distribution

$$\mathrm{T}_i(r,t) = \sum_{i=1}^{n} \frac{e^{-\beta_n^2 \alpha t}}{\mathrm{N}_n} \cdot \varphi_{in}(r) \cdot \sum_{i=1}^{\mathrm{M}} \frac{K_i}{\varepsilon_i} \cdot \int_{r_i}^{r_{i+1}} \varphi_{in}(r) \cdot f_i(r) \ r dr, \tag{47}$$

where $\mathrm{K}_i = \lambda_i$ which is thermal conductivity of composite structure.

Therefore, the Analytical solution to temperature distribution for these two concentric spheres can now be express as follows:

For the first sphere which is Polystyrene (PS) polymer matrix:

$$\mathrm{T}_1(r,t) = \sum_{i=1}^{n} \frac{e^{-\beta_n^2 \alpha t}}{\mathrm{N}_n} \cdot \left\{ J_n\left(\frac{\beta_n}{\sqrt{\varepsilon_1}} r \right) + \mathrm{B}_1 \cdot n_n\left(\frac{\beta_n}{\sqrt{\varepsilon_1}} r \right) \right\} \cdot \mathrm{X} \tag{48}$$

For the second sphere which is silver nanoparticle (AgNPs)

$$\mathrm{T}_2(r,t) = \sum_{i=1}^{n} \frac{e^{-\beta_n^2 \alpha t}}{\mathrm{N}_n} \cdot \left\{ \mathrm{A}_2 \cdot J_n\left(\frac{\beta_n}{\sqrt{\varepsilon_2}} r \right) + \mathrm{B}_2 \cdot n_n\left(\frac{\beta_n}{\sqrt{\varepsilon_2}} r \right) \right\} \cdot \mathrm{X}, \tag{49}$$

where X is the sum of equation (45) and (47).

.

RESULTS AND DISCUSSION

A MATLAB simulation code based on the temperature distribution described by equations (48) and (49) has been developed. The primary interest is in the heat conduction in a spherical nanocomposite, and then the simulation system will consists of silver nanoparticles randomly distributed in a sphere of polystyrene. Initially, the temperature of PS sphere was set to 500 ºC and the time step size was varied from 10.0 second to 100.0 second in different runs. An equilibrium run of 10 steps was first performed, and followed by a production run of another 10 steps. For illustration, the numerical analysis demonstrated from equation (48) and (49) above is applied to analyze temperature rise in a composite material along its width direction and an axially symmetric temperature change in the two concentric spheres along its radial direction$R$are given for a sudden constant temperature change applied at $r_1$ranges from 0.5 m to 5.5 m and r_2 ranges from0.11 m to 0.20 m. The values obtained from heat conduction equation are shown in Table 1.

Table1: Show the values of temperature distribution obtained from analytical expression with their radial position and time

Radius of (AgNPs) $\mathbf{r_1}$	**Radius of PS) $\mathbf{r_2}$**	**Time (s)**	$\mathbf{T_1}(℃)$	$\mathbf{T_2}(℃)$
0.5	0.11	0.10	475	26
1.0	0.12	0.20	105	388
1.5	0.13	0.30	96	423
2.0	0.14	0.40	96	385
2.5	0.15	0.50	95	289
3.0	0.16	0.60	94	226
3.5	0.17	0.70	92	135
4.5	0.18	0.80	90	76
5.0	0.19	0.90	83	17
5.5	0.20	0.100	22	21

The numerical values of the temperature obtained from the analytical expressions are represented in the Figures 3 and 4, for the both spheres, respectively. It can be seen from Figures 3 and 4 that the temperature $T1\ (r,t)$ decreases from 475 ºC at t = 10.0 second, to 105 ºC at t = 20.0 second. On the other hand, the temperature $T2\ (r,t)$ increases from 26 ºC at time t = 10.0 second reaching a maximum value of 423 ºC at time t = 30 second, then it decreases to reach the temperature limiting value of 21 ºC at time t =100 second

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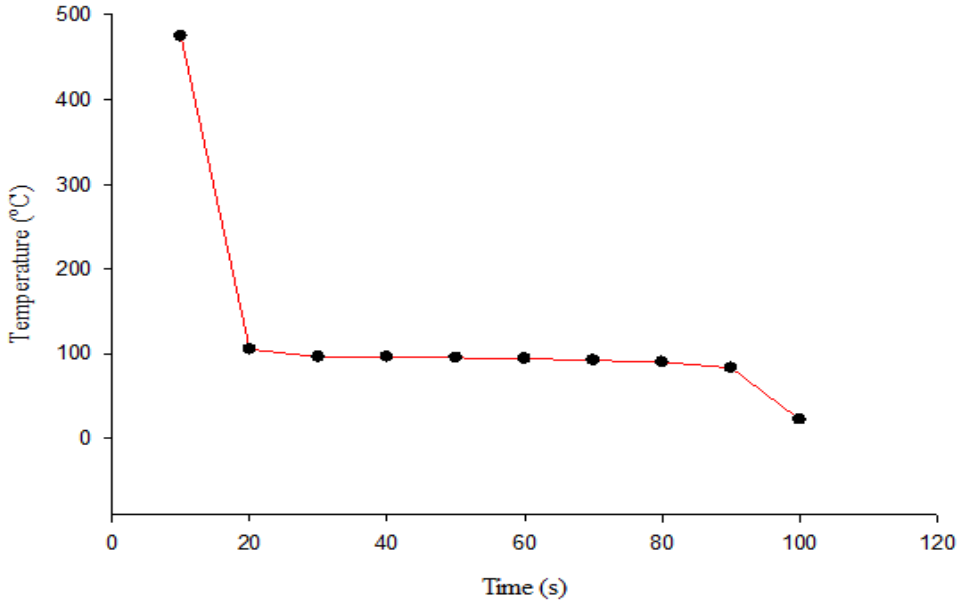

Figure 3: Temperature of the first sphere composed of Polystyrene (PS) via the time, for two concentric spheres

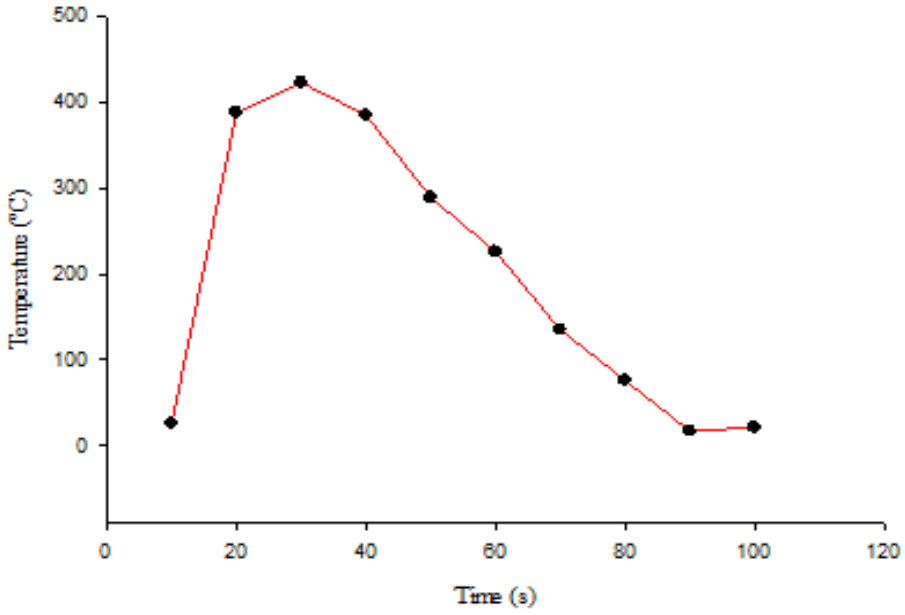

Figure 4: Temperature of the second sphere composed of silver nanoparticle (AgNPs) via the time, for two concentric spheres

As the temperature increases, the silver nanoparticle (AgNPs) scattering rates increase, which leads to increase in the thermal respond of the nanoparticle within the polystyrene (PS) sphere, owing to the scattering of nanoparticle the polymer matrix. It also shows that the volume fraction of particle increases and the concentration of the thermal conductivity are obtained; thus, the thermal conductivity of particle is responsible for the temperature induced from the composite.

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Contour Representation on Temperature Distribution of (PS/AgNPs) Composite

The Sufer 16 software was used to further show the 2-D Contour of the temperature distribution of polystyrene silver nanoparticle PS/AgNPs composite. Figure 5 show the contour map and summary of the temperature variation with time and the radius of polystyrene silver nanoparticle PS/AgNPs composite.

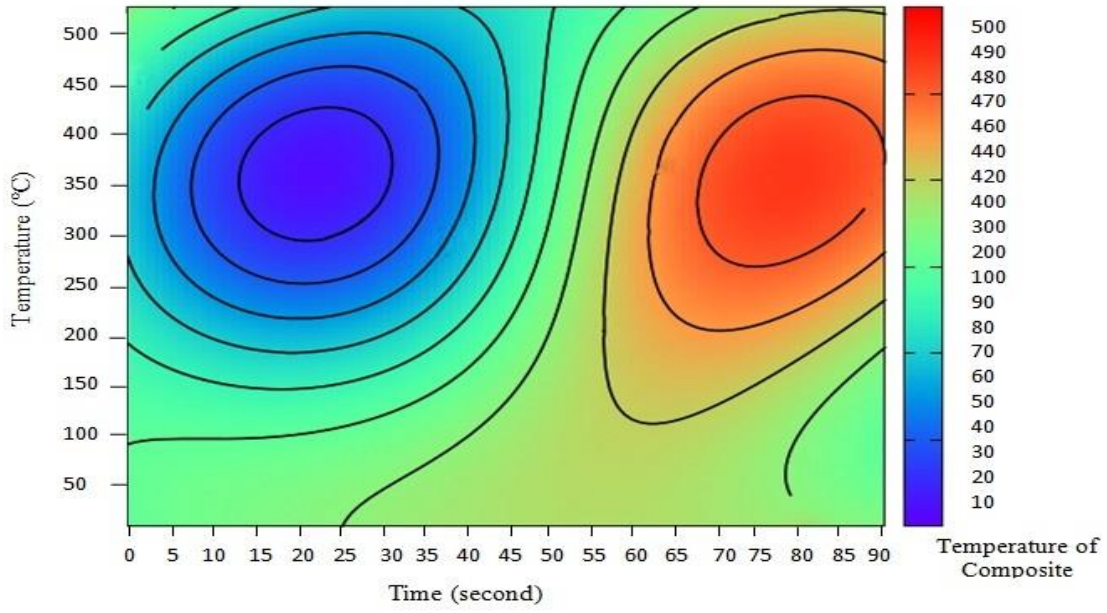

Figure 5: 2-D Contour of Temperature variation of PS/AgNPs Composite with time

Figure 5 shows a representative contour of the simulation system at thermal equilibrium. The radius and position of AgNPs are coded by the instantaneous temperature, and observation show that the temperature of Ag particles is not uniform at thermal equilibrium. It can also be observed that the relative scattering of nanoparticle with respect to polystyrene sphere becomes smaller as the temperature of inner sphere decreases from 475 ºC to 105 ºC and maintain the same temperature variation of 105 ºC at time rate of 0.20 second to 0.95 second which shows that silver nanoparticle (AgNPs) cannot be found within the specified position of polystyrene sphere. The narrower distribution of the internal heat can be understood by observing that, as temperature increases, the AgNPs become more coarse-grained, and thus exhibit less fluctuation in its internal heat transfer to the PS sphere (Willemsen*et al.,* 2000)

CONCLUSION

In this paper, an analytical solution to heat conduction equation was obtained by using separation of variable method. The analytical solution is valid for a variety of spherical geometries. Simulations of heat conduction in nanocomposite were successfully carried out using a MATLAB code to investigate the selection of time step in the simulations of the temperature distribution. With light weight and improved heat conduction capability, this PS/AgNPs nanocomposite can be used for applications such as electronic packages, communication device, thermal grease, thermal interface material and electric cable insulation

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Digestive Enzyme Inhibitory Effect and Antioxidant Activities of a Ferulic Ester and Other Phenolic Constituents from *Entada Spiralis* Ridl.

[*1]Roheem F. O., [2]So'ad S. Z. M & [2]Ahmed Q. U.

[1]Department of Chemistry, Faculty of Science, Airforce Institute of Technology, Kaduna, Nigeria.
[2]Department of Pharmaceutical Chemistry, Kulliyyah of Pharmacy, International Islamic University Malaysia, Bandar Indera Mahkota, 25200 Kuantan, Pahang DM, Malaysia.

*Correspondence Email: fatimaroheem20@gmail.com, fatimaroheem20@afit.edu.ng,

Phone No: 09078544275, 09051264496

ABSTRACT

Entada spiralis is a well-known medicinal plant in herbal medicine due to its various traditional and medicinal applications. The crude extracts from the stem bark were evaluated for their digestive enzyme inhibitory effect and antioxidant activities. Fractionation and purification from the most active methanol extract led to the isolation of a ferulic ester **1** namely hexyl 3-(4-hydroxy-3-methoxyphenyl) acrylate together with five known phenolic compounds (2-6). The compounds were characterized using NMR (1D and 2D) and ESI-MS spectroscopic techniques. All the isolated compounds were assessed for their inhibitory activities against digestive enzymes and free radicals. Compounds **1**, **5** and **6** showed good antioxidant and α-glucosidase inhibitory activities but were found inactive against α-amylase while compounds **2**, **3** and **4** displayed potent inhibitory capacity against both enzymes and free radicals.

Keywords: *Entada spiralis*; hexyl 3-(4-hydroxy-3-methoxyphenyl) acrylate; antioxidant activity; α-amylase inhibitory activity; α-glucosidase inhibitory activity

INTRODUCTION

Entada spiralis Ridl. (synonym: *Entada scheffleri*) which belongs to the family of Leguminosae is a woody climber that spines around other higher plants for support and can grow up to 25 m tall. It grows widely in the rain forest and widely spreads across Southeast Asia such as Malaysia, Indonesia and Thailand. Many other species from the same genus have been reported to exhibit potent digestive enzyme inhibitory and antioxidant activities (Diallo *et al*. 2001, Ruangrungsi *et al*. 2008, Guissou and Nacoulma, 2010, Njayou *et al*. 2013, Gautam *et al*. 2011). Previous study on this plant has shown the potency of methanol extract as an anti-bacterial and anti-fungal agent (Harun *et al*. 2014). Compounds isolated so far from this plant include ester saponin, triterpenoidal glycosides, and diterpenoidal glycosides and sugars (Harun *et al*. 2015). Other pharmacological activities are yet to be studied with respect to their true medicinal potential particularly on their enzyme inhibitory potential and as an antioxidant agent. Hence, we report herein, the inhibitory activity of *Entada spiralis* stem bark on digestive

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enzymes and free radicals and isolation of the active principles responsible for these effects.

GENERAL METHODOLOGY

E. spiralis Ridl. stem barks obtained from Tasik Chini forest Pahang, identified and authenticated by a taxonomist at Universti Kebangsaan Malaysia, and deposited at the hebaruim UKM (voucher specimen KMS-5228). The stems were cut into small pieces, air-dried at room temperature and pulverized into powdered form (4.5 kg). Due to its soapy nature, it was macerated successively using petroleum ether, chloroform and methanol. Methanol extract (100 g) was fractionated on a silica gel column with gradient mixtures of CHCl3: MeOH (2.5 L: 9:1→6:4) being used as eluent Each fraction was concentrated in vacuo and kept in a glass bottles and kept in the fridge until further analysis. All the four fractions were evaluated for bioactivity and the most active fractions (F1 and F2) were subjected to different purification processes to obtain active principles. The isolated compound was elucidated through different spectroscopic methods. DPPH assay was conducted using DPPH• by modified methods from Sulaiman et al., (2011) and Ahmed et al., (2015) while for ABTS assay, Zheleva-Dimitrova et al (2010) method was adopted with modifications. α-amylase assay inhibitory assay was measured by hydrolysis of starch in the presence of α-amylase enzyme, quantified by iodine which shows blue- black colour with starch. Johnson et al., (2011) was adopted and modified to microplate based method using Xiao et al., (2013). α-glucosidase inhibitory assay was assayed using method adapted from Johnson et al., (2011) with slight modifications using α-glucosidase (enzyme) obtained from Saccharomyces cerevisiae (1 U/mL) dissolved in 50 mM phosphate buffer (pH 6.9).

RESULTS AND DISCUSSION

Identification of the isolated compounds

Compound **1** was obtained as a white amorphous powder. mp: 80-88 ^{o}C; UV (λ_{max} MeOH) 365 nm; FT-IR (cm^{-1}): 3551, 2914, 2848 1624, 1681 and 1681 ESI-MS $[M+H]^{+}$ ion peak was observed at 279.16 corresponding to $C_{16}H_{22}O_4$. ^{1}H-NMR [600 MHz, $CDCl_3$-δ_H (ppm), J (Hz)]: 0.90 (d, J=7.14, 3H, H8′) 1.42 (m, 8H, $(CH_2)_4$), 4.20 (d, J=6.8, 2H (OCH_2), 6.33 (d, J=15.9, 1H, H-2′), 7.60 (d, J=15.9, 1H, H-1′), 6.95 (d, J=6.8, 1H, H-5), 7.06 (d, J=1.5, 1H, H-2), 7.10 (dd, J=6.8, 1.4, 1H, H-6), 3.95 (s, 3H, OCH_3), 5.95 (s, 1H, O-H). ^{13}C-NMR [150 MHz, $CDCl_3$,-δ_C (ppm)]: 64.6 (OCH_2), 55.9 (OCH_3), 144.6 (C-1′), 115.7 (C-2′), 127.05 (C-1), 109.32 (C-2), 146.76 (C-3), 147.91 (C-4), 114.72 (C-5), 123.03 (C-6), 22.69 (C-4′), 26.01 (C-5′), 29.71 (C-6′), 31.93 (C-7′), 14.11 (C-8′), 167.42 (C=O). All the carbon values were assigned based on HSQC spectrum. In COSY, protons at δ 1.42 ppm strongly correlated with the terminal primary methyl proton in the fatty acid ester chain at δ 0.90 ppm and methylene protons at δ 1.72 ppm whereas, δ 1.72 ppm was also observed to show correlation with oxymethine protons at δ 4.20 ppm as shown in Figure 1. Similarly, J_H- J_H correlation was also observed between the two olefinic protons at δ 6.33 and 7.65 ppm. In the aromatic ring, δ 6.95 (H-5) showed correlation with δ 7.10 (H-6). In HMBC spectrum, correlations were observed between protons at δ 6.33 and δ 7.60 ppm (H-1′ and H-2′) with δ 127.03 (C-1), 123.03 (C-6), and 167.41 (C=O). These further confirmed the correlation between long chain fatty acid ester and that of the aromatic ring (Figure 1). Moreover, the link between the long methylene chain with COO was established by the correlation between the oxymethine proton at δ 4.20 ppm with both methylene carbons at δ

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26.06 (C-5'), 28.87 (C-6') and that of C=O at δ 167.42 ppm. Methoxy protons at δ 3.95 ppm displayed strong correlation with carbon at δ 146.72 suggesting that the methoxy group was attached to C-3. Low intensity broad singlet at δ 5.95 indicated O-H proton signal which showed correlation with δ 147.91 (C-4) and δ 114.72 (C-5). Furthermore, proton at δ 6.95 ppm (H-5) showed correlation with δ 123.03 (C-6), 127.05 (C-1) and 146.70 (C-4) as illustrated in Figure 1. Based on these spectral data interpretation, compound **1** was unambiguously identified as hexyl 3-(4-hydroxy-3-methoxyphenyl) acrylate, a natural product isolated for the first time from this plant.

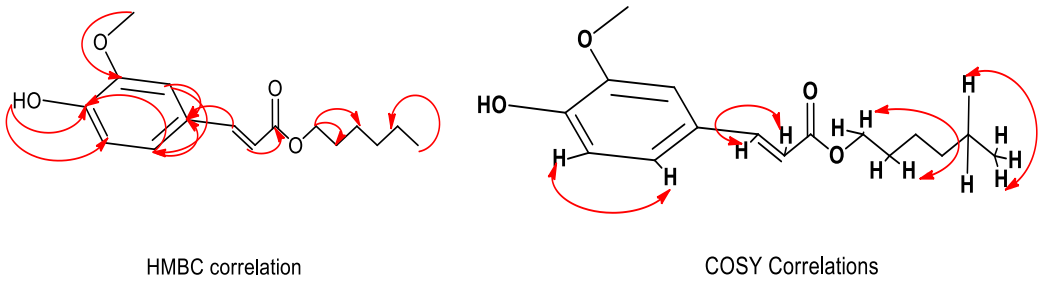

Figure 1. Significant HMBC and ^{1}H-^{1}H COSY correlations for compound **1** isolated from the methanol extract of *E. spiralis* stem bark.

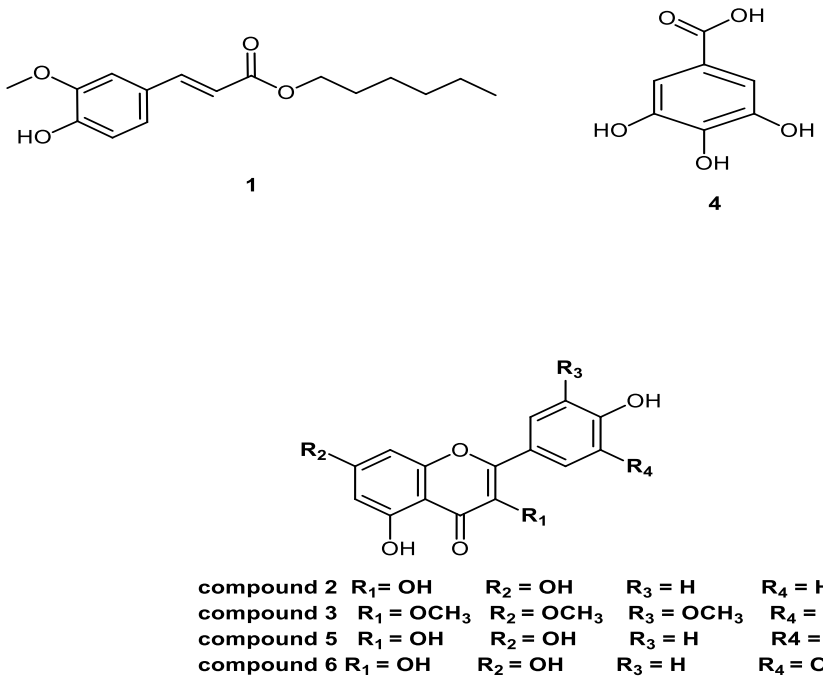

Figure 2. Structure of isolated constituents from *Entada spiralis* stem bark methanol extract.

Additionally, other compounds isolated were also characterized using FT-IR, melting point, 1D NMR and direct comparison with those previously reported in the literature. The compounds were therefore identified as kaempferol (**2**) (Emam *et al*. 2010), pachypodol (**3**) (Ali *et al*. 2008), gallic acid (**4**), (Eldahshan

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2011), (+)-catechin (**5**) (Watanabe 1988) and (-)-epicatechin (**6**) (Watanabe, 1988).

***In vitro* Antioxidant Activity**

Analysis of antioxidant activities of various extracts and fractions at different concentrations (7.81 µg -125 µg/mL) on DPPH and ABTS radicals with their corresponding IC_{50} values is shown in Table 1. Methanol extract showed the highest radical inhibitory activity against both radicals with least IC_{50} values of 42.67 ± 4.10 µg/mL and 37.39 ± 0.05 µg/mL, respectively. Similar activity of methanol extracts of *Entada pursaetha* and *Entada africana* have been previously reported (Tibiri *et al*. 2007, Pakutharivu and Suriyavadhana 2010). Among the fractions, F1 and F2 obtained from the methanol extract displayed good radical scavenging capacity as shown in Table 1. Meanwhile, among the isolated compounds**, 2, 4, 5** and **6** demonstrated the highest activity against DDPH and ABTS radicals. Compound **1** also showed good antioxidant activity against DDPH and ABTS radicals with IC_{50} of 13.79 ± 2.13 and 4.69 ± 1.25, respectively. Compound **3** exerted the least activity. The good radical scavenging activity of these compounds is a manifestation of the fact that phenolic compounds are well known antioxidants due to their electron-donating capacity (Rice-Evans *et al*. 1997).

Table 1. Radical Scavenging and Enzyme Inhibitory activity of Extracts, Fractions and Isolated compounds from *Entada spiralis* stem bark.

Sample	IC_{50} (µg/mL)			
	DPPH assay	ABTS Assay	α-amylase	α-glucosidase
Methanol extract	42.67 ± 4.10^{c}	37.39 ± 0.05^{c}	98.15 ± 6.04^{c}	20.63 ± 0.44^{a}
Chloroform extract	472.83 ± 11.20^{f}	90.84 ± 3.12^{d}	405.29 ± 7.36^{f}	74.96 ± 24.77^{C}
Petroleum Ether extract	1050.08 ± 23.21^{g}	625.16 ± 10.58^{f}	-47.60 ± 9.25^{g}	172.93 ±1.77^{f}
F1	37.09 ± 0.88^{c}	19.92 ± 1.11^{a}	312.14 ± 8.78^{e}	24.17 ± 1.24^{b}
F2	30.18 ± 1.91^{b}	30.50 ± 0.21^{b}	13.19 ± 0.18^{b}	28.15 ± 2.25^{b}
F3	78.52 ± 5.21^{d}	29.45 ± 4.12^{b}	215 ± 86 ± 16.62^{d}	123.18 ± 2.05^{d}
F4	326.63 ± 13.93^{e}	303.75 ± 12.29^{e}	-67.45 ± 8.74^{h}	143.76 ±21.43^{e}
Compound 1	13.79 ± 2.13^{b}	4.69 ± 1.25^{d}	ND	149.00 ± 9.71
Compound 2	11.29 ± 0.26^{b}	2.10 ± 0.51^{c}	95.27 ± 3.48	21.91 ± 3.24
Compound 3	66.17 ± 8.03^{c}	35.26 ± 1.19^{e}	478.12 ± 15.39	75.30 ± 9.83
Compound 4	6.15 ± 0.38^{a}	1.28 ± 0.89^{b}	87.14 ± 5.31	6.49 ± 1.52
Compound 5	12.48 ± 1.55^{b}	1.86 ± 0.15^{b}	ND	101.84 ± 7.24
Compound 6	11.75 ± 33.82^{b}	1.12 ± 0.09^{b}	ND	99.05 ± 5.20
Qc	29.82 ± 3.73^{a}	-	-	4.85 ± 1.05
Ac	24.67 ± 0.45^{a}	16.74 ± 1.76^{a}	-	-
Acarbose	-	-	0.85 ± 0.19	-
Tx	-	15.23 ± 2.15^{a}		

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Values are expressed as mean ± SEM of triplicat e measurement. Samples were analyzed using o ne-way ANOVA. Values of different samples with similar superscript are n ot different significantly as measured by Turkey's HSD post Hoc test (at $p < 0.05$). Ac (ascorbic acid), Tx (trolox), Qc (quercetin), ND (not detected), 1 (hexyl 3-(4-hydroxy-3-methoxyphenyl) acrylate), 2 (kaempferol), 3 (pachypodol), 4 (gallic acid), 5 ((+)-catechin), 6 ((-)- epicatechin). IC_{50} (concentration of the sample required to scavenge 50% of the radicals).

***In vitro* Digestive Enzyme Inhibitory Activity**

α-amylase inhibitory activity of *E. spiralis* extracts and methanol fractions using acarbose as positive control was investigated using starch-iodine method which has a characteristic blue colour. Intensity of iodine colour decreases as the starch is hydrolyzed into monosaccharaides by α-amylase. However, in the presence of active sample, the hydrolysis is forestalled as the colour remains unchanged (Tunna *et al*. 2015). Enzymes inhibitory activity of the extracts and fractions and isolated compounds are shown in Table 1. Methanol extract showed good inhibition with IC_{50} value of 53.12 ± 3.42 µg/mL while fraction F2 among the fractions was considered the most active with IC_{50} of 13.19 ± 0.18 µg/mL. Fraction F4 was observed to have negative IC_{50} (-67.45 ± 8.74 µg/mL respectively), revealing the fact that α-amylase enzyme may have been activated rather than being inhibited and therefore could shoot-up the rate of glucose absorption thereby aggravating post-prandial hyperglycemia in diabetes condition if ingested (Oyedemi *et al*. 2017). Only compounds **2** and **4** showed moderate inhibitory activity with IC_{50} values of 95.27 ± 3.48 and 87.14 ± 5.31 µg/mL, respectively. Compound **3** showed weak activity having IC_{50} close to 500 µg/mL. Compounds **1**, **5** and **6** did not show any observable α-amylase inhibition. This outcome corroborates with the result obtained from inhibitory activity of the water extract of Qingzhuan dark tea which showed that extract rich in catechin **5** and epicatechin **6** does not have any significant α-amylase inhibition (Liu *et al*. 2016).

However, for α-glucosidase, all the extracts and fractions showed different degrees of inhibition in a concentration dependent manner. Methanol and chloroform extracts, fractions F1 and F2 showed good α-glucosidase inhibitory activity having IC_{50} less than 100 µg/mL. Meanwhile, methanol extract which had the least IC_{50} (20.63 ± 0.44 µg/mL) among these samples was considered most potent α-glucosidase inhibitor. Among the isolated compounds, compound **4** showed the highest inhibitory activity (IC_{50}, 6.49 ± 1.52 µg/mL) followed by compound **2** with IC_{50} of 21.91± 3.24 µg/mL as compared to commercial quercetin, reference standard (with IC_{50} 4.85± 1.05 µg/mL). Compound **1** exhibited the least inhibition with IC_{50} of 149.00 ± 9.71 µg/mL. Compound **3** was observed to show moderate inhibitory activity with IC_{50} values of 75.30 ± 9.83 µg/mL while compounds **5** and **6** showed weak inhibition with IC_{50} values of 101.84 ± 7.24 and 99.05 ± 5.20 µg/mL, respectively. (Tadera *et al*. 2006) also reported similar weak α-glucosidase inhibitory activity for catechin and epicatechin with IC_{50} greater than 200 µM. Enzyme inhibitory activity of phenolic acids such as gallic acid (compound **4**) is based on the conjugation and hydroxyl group in the ring, however, esterification of phenolic acid as the case of compound **1** significantly abrogates the inhibitory activity (Xiao *et al*. 2011) due to steric hindrance caused by the long ester chain, which is farther away from the resonance and hydrogen bonding effect of the phenolic ring.

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Consequently, gallic acid and ferulic acid are expected to be more active than their derivatives (Jeong et al. 2012; Xu *et al*. 2016).

CONCLUSION

Results obtained through this research work vividly validate *E. spiralis* stem bark's methanol extract as potent free radicals and digestive enzymes inhibitor. Repeated fractionation and purification of the active methanol extract of *E. spiralis* stem bark has resulted in the isolation of a ferulic ester (hexyl 3-(4-hydroxy-3-methoxyphenyl) acrylate) together with five biologically active phenolic compounds comprising a phenolic acid and four flavonoids. All these compounds were found active either as free radical quenchers or/and digestive enzymes inhibitors. Interestingly, this is the first scientific report on the isolation of kaempferol, catechin and epicatechin from *E. spiralis* Ridl. To the best of our knowledge, pachypodol and gallic acid have been isolated from the entire *Entada* genus for the first time through this research work.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Investigating the Effects of Tarmarind, *Tamarindus indica* Pulp and Tetracycline on Serum Biochemical Parameters in Broiler Chickens

Azaki T. S. & Mohammed A.

Department of Biochemistry & Biotechnology, IBB University Lapai.

Email: azakitayawoza@gmail.com; *Phone number*: +2347037761677

ABSTRACT

Evaluation of blood biochemical, electrolyte and enzyme functions involved in metabolic processes could provide information about the health or disease status of an animal. This study was undertaken to compare the effect of Tetracycline (AT), and Tarmarind (TD) dissolved in drinking water on serum biochemical parameters of broiler chickens, compared to a control (C). 120 acclimated broiler chickens were randomly divided into 3 groups, with each group containing 40 birds each and labelled AT, TD and C. Each group was further divided into 4 replicates of 10 birds each. Commercial Feed and water were offered to the birds in all the different groups *ad libitum*, and no vaccine was used during the study. After 6 weeks, Blood was collected and the hepatic and renal function biomarker enzymes of broiler chickens assessed. Animals fed with Tetracycline (AT) and Tarmarind (TD) showed AST, K and Total Protein values not significantly varied ($P>0.05$) across all the treatments. Notably however, the ALT for the TD were numerically higher and significantly ($P<0.05$) higher when compared with the control C and AT groups. All the biochemical parameters evaluated across all the treatment groups were still within the normal range in chickens. The results of this study suggests that there was no adverse effect on blood metabolic activities and that using Tamarind has no adverse effect on liver and kidneys of broiler chickens. Therefore, tarmarind could be useful as a substitute for Tetracycline in broiler chicken to Boost immune function of broiler Chickens.

Key words: Broiler, Tamarind pulp, Antibiotic, Immunity, Hepatic and renal Biomarker.

INTRODUCTION

For over six decades dietary antibiotics have been used not only as a means to control infectious diseases or improve health but also to improve growth performance and feed efficiency (Birmani *et al.*, 2019). The continuous use of antibiotics as feed additives in the long run can contribute to the development of bacteria resistance to drugs used to treat infections, which are of potential risk if they are transferred to humans. In addition, antibiotics cause some other problems such as destruction of some beneficial bacteria in poultry gut (Alagawany *et al.*, 2018).

For these reasons, the World Health Organization in 1997 and the Economic and Social Committee of the European Union in 1998 concluded that the use of antimicrobials in food

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animals is a public health issue, therefore the European Union banned the use of all in-feed antibiotics as growth promoters in 2006 (Palamidi *et al.*, 2016). In 2009, government agencies in the USA such as the Food and Drug Administration (FDA) testified that the use of antibiotics for growth promotion should be eliminated (Al-Khalaifah, 2018). In some countries, due to the human health concern on the use of antimicrobial agents and their effects on antimicrobial resistance in humans, certain Antibiotic Growth Promoters (AGP) have already been banned, and there is a possibility of future restrictions on their use worldwide (Al-Fatah, 2020).

In light of these growing concerns, it became very necessary to find alternative method to improve gut health and reduce the productivity losses (Al-Fatah, 2020). Scientists are now focused on finding new, cheap, harmless and efficient feed additives, which can increase the overall immunological resistance of the chicken and improve the quality of the diet (Yadav *et al.*, 2013). Several alternatives to antibiotic growth promoters in feeds have been proposed such as organic acids, probiotics, phytobiotic or phytogenic feed additives and enzymes (Fascina *et al.*, 2017). Studies are been conducted to use phytobiotics or Alternative Growth Promoters from herbal plants and their bioactive compounds. Example of such a plant is tamarind which is rich in minerals and amino acids, it has good nutritive values, available in abundance containing some active compounds such as phenols flavonoid anthocyandine (Omar, Hejazi and Badran, 2016). Tamarind or *Tamarindus Indica L*. belongs to the family Fabaceae (Leguminosae), it is called “tsamia” in the northen part of Nigeria, it is an important food in the tropics. It is a multipurpose tree of which almost every part find some use either nutritional or medicinal properties. It is a tree which is indigenous to tropical Africa where it still grows in wild, although Tamarind is indigenous to tropical Africa it has been introduced and naturalized worldwide in over 50 countries (Ferdouse *et al.*, 2020). Tamarind has been reported to have anti-diabetic, anti-inflammatory, cholesterol lowering, anti-obesity, antifungal, antioxidant, antipyretic and antimicrobial properties. In addition, it has appetizing and stimulatory effect in the digestive process. Polyphenolic compound in the extracts could reduce heat stress in broiler chickens. T. indica leaves contains good amount of protein, fiber, fat and different types of vitamins such as B1, B2, B3, Vit. C, β-carotene and also flavonoid and polyphenols which have a proven record as antimicrobial activity (Attia, Al-Harthi and Hassan, 2017)

Tamarind is classified within the legume family and its scientific name is *Tamarindus indica*. Tamarind contains 13.3-26.9% raw protein, which has high basic amino acids such as Lysine, Arginine and Threonine and rich in fat acids especially in seed nucleus rich in palmitic, oleic and linoleic and 4.5-16.2% fat (Bushra, Basil and Saja, 2019). Tamarind is highly valued for its fruits, especially the pulp which is used for a wide variety of domestic and industrial purposes, especially for food and beverages. The most outstanding characteristics of tamarind pulp is its sweet acidic taste, the acid is due to mostly tartaric acid (10%) while the sweet taste is as a result of its high reducing sugars. Tamarind pulp improves performance of broiler chickens (Omar, Hejazi and Badran, 2016).

This study was carried out to investigate dissolving *Tamarindus indica* pulp in water and how it improves the immune system in broiler

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chickens. The serum biochemical parameters were measured in an attempt to see what was happening to the immune system of chickens.

METHODOLOGY

The tamarind pulp was all purchased from a single seller in Paiko, Niger State, Nigeria. The tamarind pulps were aggregated into an average of 50g each. 120 day-old broiler starter birds were purchased from Zartech Farms limited in Ibadan, Oyo State Nigeria. Chicks were reared in cages of dimension 1.5m x 1.5m x 2.0m; length x breadth x height, that were previously cleaned and disinfected. The acclimatized broiler chicks were weighed with an electronic digital scale. The birds were randomly divided into three groups of 40 birds each. Each group was assigned into four replicates of 10 birds each. The birds were fed commercial Ultima starter feed and the tamarind pulp (25g/L of water) dissolved in their drinking water for the six weeks starter period. Feed and water were offered to the birds in all the different treatments *ad-libitum*. All birds were reared without vaccination. Chicks were reared under approximately natural photoperiod of 12/12 hours of light/dark cycles for period of six weeks and cages were cleaned at the end of every week.

COLLECTION OF BLOOD FROM EXPERIMENTAL BIRDS

The experimental sampling procedure involved the collection of blood from the test broiler chickens. At the end of the sixth (6th) week, nine birds were randomly selected from each treatment. The blood sample was obtained by venipuncturing the brachial veins (wing veins) of the experimental birds (broilers), according to method described by (Lisa and Leanne, 2013). This was done by plucking a few feathers from the ventral surface of the humeral region of the right wing to aid visualization. The skin was then sterilized with methylated spirit, and was afterwards punctured with 2mm syringe to obtain 2ml of blood sample. The blood sample was transferred into appropriate sample bottles; some with anticoagulants and some plain. The serum was obtained by centrifugation using an electric desktop lab centrifuge (EU Plug 220V, Jersey, UK) for 10 minutes at 3,000 rpm. The serum was collected by the use of micro pipette and transferred into anticoagulant free test-tube and stored in refrigerator (4°C) for subsequent analyses.

Determination of Aspartate aminotranferase (AST)

(Reitman and Frankel, 1957)**. (DNPH Method)**

Principle:

$$\text{L-Aspartate + 2-Oxogluatarate} \xleftrightarrow{\text{SGOT}} \text{Oxaloacetate + L-Glutamate.}$$

$$\text{Oxaloacetate + 2,4 DNPH} \longleftrightarrow \text{Brownish red coloured complex.}$$

SGOT (AST) catalyses the transfer of amino group from Aspartic acid to 2-Oxoglutarate to form Oxaloacetate and L-Glutamate. The Oxaloacetate thus formed reacts with 2,4 Dinitrophenyl Hydrazine (2,4 DNPH) to form a corresponding Hydrazone, a brownish red colored complex in an alkaline medium. The color intensity is directly proportional to the SGOT concentration in the serum and is measured photometerically at 505 nm (490-546) (Reitman and Frankel, 1957).

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Determination of Alanine aminotranferase activity (ALT)

(Reitman and Frankel, 1957)**.** (Colorimetric DNPH Method)
Principle:

$$\text{L-Alanine + 2-Oxoglutarate} \xrightarrow{\text{SGPT}} \text{Pyruvate + Glutamate}$$
$$\text{Pyruvate + 2,4 DNPH} \longrightarrow \text{Brownish red coloured complex.}$$

SGPT (ALT) catalyses the transfer of amino group from L-Alanine to 2-Oxoglutarate to form pyruvate and LGlutamate. The Pyruvate thus formed reacts with 2,4 Dinitrophenyl Hydrazine (2,4 DNPH) to form a corresponding Hydrazone, a brownish red colored complex in an alkaline medium. The color intensity is directly proportional to the SGPT concentration in the serum and is measured photometerically at 505 nm (490-546) (Reitman and Frankel, 1957).

Determination of Alkaline Phospatase activity (ALP)

Principle:
Serum ALP hydrolyzes phenyl phosphate into phenol and disodium hydrogen phosphate at pH 10.0 The phenol so formed reacts with 4-Aminoantipyrine in alkaline medium in presence of oxidizing agent Potassium ferricyanide to form a red colored complex whose absorbance is proportional to the enzyme activity (KIND and KING, 1954)

Determination of Serum Albumin

BCG Method

Principle

The method is based on the specific binding of bromocresol green (BCG), an anionic dye, and the protein at acid pH produce a color change of the indicator from yellow –green to green –blue with the resulting shift in the absorption wavelength of the complex. The intensity of the color formed is proportional to the concentration of albumin in the sample (Bartholomew and Delaney, 1966).

$$\text{BCG + Albumin} \xrightarrow[\text{pH 4.3}]{} \text{BCG-albumin complex}$$

Determination of Total protein

The concentration of total protein in the chickens serum was determined spectrophotometrically following the manufacturer's instructions of BIOLABO SAS, Maizy France.

Principle: The determination was based on the principle of the biuret reaction (copper salt in an alkaline medium) described by (Gormall, Bardwill and David, 1949). Protein in serum sample forms a blue colored complex when treated with cupric ions in alkaline solution. The intensity of the blue color is proportional to the protein concentration.

Determination of Serum Creatinine

The level of creatinine in the chicks' serum was determined spectrophotometrically following the manufacturer's instructions of DIALAB Diagnostics, Wiener Neudorf, Austria.

Principle: The determination was based on the method, which uses the Jaffe reaction, according to the work of (Bartels, Böhmer and Heierli, 1972). In an alkaline medium, creatinine forms

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an orange-red colored complex with picric acid. The rate of color formation is proportional to the concentration of creatinine present, as shown in the following reaction;

$$\text{Creatinine + Picric acid} \xrightarrow[37^{O}C]{\text{pH}>12} \text{Red addition complex}$$

Procedure: Cold reagents and samples were brought to ambient temperature. To three (3) sets of test-tubes labeled; blank, sample and standard, 1000µl of working reagent (equal volume of Picric acid 35mmol/l and sodium hydroxide 0.32mol/l) was added. Thereafter, 50µl of sample, water and standard (2.0mg/dl) was added to sample, blank and standard labeled test-tubes respectively. The solvents were mixed and allowed to stand for one minute. The absorbance (A) of sample and standard were measured against the blank (distilled water) with spectrophotometer (VWR UV-3100PC UV-VIS, Version 1.0.1, China) at 492nm after 30 seconds (A_1) and exactly 90 seconds later (A_2). The level of creatinine in the sample was calculated with the following formula:

$$\text{Concentration of serum creatinine } \left(\frac{\text{mg}}{\text{dL}}\right) = \frac{\Delta\text{A of sample}}{\Delta\text{A of standard}} \times \text{conc. of standard[mg/dl]}$$

(Bartels, Böhmer and Heierli, 1972)

Determination of Serum Urea

The quantity of urea in the chicks' serum was determined spectrophotometrically following the manufacturer's instructions of DIALAB diagnostics, Wiener Neudorf, Austria.

Principle: The determination was based on the principle suggesting that Urea is hydrolyzed by water and urease to form carbondioxide (CO_2) and ammonia. In a modified Berthelot reaction, ammonium ions react with hypochlorite and salicylate to form a green dye (Bartels, Böhmer and Heierli, 1972). The increase in absorbance at 578 nm is proportional to the urea concentration in the sample.

Determination of Serum Potassium

The concentration of potassium in the chicks' serum was determined spectrophotometrically following the manufacturer's instructions of AGAPPE diagnostics, Cham, Switzerland.

Principle: The determination was based on the principle suggesting that sodium tetraphenylboron in a specifically prepared mixture produces a colloidal suspension (Gormall, Bardwill and David, 1949), the turbidity of which is proportional to potassium concentration.

Determination of Serum Chloride

The concentration of potassium in the chicks' serum was determined spectrophotometrically following the manufacturer's instructions of DIALAB diagnostics, Wiener Neudorf, Austria.

Principle: The determination was based on the principle suggesting that chloride ions form a soluble, non-ionized compound, with mercuric ions and will displace thiocyanate ions from non-ionized mercuric thiocyanate. The released thiocyanate ions react with ferric ions to form a colour complex that absorbs light at 480nm (Bartels, Böhmer and Heierli, 1972). The intensity of the colour produced is directly proportional to the chloride concentration, according to the reaction;

$$3\ Hg(SCN)_2 + 6\ Cl^- \longrightarrow 3\ HgCl_2 + 6\ SCN^-$$

$$6\ SCN^- + 2Fe^{3+} \longrightarrow 2\ Fe(SCN)_3 \text{ red complex}$$

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Procedure: Cold samples and reagents for the determination were brought to ambient temperature. To three (3) sets of test-tubes labeled: standard, sample and blank, was added 1.5ml of chloride reagent (mercuric nitrate 0.058mM, mercuric thiocyanate 1.75 mM, mercuric chloride 0.7 mM, ferric nitrate 22.3 mM, non-reactive ingredients and stabilizers in dilute acid and methanol). Thereafter, 10 µL of sample, distilled water and standard (chloride calibrator: sodium chloride 100 mEq/L) was added to sample, blank and standard test-tubes respectively. The solvents were allowed to stand for 5 minutes at ambient temperature. The absorbance (A) of sample and standard were measured against the blank with a spectrophotometer at 480nm. The concentration of chloride in the sample was calculated using the following formula;

Concentration of chloride (mEq/L) = $\frac{\text{Abs. of sample}}{\text{Abs.of standard}}$ × conc. Of standard [mEq/L]

Determination of Serum Sodium

The concentration of sodium in the chicks' serum was determined spectrophotometrically following the manufacturer's instructions of TECO diagnostics, Anaheim, USA.

Principle: The determination was based on the modified principles described by (Bartels, Böhmer and Heierli, 1972) in which sodium is precipitated as the triple salt, sodium magnesiumuranyl acetate, with the excess uranium then reacted with ferrocyanide, to produce a chromophore, the absorbance of which varies inversely as the concentration of sodium in the test specimen.

RESULTS AND DISCUSSION

Table 1.0; Results for hepatic and renal function biomarkers; Serum metabolic parameters of broiler chickens fed tamarind pulp and tetracycline dissolved in their drinking water for 6 weeks starter period.

	C	AT	TD
Albumin (g/ml)	2.13±0.09^{a}	2.77±0.30^{b}	2.63±0.12^{b}
AST (u/l)	5.00±0.58^{a}	5.00±0.58^{a}	7.00±1.73^{a}
ALT (u/l)	31.00±2.08^{a}	35.00±2.08^{a}	63.33±1.33^{b}
ALP (u/l)	22.67±1.76^{a}	21.00±.58^{a}	25.00±2.08^{a,b}
Urea (mmol/l)	8.33±1.94^{a}	7.36±0.62^{a}	13.03±0.30^{b}
Na (mmol/l)	121.33±2.60^{a}	133.00±6.23^{b}	126.00±3.06^{a,b}
K (mmol/l)	4.93±0.13^{a}	5.20±0.26^{a}	5.13±0.18^{a}
Cl (mmol/l)	66.00±3.05^{a}	71.67±1.45^{b}	73.33±6.36^{a,b}
Creatine (u/l)	4.67±0.67^{a}	5.67±0.88^{a,b}	6.67±0.67^{b}
Total pro (g/ml)	5.17±0.65^{a}	5.87±0.18^{a}	5.87±0.18^{a}

C = Control group, AT = Tetracycline group, TD = Tamarind group, SEM = Standard error of mean, Data are expressed as mean ± SEM (n = 3), Mean ± SEM followed by different letters within a row are significantly different ($P < 0.05$)

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Animals fed with Tetracycline (AT) and Tarmarind (TD) showed AST, K (mmol/l) and Total protein (g/ml) values not significantly varied (P>0.05) across all the treatments. , ALT and ALP values were significantly (P<0.05) higher in TD compared to AT and the control C (P>0.05). The Albumin values of the AT and TD treatments were not significantly different but are both significantly higher compared to the negative treatment (C). The Na and Cl values of the C and TD groups were not significantly (P>0.05) different as the AT and TD were also not significantly different. However, the AT group has a value significantly higher (P<0.05) than the C group. The creatine value of the AT and TD groups were statistically similar though the C result was also similar to the AT but not the TD groups. Lastly, the TD group had a statistically higher (P<0.05) value compared to the other two treatments.

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Phytochemical Composition and *In vitro* Antioxidant Potential of Different Solvent Extracts of *Terminalia schimperiana* Leaves Extract

***Yakubu N., Oloyede A. A., Abubakar H., Gogo M. F., Hamzah B. U., Suleiman R., Abu M. L & Amuzat A. O.**

Department of Biochemistry, Faculty of Natural Sciences, Ibrahim Badamasi Babangida University, P.M.B. 11, Lapai, Niger State, Nigeria

*Corresponding author: Email: yndatsu2011@gmail.com (+2348065940069)

ABSTRACT

Traditionally, *Terminalia schimperiana* have been used in treating different abnormalities in human and could be good sources of important active compounds. This study aimed at evaluating the different solvents of the plant leaves for its qualitative and quantitative phytochemical contents as well as its *in vitro* antioxidant properties. Soxhlet extraction using distilled water, methanol, ethanol, acetone, Dichloromethane, and Ethyl acetate was performed for 48 hours. The results revealed that methanol showed better for the extraction of phytochemicals as well as a higher level of phenols (135.12mg/g), flavonoids (118.15 mg/g), saponins (86.50 mg/g), tannins (75.15 mg/g) and alkaloids (54.20 mg/g) contents compared to others. Likewise, higher antioxidant property of the free soluble phenols (FSP), free bound phenols (FBP) were recorded in the methanol extract compared to others. Conclusively, methanol as a solvent is a better extracting solvent if maximum bioactive compounds are desired from *T. schimperiana* leaves.

Keywords: Antioxidant, extraction, phytochemicals, solvents, *Terminalia schimperiana*

INTRODUCTION

Africa countries are endowed with medicinal plants which serve a vital therapeutic agents and raw materials in industries for the production of therapeutic agents (Muluh *et al*., 2019). Plants with therapeutic properties are still the source of conventional medicine development and are still been explored globally for the treatment of diverse medical conditions (Muluh *et al*., 2019). The safety of phytomedicine as compared to conventional medicines has been demonstrated through their toxicity profiles assessment (Armijos *et al*., 2018).

The increasing awareness to look for new natural sources that possess antioxidant properties is as a result of their health benefits recognition (Armijos *et al.*, 2018). Elajalde (2001), and Halliwell and Whitman (2004) have demonstrated series of oxidative stress caused by reactive oxygen species (ROS) related ailments to include; atherosclerosis, cancer, diabetes, cataracts, asthma, cardiovascular problems, and ageing as well as several *in vitro* studies of medicinal plants have justified their bioactive component as good protective agents

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against the ROS generated in the system (Armijos *et al.*, 2018).

Terminalia schimperiana belongs to the Combretaceae family and popularly called the '*Tuit*" plan It is known as '*Buashe* and *Idi"* in both Hausa and Yoruba languages (Muluh *et al.*, 2019). In the African continent, *T. schimperiana* parts are been used as medicine in the treatment of different as the preparations of its stem and root bark are used in the treatment of wounds burns, ulcerations, skin disorders like leprosy and epilepsy, skin and brain disorders, respectively (Arbonmier, 2004). The root prepared in form of chewing stick is used as oral hygiene enhancer and dental care ref. The decoctions of barks are used in treating catarrh, diarrhea, and worms and malaria infections (Toma *et al*., 2009). Boiled leaf concentrates are taken to prevent stomach-ache, bronchial asthma, hepatitis, cough, and to combat inflammation (Khan *et al*., 2019). This study therefore aim to evaluate the qualitative and quantitative phytochemical screening as well as to ascertain the *in vitro* antioxidant property of the different solvent leaf extracts.

MATERIALS AND METHODS

Collection of sample

The fresh leaves of *T. schimperiana* were collected from Ibrahim Badamasi Babangida University, Lapai Farm, Niger State, Nigeria. The leaves were taxonomically identified in the Department of Biological Sciences, Ibrahim Badamasi Babangida University, Lapai, Niger State, Nigeria. A herbarium specimen was deposited and voucher (0031) issued.

Preparation of plant materials

The materials collected were washed in cleaned water to remove debris. The leaves were air-dried at room temperature for four (4) weeks and pulverized into fine powder using an electric milling machine (Jehmlich, Germany). The grounded sample was then stored in an air tight container until needed for experiment.

Sample extraction for phytochemical analysis

One gram (1.00g) pulverized *T. schimperiana* leaves were measured into round bottom flasks containing 50 mL of distilled water, methanol, ethanol, acetone, dichloromethane, and ethyl acetate, respectively, for 48 h at room temperature. The extraction was successively via homogenization by homogenizer (IKA, Germany) at room temperature for 4 h and each mixture was filtered using Whatman filter paper No.1. The rotary evaporator (Polylab, India) was used to evaporate the filtrates, separately, and re-concentrated using water bath. The extracts obtained were stored in the freezer at 4^{o}C until needed for experiment. The extraction yield for each solvent was determined using the expression:

$$\%\ \text{yield} = \frac{\text{Mass of concentrated extract}}{\text{Mass of the sample}} \times 100$$

Free Soluble Polyphenols

This was performed by modified method of Nwanna and Oboh, (2007). Exactly, 0.2g of each sample extract was introduced into a 100 mL beaker containing 80% acetone, separately, homogenized by chilled waring blender for 10 min, and the homogenate was filtered using Whatman paper number 2 on a funnel. The obtained residue was used for bound polyphenols extraction. The filtrate was evaporated at 45^{o}C under vacuum using a rotary evaporator and the remained extracts were kept under freezer at 4 ^{o}C.

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Extraction of Bound Polyphenols

This was done by the modified method of Nwanna and Oboh, (2000). The bound polyphenol of each was extracted from their various residues of free soluble polyphenol extract, separately.

Phytochemical screening

Qualitative phytochemical screening

The qualitative phytochemical analysis to detect for the presence of alkaloids, flavonoids, phenols, saponins, tannins, carbohydrate, glycoside, anthraquinone, and terpenoids was performed using standard methods (Sofowra, 1993; Trease and Evans, 1999; and Harborne, 1993; El-Olemmy *et al*., 1994).

Quantitative phytochemical screening

Total phenol

The Edega *et al.* (2005) method was used for phenols determination. Soxhlet apparatus was used for 2 h to defat 1.00 g of each extract in a conical flask containing 100 mL of methanol. The phenol component was extracted by boiling the defatted sample in 50 mL of ether for 10 min. In a 50 mL flask, 5 mL of the extract, distilled water (10 mL), ammonium hydroxide solution (2 mL), and concentrated amyl alcohol (5 mL) were introduced. The all mixtures were left standing for 30 min to react for colour development. The total phenol content was measured at 765 nm using a UV spectrophotometer (Jasco, USA). They were expressed as gallic acid equivalent (mg/g).

Total flavonoid

These were assessed by aluminium chloride colorimeter method described by Chamg *et al.*, 2002). One millilitre (1.00 mL) of each extract was introduced into 100 mL beaker containing 1.5 mL of methanol, 10 % aluminium chloride (1 mL), 1M sodium acetate (1 mL), and distilled water (2.5 mL), separately. The solutions were left standing for 25 mins at room temperature and the absorbance read at 415nm using UV-visible spectrophotometer (Waltham, MA, USA). The flavonoid content of each extracts was extrapolated from the calibration curve prepared using quercetin solution at the concentrations (12.5 - 100 µg/mg) in methanol.

Saponins

This was performed using the method described by Oloyed, (2005). About one gram (1.00 g) of each solvent extract was taken into a 100 mL conical flask containing 20 mL of 1.0 N HCl, boiled for 4 h, cooled and filtered, separately. Five millilitre (5.0 mL) of each was mixed with 20.0 mL of petroleum ether and 5.0 mL of acetone-ethanol ratio (1:1). This was followed by introducing 0.4 mL of each mixture into three (3) different test tubes containing 5.0 mL of ferrous sulphate reagent, 2.0 mL of H_2SO_4. The mixtures were thoroughly shaken for 10 min and the absorbance measured at 490nm using UV-visible spectrophotometer (Waltham, MA, USA). The saponin content was expressed against saponin standard equivalent.

Tannins

The analysis was determined using the method described by AOAC, (1984). Exactly, 0.5g of each extract was introduced into 50ml of beakers containing 20 mL of 50% methanol, covered with Paraffin, separately, boiled in a water bath at 80 ^oC for 1h and then filtered. To each filtrate, 20 mL of distilled water, 2.5 mL of Folin-Denis reagent, and 10 mL of 17% Na_2CO_3 were added and mixed thoroughly. Each solution was rise up

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to mark using water, mixed and left for standing for 20 min until a bluish-green colour was formed. UV-visible spectrophotometer (Waltham, MA, USA) was used to read the absorbance at 740nm.

Alkaloids

This was determined using the method of Harborne, (1973). Briefly, 2.0 g of each extract was measured into a 200 mL beaker containing 50 mL 0f 10% acetic acid and ethanol, separately, covered using aluminium foil, and left standing for 2 hr. Each of the mixture was filtered, concentrated in a water bath for 2 hr and concentrated ammonium hydroxide was added drop by drop to each beaker for precipitation to form. All solutions were allowed to stand for 10 min and the formed precipitate from each sample was collected, washed using ammonium hydroxide and filtered. The residue from each extract was the alkaloid, which was dried and weighed.

Antioxidant capacity

Free Radical Scavenging Ability

The DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging ability of both free soluble and bound phenols were determined using the procedures outlined by Ursini et al. (1994) with slight modification by Ndatsu *et al.* (2013). Briefly, 1.00 mL of each sample extract was added into a 100 mL beaker containing 20 mL of methanol and 1.00 mL of 0.4M methanolic solution containing DPPH-radicals, separately. The mixtures were thoroughly stirred, kept standing for 30 min, and the absorbance of each was taken at 516 nm.

Reducing Property

The reducing property of free soluble and bound phenols was determined by the method of Nwanna and Oboh, (2007). This was done by assessing the ability of each sample extract to reduce $FeCl_3$ solution. Briefly, 2.5 mL of each extract was introduced into a 100 mL beaker containing 20 mL of methanol, mixed with 2.5 mL of 150 M sodium phosphate buffer (pH6.6), 2.5 mL of 100% potassium ferricyanide and all mixtures were incubated at 50 ^{o}C for 20 mins. Thereafter 2.5 mL of 10%, trichloroacetic acid was added, centrifuged at 650 rpm for 10 mins, then mixed 5.0 mL of the supernatant with 5.0 mL of distilled water, and 1mL of 0.1% ferric chloride. The absorbance of each was read at 700 nm.

RESULTS

The results of presence of different bioactive compounds in *T. schimperiana* leaves extracted using different solvents are presented (Table 1). It reveals the presence of all the phytochemical constituents under study. The evident of absence of glycosides in ethanol extract and steroids in both dichloromethane and chloroform extracts of *T. schimperiana* leaves were also revealed.

The results of effect of various solvents on the yield of *T. schimperiana* leaves extract are presented in Table 1. It revealed significant differences on the extraction yield among the different solvents used. Methanol (28.5%) had the highest % yield of extraction, followed by ethanol (25.0%), distilled water (18.5%), Acetone (10.3%), Dichloromethane (8.5%), and Chloroform (5.4%) (Figure 1), showing that high polar solvents are more promising in plant phytochemical extraction than the non-polar solvents.

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Table 1. Phytochemical screening of *T. schimperiana* leaf extracts

Phytochemicals	DW	M	E	A	DM	C
Phenols	*	*	*	*	*	*
Flavonoids	*	*	*	*	*	*
Tannins	*	*	*	*	*	*
Saponins	*	*	*	*	*	*
Terpenoids	*	*	*	*	*	*
Glycosides	*	*	-	*	*	*
Alkaloids	*	*	*	*	*	*
Steroids	*	*	*	*	-	-
Carbohydrates	*	*	*	*	*	*
Anthraquinones	*	*	*	*	*	*
% Yield	18.5 ±1.02^{c}	28.5±1.25^{a}	25.0±0.21^{b}	10.3±0.25^{d}	8.5±0.13^{e}	5.4±0.35^{f}

-: Absent, *: present DW: Distilled water, M: Methanol, E: Ethanol, A: Acetone, DM: Dichloromethane, C: Chloroform.

The phytochemical contents of *T. schimperiana* leaf extracts as influenced by different solvents are presented in table 2.

Table 2. Phytochemical contents of *T. schimperiana* leaf extracts

Phy	DW	M	E	A	DM	C
Ph (mg/g)	42.30±0.21^{f}	135.12±0.12^{a}	85.71±0.12^{d}	100.20±0.14^{c}	21.21±0.04^{b}	23.46±0.02^{e}
Fl (mg/g)	76.71±0.16^{e}	118.15±0.15^{a}	85.20±0.05^{c}	85.15±1.02^{b}	55.89±0.20^{b}	71.25±0.21^{d}
S (mg/g)	38.51±0.20^{e}	86.50±0.20^{a}	79.67±0.12^{c}	75.35±0.12^{d}	25.12±0.12^{d}	22.10±0.07^{b}
T (mg/g)	45.23±0.21^{f}	75.15±0.25^{a}	65.54±0.23^{d}	60.42±0.23^{e}	33.25±0.05^{b}	40.54±0.06^{c}
Al (µg/g)	37.81±0.05^{e}	54.20±0.12^{a}	40.54±0.06^{d}	51.55±0.25^{b}	16.27±0.23^{c}	25.76±0.04^{c}

Phy: Phytochemicals, Ph: Phenols, Fl: Flavonoids, S: Saponins, T: Tannins, Al: Alkaloids, DW: Distilled water, M: Methanol, E: Ethanol, A: Acetone, DM: Dichloromethane, C: Chloroform. Data are expressed as mean±SD. Letters on the same row represent the level of significance at $p<0.05$.

It shows that methanol influenced maximal extraction of all bioactive compounds from *T. schimperiana* leaves as the highest composition of phenol (135.12 µg/ml), flavonoids (118.15 µg/ml), tannins (75.15 µg/ml), Saponin (86.50 µg/ml) and alkaloids (54.12 µg/ml) were achieved. Also, higher levels of phenols (100.20, 85.71 µg/ml), flavonoids (85.15, 85.20 µg/ml), Saponins (75.35, 79.67 µg/ml) were obtained in acetone and ethanol extracts respectively (Table 2). In chloroform extract, large quantity of flavonoid was found. However, low levels of all bioactive compounds extracted were obtained in both distilled water and dichloromethane extracts (Table 2).

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In table 3 the results of *in vitro* antioxidant properties were presented. It reveals higher significant ($p<0.005$) values of free radical scavenging ability of free soluble phenols (FSFP) (49.20%) and free radical scavenging ability of bound phenols (FSBP) (60.15%), reducing power of free soluble phenols (RPFP) (0.78%) and reducing power of bound phenols (RPBP) (1.34%) were detected in methanol extracts. These are followed by FSFP (45.20, 43.25%), FSBP (51.15, 50.11%) contents obtained in acetone and ethanol extracts, respectively, and RPFP (0.67, 0.61%) and RPBP (0.95, 0.45%) contents in dichloromethane and acetone extracts, respectively (Table 3).

Table 3. Antioxidant property of *T. schimperiana* leaf extracts

Ant (%)	DW	M	E	A	DM	C
FSFP	28.35 ± 0.04^{f}	49.12 ± 0.12^{b}	43.25 ± 0.55^{c}	45.20 ± 0.14^{a}	40.05 ± 0.53^{d}	32.65 ± 0.24^{e}
FSBP	36.34 ± 0.13^{e}	60.15 ± 0.15^{b}	50.11 ± 1.41^{c}	51.15 ± 1.02^{a}	48.50 ± 1.02^{d}	48.35 ± 1.42^{d}
RPFP	$0..23\pm1.45^{f}$	0.78 ± 0.20^{a}	0.45 ± 0.32^{e}	0.61 ± 0.12^{c}	0.67 ± 0.35^{b}	0.54 ± 0.52^{d}
RPBP	0.28 ± 0.12^{f}	1.34 ± 0.25^{a}	0.31 ± 1.23^{e}	0.45 ± 0.23^{c}	0.95 ± 1.21^{b}	0.35 ± 0.25^{d}

Ant: Antioxidant, FSFP: Free radical scavenging ability of free soluble phenols; FSBP: Free radical scavenging ability of bound phenols; RPFP: Reducing power of free soluble phenols; RPBP: Reducing power of bound phenols, DW: Distilled water, M: Methanol, E: Ethanol, A: Acetone, DM: Dichloromethane, C: Chloroform. Data are expressed as mean±SD. Letters on the same row represent the level of significance at $p<0.05$.

However, lower significant ($p<0.005$) levels of both FSFP and FSBP were obtained in acetone, dichloromethane and distilled water extracts (Table 3).Also, lower levels of RPFP and RPBP are recorded in chloroform and distilled water (Table 3).

DISCUSSION

Extraction is one of the vital methods used in recovering and isolating phytochemicals from the plant materials. The extraction method, temperature, time allowed, bioactive compounds presence and the type of solvent utilized could greatly effluence the extraction efficiency (Dieu-Hien et al., 2019, Ngo *et al*., 2017). Significant high amount of phenols and flavonoids detected in methanol extract attributed to its highest extraction yield compared to other extracts (Table 1). Higher extraction yield recorded in methanol and ethanol extracts compared to the others signifying that high polar solvents influence plant bioactive compounds extraction than the non-polar solvents. These variations recorded in extraction yield due to solvent type used could be as a result of differences in the polarity of the extraction solvents. Higher polar solvents favour the extraction yield of bioactive compounds. That is higher concentrations of polar compounds in plant materials are more soluble in higher polar solvents, such as ethanol, water and methanol (Turkmen *et al*., 2006, Dieu-Hien *et al*., 2019). This result is in line with what was reported by Kuppusamy *et al*., (2015) in some medicinal plants.

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In order to gain more knowledge on the effect of different solvents on the yield of phytochemical extraction, the content of the phytochemicals in the extracts were measured. As a result of these, the composition of the bioactive compounds tested in various extracts varied. High percentage content of phenols and flavonoids exhibited in methanol extract than the other extract could reflect its higher extraction yield. It could also signify that the solubility of these compounds in methanol compared to others is intense. Solvent type influences extraction yield and phytochemical contents and thereby significantly affects the activity of the bioactive components of the extract (Ngo *et al*., 2017 and Dieu-Hien *et al.*, 2019).

Furthermore, the extracts used in this study were subjected to antioxidant activity determination using DPPH scavenging activity and $FeCl_3$ reducing power assays. For the all extracts evaluated, increase significant values of FSFP, FSBP, RPFP and RPBP recorded in methanol extract than other extracts could be attributed to its higher levels of both phenol, flavonoid compounds (Kuppusamy *et al*., 2015). These compounds are powerful protective agents that can prevent the free radicals from molecular cell damages. This may suggest that methanol extract of T*. schimperiana* leaf processes high antioxidant agent which could be used for drug development (Dieu-Hien *et al*., 2019, Ngo *et al*., 2017). The most important types of phytochemicals in plants are flavonoids and phenolic acids are the most important groups of secondary metabolites and bioactive compounds in plants. Flavonoids enhance the protection of biological systems against the oxidative stress damages on the macromolecules, such as carbohydrates, proteins, lipids and DNA (Rabiat *et al*., 2013).

CONCLUSION

Conclusively, this study has demonstrated that methanol extract exhibited high levels of percentage extraction yield, concentrations of phytochemicals extracted and antioxidant from the *T. schimperiana* leaves compared to other solvents used in this study. Therefore, methanol could be the best extraction solvents for the extraction of different phytochemicals from *T. schimperiana* leaves and a good source for antioxidant agents.

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Verification of a Scalable Convolutional Neural Network (CNN) in Android Malware Detection

***Adamu A. I., Aliyu U. S., Haruna A., Bashir Y. S. & Amina M. T.**

Computer Science Department, Ibrahim Badamasi Babangida University Lapai Niger State Nigeria

*Correspondence: adamuaisah@ibbu.edu.ng, adamuaisah88@gmail.com

ABSTRACT

Android malware is installed and launched on cellphones without the owners' knowledge or permission. Forced installation, browser hijacking, stealing and manipulating user data, harmful collecting of user information, malicious installation, malicious bundling, and other malicious behaviors are all common characteristics. There have been numerous studies on the area of android malware detection and classification using machine learning approach, but must of these algorithms were not evaluated over large-scale datasets. This research is aimed at verifying the scalability of convolutional neural network on android malware detection over large datasets. For the reverse engineering procedure, a lab architecture with an isolated environment (no outgoing connection) will be set up. Open source applications would be employed in this lab setting, and they will be examined statically without the program being run. The result expected at the end of this research is to have the scalability of Convolutional Neural Network (CNN) in the detection of android malware over large-scale datasets verified.

***Keywords**: Malware, Verification, Android and Convuolutional Neural Networks.*

INTRODUCTION

An Operating System is a software interface that is responsible for managing and operating hardware units and assisting the user to use those units as suggested by Tarfa et. al, (2017). For mobile phones, Operating Systems have been developed to enable users to use phones in much the same way as personal computers were used 1 or 2 decades ago. Android is a Google-developed open-source mobile operating system that was released in 2008, the study was explained in P. Faruki *et al*. (2015). Android is a Linux-based operating system that includes security, memory management, process management, a network stack, and a driver model. It provides a diverse set of libraries that allow app developers to create a variety of applications.

The Java programming language is commonly used to create Android applications by M. Yesilyurt and Y. Yalman, (2016). Android has grown to become the most popular clever operating system in the last decade, thanks to the spectacular rise of the mobile industry. So far, Android systems have accounted for more than 72 percent of the total smartphone market share and this was a workshop by StatCounter (2021). When there are a lot of app developers and users, the Android operating system becomes a favorite attack target for bad people.

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Android applications have become commonplace in everyday life. As of June 2021, there are over 2.9 million Android applications available for consumers, according to Google Play as confirmed in AppBrain, (2021). Many harmful applications, on the other hand, are disguised in the Android market, posing a serious hazard to consumers. In the first half of 2021, experts at the AV-TEST Institute, (2021) estimated that there were about 1.8 million new harmful apps. The most ravaging current key issues in Android in 2021 are Social Engineering, Data Leakage via Malicious Apps, Unsecured Public WiFi, End-to-End Encryption Gaps, Internet of Things (IoT) Devices, Spyware, Poor Password Habits, Lost or Stolen Mobile Devices, Out of Date Operating Systems, Malware Attacks and Drive by Download Attacks and this study was observed by Martin Gontovnikas, (2021).

As much as the above key current issues exist in Android, this research will only focus on malware attacks. The first time malware was discovered in the Android system occurred in September 2010. Android malware is installed and launched on cellphones without the owners' knowledge or permission. Forced installation, browser hijacking, stealing and manipulating user data, harmful collecting of user information, malicious installation, malicious bundling, and other malicious behaviors are all common characteristics. These actions would substantially infringe on users' legitimate rights, as well as cause them to lose a significant amount of money. Malware installation (e.g., repacking, update attack, and drive-by download), malware activation, malicious payloads (e.g., privilege escalations, remote control, finance charge, and information collection), and permission abuse are the four categories of Android malware by Y. Zhou and X. Jiang, (2012).

This research will employ the use of convolutional neural network (CNN); an approach of machine learning methods in the detection of android malware.

LITERATURE REVIEW

In a Survey of Android Malware Static Detection Technology Based on Machine Learning, Qing Wu et. al, (2021) investigated Android applications' structure, as well as several sources of static features. Machine learning approaches for detecting Android malware were evaluated, as well as the merits and drawbacks of these methods, and future possibilities in this field were considered. This research revealed the following current state of researches in area of convolutional neural network technique of machine learning.

A. Pekas and T. Acarman (2020) analysed and recommended using pseudo-dynamic analysis of Android apps, constructing API call graph, and embedding graphs into a low dimension feature vector using GE and CNN. The sources of datasets collection were AMD, AndroZoo, Drebin, ISCX Android and Botnet Dataset were 33,139 malwares and 25,000 benign data were collected. The performance measurement of the method was taken to be:

Accuracy	=	98.86
Recall	=	98.47
Precision	=	98.84
*F*1	=	98.65

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W. Y. Lee et. al, (2019) suggested and approved a classification method for using stacked RNNs and CNNs. They considered Package name, Certificate owner name, Permissions and Intent actions as features. Total number of datasets used was 2,000,000 Apps collected from VirusTotal. The proposed method gave a measurement of:

AUC	=	0.9986
TPR	=	0.977
FPR	=	0.01

M. Amin et. al, (2020) used the approach of BiLSTMs for Android malware classification which achieved excellent results as compared to LSTM, CNN, and DBN. They only considered opcodes as features, with 5560 malwares and 123,453 benign data all drawn up from AMD, Drebin and VirusShare. Their system was measured with the following performances:

Accuracy	=	0.999
*F*1	=	0.996

R. Nix and J. Zhang (2017) both proposed a classification method for android malware. Their work is one of the first attempts to construct CNNs for Android application/malware classification which only considered API sequences as features. Contagio and Third party app stores were the sources of data collection where 216 malwares and 1016 benign data were collected. Their system had the following performance measures:

Accuracy	=	0.994
Precision	=	1
Recall	=	0.983

M. Ganesh et. al, (2017) worked with a proposed a CNN-based model extracts the patterns of Android malware which only considered permission features. Sources of dataset collection are MalGenome, Debrin, Apk mirror, and Apk4fun where 2000 malwares and 500 benign data were collected. The performance metrics used was Accuracy with a measure of 0.93.

Yi Zhang et. al, (2018) used the concept of a Deep Classify Droid based on CNN model. Permissions, Intent filters, API calls and Constant strings are the feature sources. The used 5546 malwares, and 5224 benign data both from Drebin and Chinese App Market. For the performance metrics used the measurements are:

Precision	=	0.966
Recall	=	0.983
Accuracy	=	0.974
*F*1	=	0.974

The gap identified in Qing Wu et. al, (2021) is the likelihood of detection methods to support detection on a big scale. Many methods have been tested on small datasets in previous studies. Although these algorithms produced great performance metrics, their scalability was not tested on big datasets. With the expanding number of applications, there will be a greater demand for quick detection methods to handle large applications in the future.

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PROBLEM STATEMENT

The detection method will be more likely to support detection on a big scale. Many methods have been tested on small datasets in previous studies. Although these algorithms produced great performance metrics, their scalability was not tested on big datasets. With the expanding number of applications, there will be a greater demand for quick detection methods to handle large applications in the future as suggested by Ya Pan et. al, (2020) . One of these machine learning algorithms that were only tested on small scale datasets is the Convolutional Neural Network. Its scalability to work on large datasets has not been verified, hence its performance metrics in android malware detection was not determined on large scale of data.

AIM OF THE RESEARCH

The aim of this research is to verify the scalability of Convolutional Neural Network (CNN) in the detection of android malware over large-scale datasets.

SIGNIFICANCE OF THE RESEARCH

With the expanding number of applications, and the need for quick detection methods to carter for these increasing number of android applications, the results of this study will be of great benefits in the aspect of detecting malware in the android platforms. The verification of the scalability of one of the efficient algorithms in detecting android malware; convolutional neural network (CNN) will help in the determination of the speed, accuracy and precision of this technique over large datasets and will pave way for further improvement in the algorithm in the future if the performance is not as excellent as it recorded over small datasets.

METHODOLOGY

For the reverse engineering procedure, a lab architecture with an isolated environment (no outgoing connection) will be set up. Open source applications would be employed in this lab setting. The training dataset for this study is made up of several varieties of mobile malware and may be downloaded from the Android Malware Genome Project, while the testing and evaluation dataset comes from Drebin. Static analysis will be carried out in this lab setting, where the files and material connected with the mobile network will be examined without the program being run. The static analysis will be based on the following five steps:

- Data gathering: This is to acquire both benign and dangerous datasets. The more experimental datasets there are, the more credible the results become.
- Feature extraction: This will try to extract features from APKs using static analysis assistance tools.
- Feature reduction: This step uses feature reduction techniques to determine and choose significant characteristics.
- Model selection: This seeks to find an acceptable model for distinguishing malicious from benign applications. Statistical and machine learning models are examples of these models.
- Model evaluation: This is concerned with determining the generalization of models using performance metrics.

COMPARISONS WITH OTHER SYSTEMS

Because of the characteristic of openness and flexibility, Android has become the most popular mobile platform. However, it has also become

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the most targeted system by mobile malware. It is necessary for the users to have a fast and reliable detection method. In this paper, we proposed a two-layer methods to detect malware.

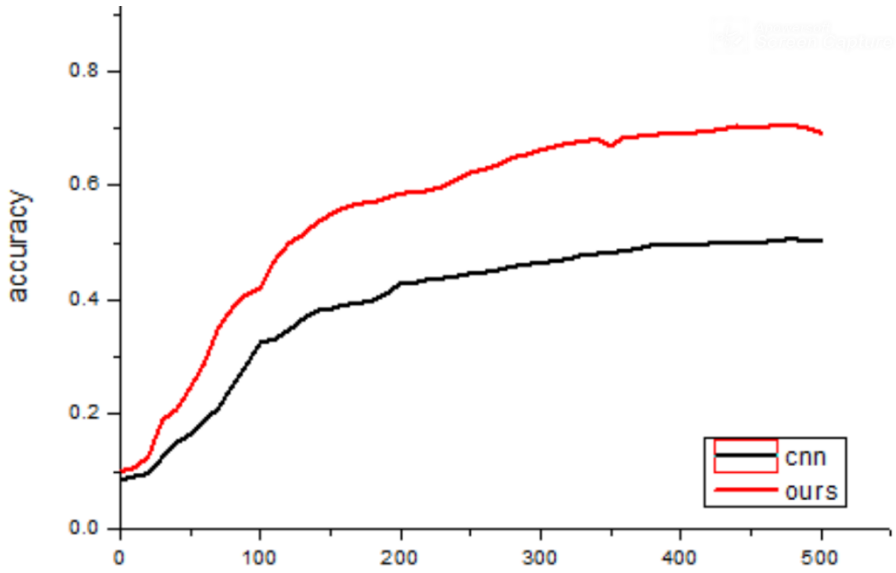

Figure 1: Comparisons of our method and CNN

RESULTS AND DISCUSSION

The results obtained after the samples were implemented on android devices showed that scalability of Convolutional Neural Network (CNN) in the detection of android malware over large-scale datasets were verified. And this determined the performance measures of this technique and its time efficiency when applied to large datasets during android malware detection.

Figure 2: Training the model before detection

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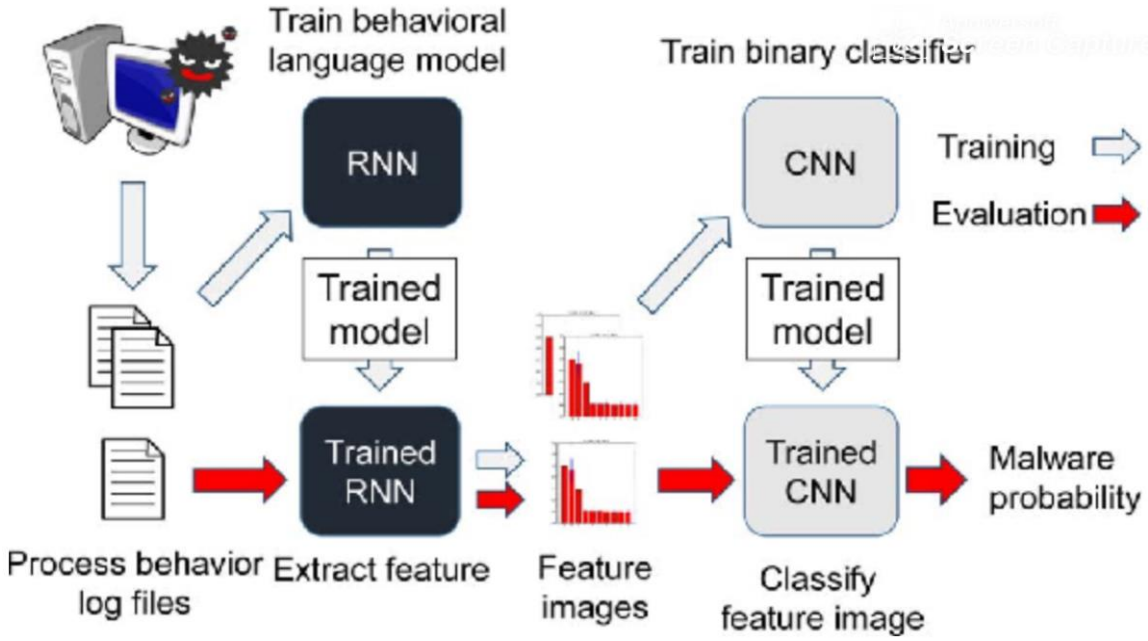

Figure 3: Results of the malware detection after undergoing CNN states

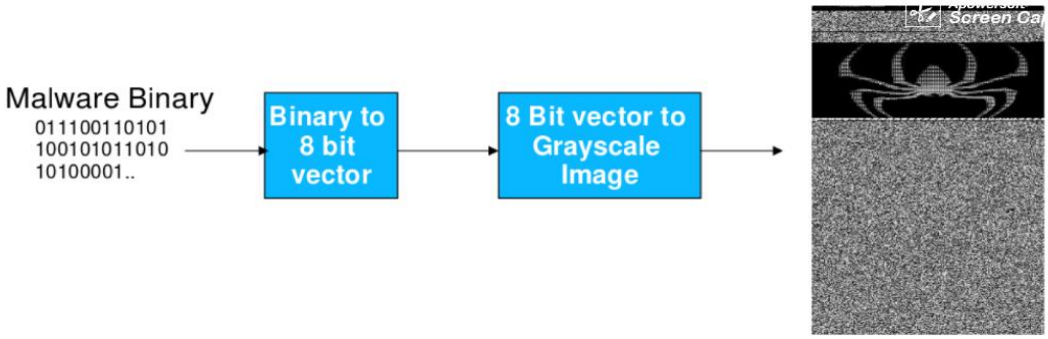

Figure 4: Malware Detection with CNN using Binary classifiers

CONCLUSION

The continuous development and fast change of the smart devices market has promoted an increase in the number of services and applications offered. As these devices integrate to the users every day activities, they become very attractive targets for cyber criminals. In this sense, malicious software (malware) has become a main security issue in this area. Although malware is not a new problem in the IT industry, differences between PC and smart devices make smart devices security a different problem bounded to the particular features of mobile devices. Moreover, the big number of stakeholders ranging from device manufactures to communication service providers creates a highly heterogeneous environment where attack surfaces characterization becomes a very complex task. In this context, this chapter aimed to present an overview of the fundamental aspects for Android malware analysis and detection.

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MINNA INSTITUTE OF TECHNOLOGY AND INNOVATION (MITI)

Address: Behind Zakkat Commission, Kpakungu-Keterangwari Road
P.M.B 246 Minna, Niger State

An Innovator is the heartbeat of any Technological Development

Briquette Making Machine

MODEL NO: MITIN0012

This machine is locally manufactured in Minna Institute of Technology and Innovation and it has the capacity of producing 250 briquette of 3.35cm length and 6cm diameter per hour.
it uses 1.5 hp electric motor.

Electric Biomass Briquette Burner

MODEL NO: MITIN007

This Biomass Briquette Burner has a thermal efficiency of cooking fabricated at Minna Institute of Technology and Innovation, using locally available standardized materials.

Features

1. Inbuilt Fan
2. On/off Buttom
3. Battery Charging Point
4. Ash Tray

Biomass Briquette

This is locally made from mixture of sawdust and starch at appropriate ratio