ANTIBACTERIAL ACTIVITIES OF THE LEAF EXTRACTS *OF* SYZYGIUM AROMATICUM, NELSONIA CANESCENS AND PARKIA BIGLOBOSA ON STAPHYLOCOCCUS AUREUS AND STREPTOCOCCUS PYOGENES

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A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA, NIGERIA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF TECHNOLOGY IN MEDICAL MICROBIOLOGY

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ABSTRACT

The development of resistance to antibiotics by infectious agents has been a continuous challenge. Thus, this study was carried out to evaluate the antibacterial activities of *Parkia biglobosa* (African Locust Bean Tree*), Nelsonia canescens* (Blue Pussy Herb) and *Syzygium aromaticum* (Cloves) on *Staphylococcus aureus* and *Streptococcus pyogenes* using agar well diffusion method. The leaves of the plants were also analyzed for their phytochemical components, Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), GC-MS (Gas Chromatography-Mass Spectrometry), antibacterial activity of their n-hexane, ethylacetate and aqueous fractions as well as their toxicity in laboratory animal (rats). The phytochemical screening showed the presence of flavonoids, saponins, tannins, phenols, alkaloids, steroids, reducing sugars and terpenes with only *P. biglobosa* extracts having all the bioactive compounds except alkaloids. The antibiogram revealed that the aqueous leaf extract of *P. biglobosa* had the highest activity with 24.0 ± 1.0mm and 22.5±1.3mm zones of inhibition on *S. pyogenes* and *S. aureus* at 120 mg/ml. *Nelsonia canescens* and *Syzygium aromaticum* on the other hand had no activity on the test organisms. The inhibitory activities of the extracts were compared to ampiclox. The MIC and MBC of *P. biglobosa* extracts on *S. aureus* and *S. pyogenes* were 60 mg/ml and 120 mg/ml respectively with only the ethanolic extract of *P. biglobosa* being 120mg/ml. The MIC and MBC of *Syzygium aromaticum* and *Nelsonia canescens* could not be determined as the test organisms were resistant to them. The antibiogram of the fractions of *P. biglobosa* revealed that the n-hexane fraction of aqeous extract had the highest activity of 22mm at 120 mg/ml on *S. aureus*. The LD_{50} of oral administration of aqeous extracts of *P. biglobosa* was found to be ≥ 1000 mg/kgbw. Rats in the acute and sub-acute toxicity groups showed no signs of toxicity except groups administered with 1000mg/kgbw of the aqueous leaf extract of *P. biglobosa.* The biochemical analysis of the serum of the rats in sub-acute toxicity groups revealed that aqueous leaf extract of *p. biglobosa* has hypoglycemic effect and lipid lowering ability. The GC-MS analysis of the n-hexane, ethyleacetate and aqueous fractions of *P. biglobosa* revealed the presence of 26 different compounds. These compounds include 1,2,3-Benzenetriol, phenol, 2, 4-bis (1, 1-dimethylethyl), Olean-12-ene-28-al and 23 others. These compounds could be used as lead molecules in the development of new antibacterial drugs that are effective against antibiotic resistant strains of *S. aureus* and *S. pyogenes.*

TABLE OF CONTENTS

Cover page	
Title page	i
Declaration	ii
Certification	iii
Dedication	iv
Acknowledgments	v
Abstract	vi
Table of Contents	vii
List of Tables	xiv
List of Figures	xvi
List of Abbreviations	xvii

CHAPTER ONE

1.0	**INTRODUCTION**	1
1.1	Background to the Study	1
1.2	Statement of the Research Problem	3
1.3	Aim and Objectives of the Study	3
1.4	Justification for the Study	4

CHAPTER TWO

2.0 **LITERATURE REVIEW** 5

2.1 Traditional Medicine 5

2.2 Bioactive Compounds 7

2.2.1 Bioactive compound - saponins 8

2.2.2 Bioactive compound - tannins 9

2.2.3 Bioactive compound - alkaloids 10

2.2.4 Bioactive compound - terpenes 11

2.2.5 Bioactive compound - flavonoids 12

2.2.6 Bioactive compound - reducing sugar 12

2.3 *Syzygium aromaticum* (cloves) 13

2.3.1 Morphology of *Syzygium aromaticum* 14

2.3.2 Phytochemical components and toxicity of *Syzygium aromaticum* 14

2.3.3 Economic importance of *Syzygium aromaticum* 15

2.3.4 Distribution of *Syzygium aromaticum* 16

2.4 *Parkia biglobosa* 17

2.4.1 Morphology of *Parkia biglobosa* 18

2.4.2 Economic importance of *Parkia biglobosa* 19

2.4.3 Phytochemical components and toxicity of *Parkia biglobosa* 20

2.4.4 Distribution of *Parkia biglobosa* 21

2.4.5 Forage management of *Parkia biglobosa* 22

2.4.6 Environmental impact of *Parkia biglobosa* 23

2.5 *Nelsonia canescens* 23

2.5.1 Morphology of *Nelsonia canescens* 24

2.5.2 Economic importance of *Nelsonia canescens* 25

2.5.3 Phytochemical components and toxicity of *Nelsonia canescens* 26

2.5.4 Distribution of *Nelsonia canescens* 26

2.6 *Staphylococcus aureus* 27

2.7 *Streptococcus pyogenes* 28

CHAPTER THREE

3.0 **MATERIALS AND METHOD** 30

3.1 Study Area 30

3.2 Collection of Plant Materials 31

3.3 Plant Identification 31

3.4 Plant Processing and Extraction 31

3.5 Source of Media, Solvents and Reagents 32

3.6 Preparation of Media 32

3.7 Qualitative Phytochemical Screening 32

3.7.1 Test for flavonoids 32

3.7.2 Test for tannins 33

3.7.3 Test for saponins 33

3.7.4 Test for alkaloids 33

3.7.5 Test for steroids 33

3.7.6 Test for terpenoids 34

3.7.7 Test for reducing sugar 34

3.7.8 Test for phenols 34

3.8 Quantitative Phytochemical Screening of the Extract 35

3.8.1 Test for tannins 35

3.8.2 Test for alkaloids 36

3.8.3 Test for flavonoids 37

3.8.4 Test for saponins 37

3.8.5 Test for phenols 38

3.8.6 Test for reducing Sugar Content (RSC) 38

3.9 Sources and Confirmation of Test Microorganisms 39

3.10 Reconstitution of the Extracts 40

3.11 Standardization of Test Organisms 40

3.12 Susceptibility Test of the Crude Extracts 40

3.12.1 Determination of zone of inhibition 40

3.12.2 Determination of minimum inhibitory concentration (MIC) 41

3.12.3 Determination of minimum bactericidal concentration (MBC) 42

3.13 Fractionation of Crude Extracts 42

3.14 Antibacterial Activity of the Fractions 43

3.15 Identification of Bioactive Compounds Using GC-MS (Gas Chromatography-Mass Spectrometry) 44

3.16 Toxicity Test of Extracts Using Laboratory Rats 45

3.16.1 Extracts reconstitution for acute and sub-acute toxicity test 45

3.16.2 Sub-acute toxicity study of rats 45

3.16.3 Acute toxicity study of rats 46

3.16.4 Weekly body weight of rats in acute and sub-acute

toxicity study 46

3.16.5 Relative organ weight of rats in acute and sub-acute toxicity study 47

3.16.6 Histopathological study of rats 47

3.16.7 Serum biochemistry of rats 48

3.17 Data analysis 48

CHAPTER FOUR

4.0 **RESULTS AND DISCUSSION** 49

4.1 Results 49

4.1.1 Qualitative phytochemical component of the extracts 49

4.1.2 Quantitative phytochemical component of the extracts 50

4.1.3 Antibacterial activities of the plant extract against *Streptococcus pyogenes* 51

4.1.4 Antibacterial activities of the plant extract against *Staphylococcus aureus* 52

4.1.5 Minimum inhibitory concentration (MIC) of plants' crude extracts against test organisms 53

4.1.6 Minimum bactericidal concentration (MBC) of plants' crude extracts against test organisms 54

4.1.7 Fractions of *Parkia biglobosa* crude extract 55

4.1.8 Antibacterial activity of the fraction of the leaf extracts of *P. biglobosa* on *S. pyogenes* and *S. aureus* 56

4.1.9 Gas chromatography-mass spectrometry (GC-MS) of *P. biglobosa* 57

4.1.10 Effects of aqueous extract of *P. biglobosa* on body weight of rat in sub-acute toxicity study 67

4.1.11 Effects of aqueous extract of *P. biglobosa* on body weight of rat in acute toxicity study 68

4.1.12 Effects of aqueous extracts of *P. biglobosa* on relative organ weight of rats in sub-acute and acute toxicity study 69

4.1.13 Acute and sub-acute toxicity study 70

4.1.14 Histopathological results 71

4.1.15 Effects of aqueous extract of of *P. biglobosa* on serum enzyme of rats in sub-acute toxicity study 73

4.1.16 Effect of aqueous extract of *P biglobosa* on plasma glucose of rats 74

4.1.17 Effect of aqueous extract of *Parkia biglobosa* on serum lipids of rats 75

4.2 Discussion 76

4.2.1 Phytochemical components of *Parkia biglobosa, Syzygium aromaticum* and *Nelsonia canescens* 76

4.2.2 Antimicrobial activities of the crude extracts 77

4.2.3 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) 78

4.2.4 Antibacterial activity of the fractions of *Parkia biglobosa* 78

4.2.5 Gas chromatography-mass spectrometry (GC-MS) 79

4.2.6 Toxicity of crude extract on rats 79

4.2.7 Histopathology of rats 81

4.2.8 Serum biochemistry 82

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS 84

5.1 Conclusion 84

5.2 Recommendations 85

5.3 Contribution to Knowledge 85

REFERENCES 87

APPENDIX 96

LIST OF TABLES

Table **Page**

4.1 Qualitative Phytochemical Components of the Extracts 50

4.2 Quantitative Phytochemical Components of the Extracts in Percentage (%) 51

4.3 Activities of Extracts against *Streptococcus pyogenes* (mm) 52

4.4 Activities of Extracts against *Staphylococcus aureus* (mm) 53

4.5 Minimum Inhibitory Concentration of Plants' Crude Extracts against Test Organisms (mg/ml) 54

4.6 Minimum Bactericidal Concentration (MBC) of Plant's Crude Extracts Against Test Organisms 55

4.7 Fractionation of *Parkia biglobosa* Crude Extracts 56

4.8 Activities of the Fractions of the Leaf Extracts of *P. biglobosa* against *S. pyogenes* and *S. aureus* 57

4.9 Compounds in *P. biglobosa* Ethanolic Fractions as Revealed by GC-MS 64

4.10 Compounds in *P. biglobosa* Aqueous Fractions as Revealed by GC-MS 65

4.11 Initial Weight of Rats Administered with Aqueous Extracts of *P. biglobosa* in Sub-acute Toxicity study 66

4.12 Initial Weight of Rats Administered with Aqueous Extracts of *P. biglobosa* in Acute Toxicity study 67

4.13 Effect of Aqueous Extracts of *P. biglobosa* on Weight of Rats in Sub-acute Toxicity Study. 68

4.14 Effect of Aqueous Extracts of *P. biglobosa* on Weight of

Rats in Acute Toxicity Test 69

4.15 Effects of the Aqueous Extracts of *Parkia biglobosa* on Relative Organ Weights of Rats in Sub-acute Toxicity Study 70

4.16 Effects of the Aqueous Extracts of *Parkia biglobosa* on Relative Organ Weights of Rats in Acute Toxicity Study 70

LIST OF FIGURES

Figure		Page
2.1	*Syzygium arom*aticum (cloves tree, fresh and dried flower buds)	13
2.2	*Parkia biglobosa* Pods and Leaves	17
2.3	Locust Beans	18
2.4	*Nelsonia canescens*	24
3.1	Map of Niger State Showing Minna	30
4.1	Chromatogram of Ethyl Acetate Fraction of Ethanolic Extract of *P. biglobosa*	58
4.2	Chromatography of N-hexane Fraction of Ethanolic Extract of *P. biglobosa*	59
4.3	Chromatography of Aqueous Fraction of Ethanolic Extract of *P. biglobosa*	60
4.4	Chromatography of Ethyl Acetate Fraction of Aqueous Extract of *P. biglobosa*	61
4.5	Chromatography of N-hexane Fraction of Aqueous Extract of *P. biglobosa*	62
4.6	Chromatography of Aqueous Fraction of Aqueous Extract of *P. biglobosa*	63
4.7	Photomicrograph of Heart Tissue of Rats	71
4.8	Photomicrograph of Liver Tissue of Rats	72
4.9	Photomicrograph of Kidney Tissue of Rats	72
4.10	Effect of the Extract of *P. biglobosa* on Liver Enzyme's Level	73
4.11	Effect of the Extract of *P. biglobosa* on the Plasma Glucose in Rats	74

4.12 Effects of the Extracts of *P. biglobosa* on Lipid Serum Profile 75

LIST OF ABBREVIATIONS

AFAE	Aqeous Fraction of Aqeous Extract
AFEE	Aqeous Fraction of Ethanolic Extract
ALP	Alanine Phosphatase
ALT	Alanine Amino Transferase
AST	Aspartate Amino Transferase
AQLE	Aqeous Leaf Extracts
CHD	Congenital Heart Disease
DMSO	Dimethyl Solfonamide
DNSA	3, 5-dinitrosalicyclic acid
EFAE	Ethanolic Fraction of Aqeous Extract
EFEE	Ethanolic Fraction of Ethanolic Extract
ETLE	Ethanolic Leaf Extract
GC-MS	Gas Chromatography-Mass Spectrometry
MBC	Minimum Bactericidal Concentration
MIC	Minimum Inhibitory Concentration
NFAE	n-hexane Fraction of Aqeous Extract
NFEE	n-hexane Fraction of Ethanolic Extract
OECD	Organisation of Economic Co-orperation and Development
RSC	Reducing Sugar Content

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Rising antibiotic resistance and the scarcity of new antimicrobials has long been acknowledged (Aastha *et al*., 2019). A major challenge in global health care is the need for novel, effective and affordable medicines to treat microbial infections, especially in developing countries of the world, where up to one-half of deaths are due to infectious diseases (Nautiyal *et al*., 2018).

Staphylococcus aureus and *Streptococcus pyogenes* involved in the pathogenesis of respiratory and skin infections, along with Pseudomonads and members of the Enterobacteriaceae causing gastrointestinal, urogenital diseases and wound contamination are resistant to virtually all of the older antibiotics (Modimola *et al*., 2018). Clinical isolates of *Staphylococcus aureus*, the leading cause of nosocomial infections, are increasingly resistant to an array of antimicrobial agents like penicillin, gentamicin, tobramycin, amikacin, ciprofloxacin, clindamycin, erythromycin, chloramphenicol, trimethoprim-sulfamethoxazole and vancomycin (Kotze *et al*., 2020).

The development of antimicrobial-resistant bacterial species stems from a number of factors which include the prevalent and sometimes inappropriate use of antibiotics, extensive use of these agents as growth enhancers in animal feed, and increased transboundary passage of antibiotic-resistant bacteria. The problem of antibiotic resistance in humans and animals will continue for a long time. Against this backdrop, the development of alternative drug classes to treat such infectious diseases is urgently required (Nautiyal *et al*., 2018).

Nature has been a source of medicinal agents from thousands of years back and an impressive number of modern drugs have been isolated from natural sources. Many use herbs traditionally (Verma *et al*., 2019). According to WHO more than 80% of the world's population rely on traditional medicine for their primary health care needs (Tuhinadri and Samanta, 2014). Herbal medicines are gaining growing interest because of their cost effective and eco-friendly attributes. Antimicrobials of plant's origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases. Despite rapid development and advanced methods of organic synthesis in laboratories, medicinal plants continue to play a significant role in modern medicine due to their inherent distinct chemical and biological properties (Murray *et al*., 2018).

Plants have an amazing ability to produce a wide variety of secondary metabolites, like alkaloids, glycosides, terpenoids, saponins, steroids, flavonoids, tannins, quinones and coumarins. These biomolecules are the source of plant-derived antimicrobial substances (PDAms). Some natural products are highly efficient in the treatment of bacterial infections (Verma *et al*., 2019).

Nigeria has a large diversity of plant species containing many useful bioactive constituents. The lack of access to Western primary health care and veterinary services in many rural parts of the world has helped sustain the use of traditional medicine to treat both humans and animals. Even where orthodox medicines are readily available, a large percentage of the population still use herbal remedies along with or in preference to conventional medicines (Eloff *et al*., 2018).

Amongst thousands of medicinal plants on the earth are *Syzygium aromaticum* (clove), *Parkia biglobosa* (Locust beans tree) and *Nelsonia canescens* (Bluepussy Leaf) which are known for their medicinal value and several other uses. This study screened the three plants for their

antimicrobial activities against *Staphylococcus aureus* and *Streptococcus pyogenes* considering the vast medical potentials of the three plants. The bioactive compounds in these plants were also determined as well as their potential toxic effects in man by the use of laboratory animals (Wister rats).

1.2 Statement of the Research Problem

Due to genetic recombination and several other gene exchange mechanisms, many microorganisms have developed resistance to contemporary antibiotics and so there is a need for alternative and effective treatment of microbial diseases. *Staphylococcus aureus* and *Streptococcus pyogenes* are common pathogens with increasing resistance to many antibiotics.

1.3 Aim and Objectives of the Study

The aim of the study was to evaluate the antimicrobial activities of *Syzygium aromaticum, Parkia biglobosa* and *Nelsonia canescens* on *Staphylococcus aureus* and *Streptococcus pyogenes.*

The objectives of the study were to:

i. determine the phytochemical constituents (both qualitatively and quantitatively) of *Syzygium aromaticum* (clove), and the leaves of *Parkia biglobosa* and *Nelsonia canescens.*

ii. determine the susceptibility of *Staphylococcus aureus* and *Streptococcus pyogenes* to the extracts of *Syzygium aromaticum* (clove), *Parkia biglobosa* and *Nelsonia canescens* (Bluepussy Leaf) leaves.

iii. determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts.

iv. identify the compounds in the active fractions of the leaf extract using GC-MS (Gas chromatography-Mass Spectrometry).

v. determine the acute and chronic toxicity of the extracts using laboratory rats.

1.4 Justification for the Study

Due to the recent trends in antibiotic resistance and emergence of multidrug resistant strains, there is a dire need to provide an alternative treatment option. Thus, this study was undertaken to test the bioactive components of cloves (*Syzygium aromaticum*), leaves of *Parkia biglobosa* as well as *Nelsonia canescens* against *Staphylococcus aureus* and *Streptococcus pyogenes*.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Traditional Medicine

Traditional medicine is the sum total of knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures that are used to maintain health, as well as to prevent, diagnose, improve, or treat physical and mental illnesses (Che *et al*., 2017). Traditional medicine that has been adopted by other populations (outside its indigenous culture) is often termed complementary or alternative medicine (CAM) (Mohamad, 2018).

The World Health Organization (WHO) reported that 80% of the emerging world's population relies on traditional medicine for therapy including Nigeria. During the past decades, the developed world has also witnessed an ascending trend in the utilization of CAM, particularly herbal remedies. Herbal medicines include herbs, herbal materials, herbal preparations, and finished herbal products that contain parts of plants or other plant materials as active ingredients. It is likely that the profound knowledge of herbal remedies in traditional cultures, developed through trial and error over many centuries, during which the most important cures were carefully passed on traditionally from one generation to another (Nunkoo and Mahodally, 2016). Indeed, modern allopathic medicine has its roots in this ancient medicine, and it is likely that many important new remedies will be developed and commercialized in the future from the

African biodiversity, as it has been till now, by following the leads provided by traditional knowledge and experiences (Akintobi *et al*., 2016).

The extensive use of traditional medicine in Africa, composed mainly of medicinal plants, has been argued to be linked to cultural and economic reasons. This is why the WHO encourages African member states to promote and integrate traditional medical practices in their health system. Plants typically contain mixtures of different phytochemicals, also known as secondary metabolites that may act individually, additively, or in synergy to improve health. Indeed, medicinal plants, unlike pharmacological drugs, commonly have several chemicals working together catalytically and synergistically to produce a combined effect that surpasses the total activity of the individual constituents. The combined actions of these substances tend to increase the activity of the main medicinal constituent by speeding up or slowing down its assimilation in the body. Secondary metabolites from plant's origins might increase the stability of the active compound(s) or phytochemicals, minimize the rate of undesired adverse side effects, and have an additive, potentiating, or antagonistic effect (Efferth and Koch, 2011).

It has been postulated that the enormous diversity of chemical structures found in these plants is not waste products, but specialized secondary metabolites involved in the relationship of the organism with the environment, for example, attractants of pollinators, signal products, defensive substances against predators and parasites, or in resistance against pests and diseases. A single plant may, for example, contain bitter substances that stimulate digestion and possess anti-inflammatory compounds that reduce swellings and pain, phenolic compounds that can act as an antioxidant and venotonics, antibacterial and antifungal tannins that act as natural antibiotics, diuretic substances that enhance the elimination of waste products and toxins, and alkaloids that enhance mood and give a sense of well-being (Mohamad, 2018). Although some may view the

isolation of phytochemicals and their use as single chemical entities as a better alternative and which have resulted in the replacement of plant extracts' use, nowadays, a view that there may be some advantages of the medical use of crude and/or standardized extracts as opposed to isolated single compound is gaining much momentum in the scientific community (Philippe, 2019).

2.2 Bioactive Compounds

The definition of bioactive compounds remained ambiguous and unclear for a long time. Very few references describe the term "bioactive". It is composed of two words bio- and -active. In etymology bio- is from the Greek (βío-) "bios" means life, while -active is derived from the Latin word "activus" which refers to dynamic, full of energy, with energy, or involved in activity. The term "bioactive" is an alternative term for "biologically active". Hence, a bioactive compound is simply a substance with biological activity (Neeraj, 2016).

A plant extract is a substance or an active substance with desirable properties removed from the tissues of a plant, frequently by treating it with a solvent, to be used for a particular purpose. The term "bioactive compounds" is generally referred to as biologically significant chemicals but not established as essential nutrients. Bioactive compounds are essential (e.g., vitamins) and non-essential (e.g., polyphenols, alkaloids, etc.) compounds that occur in nature, are part of the food chain, and can affect human health. They are derived from various natural sources such as plants, animals, microorganisms (e.g., fungi) and marine organisms (e.g., lichens). The amount of bioactive natural products in natural sources is always fairly low. Active compounds are synthesized in small quantities and different concentrations in all plant organs or parts such as leaves, roots, barks, tubers, woods, gums or oleoresin exudations, fruits, figs, flowers, rhizomes,

berries, twigs, as well as the whole plant. Further processes may be required after extraction to purify or isolate the desired compounds (Fongang, 2020). Some bioactive compounds include; saponin, alkaloids, phenol, reducing sugar, terpenes, tannins, e.t.c

2.2.1 Bioactive compound - saponins

Saponins are naturally occurring compounds that are widely distributed in all cells of legume plants. Saponins, which derive their name from their ability to form stable, soap-like foams in aqueous solutions, constitute a complex and chemically diverse group of compounds. In chemical terms, saponins contain a carbohydrate moiety attached to a triterpenoid or steroids. Saponins are attracting considerable interest as a result of their diverse properties, both deleterious and beneficial. Clinical studies have suggested that these health-promoting components, saponins, affect the immune system in ways that help to protect the human body against cancers, and also lower cholesterol levels. Saponins decrease blood lipids, lower cancer risks, and lower blood glucose response. A high saponin diet can be used in the inhibition of dental caries and platelet aggregation, in the treatment of hypercalciuria in humans, and as an antidote against acute lead poisoning (Renner, 2016).

2.2.2 Bioactive compound - tannins

Tannins (commonly referred to as tannic acid) are water-soluble polyphenols that are present in many plant foods. They have been reported to be responsible for decreases in feed intake, growth rate, feed efficiency, net metabolizable energy, and protein digestibility in experimental animals. Therefore, foods rich in tannins are considered to be of low nutritional value. However, recent

findings indicate that the major effect of tannins was not due to their inhibition on food consumption or digestion but rather the decreased efficiency in converting the absorbed nutrients to new body substances (Eloff *et al*., 2018). Incidences of certain cancers, such as esophageal cancer, have been reported to be related to consumption of tannins-rich foods such as betel nuts and herbal teas, suggesting that tannins might be carcinogenic. However, other reports indicated that the carcinogenic activity of tannins might be related to components associated with tannins rather than tannins themselves. Interestingly, many reports indicated negative association between tea consumption and incidences of cancers. Tea polyphenols and many tannin components were suggested to be anticarcinogenic. Many tannin molecules have also been shown to reduce the mutagenic activity of a number of mutagens. Many carcinogens and/or mutagens produce oxygen-free radicals for interaction with cellular macromolecules (Kansal *et al*., 2020).

The anticarcinogenic and antimutagenic potentials of tannins may be related to their antioxidative property, which is important in protecting cellular oxidative damage, including lipid peroxidation. The generation of superoxide radicals was reported to be inhibited by tannins and related compounds. The antimicrobial activities of tannins are well documented. The growth of many fungi, yeasts, bacteria, and viruses was inhibited by tannins. It was also discovered that tannic acid and propyl gallate, but not gallic acid, were inhibitory to foodborne bacteria, aquatic bacteria, and off-flavor-producing microorganisms. Their antimicrobial properties seemed to be associated with the hydrolysis of ester linkage between gallic acid and polyols hydrolyzed after ripening of many edible fruits. Tannins in these fruits thus serve as a natural defense mechanism against microbial infections. The antimicrobial property of tannic acid can also be used in food processing to increase the shelf-life of certain foods, such as catfish fillets (Ajuru *et al*., 2017)

2.2.3 Bioactive compound - alkaloids

Alkaloids are secondary metabolites originally defined as pharmacologically active compounds, primarily composed of nitrogen (Murphy, 2017). They are synthesized from one of the few common amino acids: lysine, tyrosine and tryptophan. More than 12,000 alkaloids, including more than 150 families, have been identified in plants; and abound 20% of the 'species of flowering plants' contain alkaloids. In plants, alkaloids generally exist as salts of organic acids like acetic, malic, lactic, citric, oxalic, tartaric, tannic and other acids. Some weak basic alkaloids (such as nicotine) occur freely in nature. A few alkaloids also occur as glycosides of sugar such as glucose, rhamnose and galactose, for example, alkaloids of the solanum group (solanine), as amides (piperine) and as esters (atropine, cocaine) of organic acids (Murphy, 2017).

2.2.4. Bioactive compound - terpenes

Terpenes, also known as terpenoids are the largest and most diverse group of naturally occurring compounds. Based on the number of isoprene units they have, they are classified as mono, di, tri, tetra, and sesquiterpenes. They are mostly found in plants and form the major constituent of essential oils from plants. Among the natural products that provide medical benefits for an organism, terpenes play a major and variety of roles. The common plant sources of terpenes are tea, thyme, cannabis, Spanish sage, and citrus fruits (e.g., lemon, orange, mandarin). Terpenes have a wide range of medicinal uses among which antiplasmodial activity is notable as its mechanism of action is similar to the popular antimalarial drug in use—chloroquine (Parhizgar and Azar, 2017). Monoterpenes specifically are widely studied for their antiviral property. With growing incidents of cancer and diabetes in modern world, terpenes also have the potential to

serve as anticancer and antidiabetic reagents. Along with these properties, terpenes also allow for flexibility in route of administration and suppression of side effects. Certain terpenes were widely used in natural folk medicine. One such terpene is curcumin which holds anti-inflammatory, antioxidant, anticancer, antiseptic, antiplasmodial, astringent, digestive, diuretic, and many other properties. Curcumin has also become a recent trend in healthy foods and open doors for several medical researches (Swarup *et al*., 2022).

2.2.5. Bioactive compound - flavonoids

Flavonoids, a group of natural substances with variable phenolic structures, are found in fruits, vegetables, grains, bark, roots, stems, flowers, tea and wine. These natural products are well known for their beneficial effects on health and efforts are being made to isolate the ingredients so called flavonoids. Flavonoids are now considered as an indispensable component in a variety of nutraceutical, pharmaceutical, medicinal and cosmetic applications. This is attributed to their anti-oxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme function. Research on flavonoids received an added impulse with the discovery of the low cardiovascular mortality rate and also prevention of CHD (Congenital Heart Defect). Information on the working mechanisms of flavonoids is still not understood properly. However, it has widely been known for centuries that derivatives of plant origin possess a broad spectrum of biological activity. Current trends of research and development activities on flavonoids relate to isolation, identification, characterization and functions of flavonoids and finally their applications on health benefits. (Panche *et al*., 2016)

2.2.6. Bioactive compound - reducing sugar

A reducing sugar is any sugar that is capable of acting as a reducing agent. All monosaccharides are reducing sugars, along with some disaccharides, some oligosaccharides, and some polysaccharides (Yoshida *et al*., 2020).

Some scientists have remarked that the combination of the reducing sugars constitutes a building block in the production of phytoalexins which are antimicrobial substances synthesized by plants. Phytoalexins accumulate rapidly at areas of incompatible pathogen infection. The abundance of reducing sugars in the root ethanolic extract could account for its high antibacterial activity

2.3 *Syzygium aromaticum* (Cloves)

Cloves are the dried, unopened, nail-shaped flower buds of the evergreen tree *Syzygium aromaticum*. The name “clove,” derived from the Latin word for nail, clavus (because of its shape). They are native to the Maluku Islands (or Mollucas) in Indonesia. Clove goes by many names in different languages such as *kanafuru* in Yoruba, *kanumfari* in Hausa, *kannafuru* in Nupe and *Nchara* in Igbo (Gaber *et al*., 2020). Figure 2.1 is the picture of *S. aromaticum* tree and its flower buds.

Figure 2.1: *syzygium aromaticum* (Cloves tree, fresh and dried flower buds) Retrieved from http://pintrest.com

2.3.1 Morphology of *Syzygium aromaticum*

The clove tree is evergreen that grows up to 8–12 metres (26–39 ft) tall, with large leaves and crimson flowers grouped in terminal clusters. The flower buds initially have a pale hue, gradually turn green, then transition to a bright red when ready for harvest. Cloves are harvested at 1.5–2 centimetres (0.59–0.79 in) long, and consist of a long calyx that terminates in four spreading sepals, and four unopened petals that form a small central ball (Banerjee *et al*., 2016).

2.3.2 Phytochemical components and toxicity of *Syzygium aromaticum*

Syzygium aromaticum is rich in many secondary metabolites as follows: sesquiterpenes, monoterpenes, hydrocarbon, and phenolic compounds. Eugenyl acetate, eugenol, and β-

caryophyllene are the most significant phytochemicals in clove oil. (Gaber *et al*., 2020). These compounds contribute to the fragrance and antioxidant, antibacterial, and antifungal properties, which is the reason for its enormous applications in the food and flavoring industries. Despite these beneficial properties, they may elicit some adverse reactions when administered at higher concentrations. Clove buds and their extracts containing active compounds, or the standalone compounds such as eugenol and oleoresins, have been approved by the Food and Drug Administration (FDA) as a food additive. The phenolics-rich fraction of clove has been reported to show no adverse effects on Wistar rats at 1000 mg/kg body weight/day. A few research reports are indicative of the extract affecting the reproductive indices in animal models (Gaber *et al*., 2020).

2.3.3 Economic importance of *Syzygium aromaticum*

Cloves are commonly used as a spice. They are used in the cuisine of Asian, African, Mediterranean and Middle East countries, lending flavor to meats, curries, and marinades, as well as fruit such as apples, pears or rhubarb. The clove tree is composed of leaves and buds (the commercial part of the tree) and the flowering bud production begins four years after plantation. Afterward, they are collected either by hand or using a natural phyto-hormone in the pre-flowering stage (Cortes-Rojas *et al*., 2014). Interestingly, they are commercially used for many medicinal purposes and in the perfume industry and clove are considered one of the spices that can be potentially used as preservatives in many foods, especially in meat processing, to replace chemical preservatives due to their antioxidant and antimicrobial properties. Several reports have documented cloves to possess anti-helmenthic, anti- inflammatory, anti-pyretic, anti-fungal, anti-

carcinogenic, anti-allergic, anti-viral, anti-mutagenic activity, antioxidant, insecticidal, anti-spamodic, anti-arthritis, anti-parasitic properties, carminative, to increase hydrochloric acid in the stomach and to improve peristalsis and an anaesthetic (Saikumari *et al*., 2016).

However, clove has gained much attention among other spices due to its potent antimicrobial and antioxidant activities (Miyazawa and Hisama, 2021). The effective role of clove in the inhibition of different degenerative diseases is attributed to the presence of various chemical constituents in high concentrations with antioxidant activity. Clove essential oil (CEO) is traditionally used in the treatment of burns and wounds, and as a pain reliever in dental care as well as treating tooth infections and toothache (Cross *et al*., 2016). In addition to that, its use has been documented in various industrial applications and is used extensively in perfumes, soaps and as a cleansing vehicle in histological work (Banerjee *et al*., 2016). Cloves are used in Indian and Chinese traditional medicine as a warming and stimulating agent. Traditionally, cloves have been used for centuries in the treatment of vomiting; flatulence; nausea; liver, bowel and stomach disorders; and as a stimulant for the nerves. In tropical Asia, cloves have been documented to relieve different microorganisms as scabies, cholera, malaria, and tuberculosis. As well, in America, clove has been traditionally used in inhibiting food-borne pathogens to treat viruses, worms, candida, and different bacterial and protozoan infections (Yadav *et al*., 2019). Moreover, eugenol has been widely used in dentistry because it can penetrate the dental pulp tissue and enter the bloodstream. Sesquiterpenes, isolated from clove were reported to have anti-carcinogenic activity (Yadav *et al*., 2019).

2.3.4 Distribution of *Syzygium aromaticum*

Cloves are available throughout the year due to different harvest seasons in different countries. Originating on and around the Maluku Islands of Indonesia, the historical importance of clove in international trade means it is now grown in numerous locations throughout the tropics including Indonesia, Zanzibar, Madagascar, Sri Lanka and parts of the Caribbean (Kaj Vollens 2018). More recently, clove production has begun in the Brazilian state of Bahia. Clove grows best on tropical mountain slopes at lower elevations as part of a mixed forest. Many of the islands to which they are native are volcanic (Gaber 2020).

2.4 *Parkia biglobosa*

locust beans tree (*Parkia biglobosa*) is another plant of medicinal importance. In Yoruba, it is called *ewe iru*. In hausa, it is called *ganyen dorowa, Origili* in Igbo and *Lei* in gbagi language (Edith 2021). Figure 2.2 is a picture of *P. biglobosa* tree and figure 2.3 is a picture of the seeds of *P. biglobosa*.

Figure 2.2: *Parkia biglobosa* pods and leaves. Retrieved from “https://medcraveonline.com/-and-conventional-antibiotics-against-multiple-antibiotic-resistant-uropathogenic-bacteria-marub.html.”

Figure 2.3: Locust beans, retrieved from "https://stellaonithewriter.com/2021/12/11/irufermented-african-locust-beans-smells-foul-but-tastes-heavenly/"

2.4.1 Morphology of *Parkia biglobosa*

African locust bean is a medium-sized legume tree that reaches 20-30 m high. It has a dense, widely spreading umbrella-shaped crown and a cylindrical trunk that can reach 130 cm in diameter, often branching low. The bark is longitudinally fissured, scaly between the fissures, thick, ash-grey to greyish-brown in colour. It exudes an amber gum when cut. The leaves are alternate and bipinnately compound, 30-40 cm long, bearing up to 17 pairs of pinnae (Akintobi *et al.,* 2016). Its beanets are numerous (13-60/pinna), subopposite, 8-30 mm long x 1.5-10 mm wide, rounded or obtuse at apex, glabrous but slightly ciliate near apex. The inflorescence is held on a long (10-35 cm) drooping peduncle. It is biglobose, showy, red in colour, and it looks like an electric bulb. The flowerhead is 4.5-7 cm long x 3.5-6 cm broad and it has a strong pungent smell. The many flowers are either bisexual, sterile or nectar-bearing. Bisexual flowers are pentamerous, 1-1.4 cm long, and corolla lobes are fused at their base. Sterile flowers are shorter and are borne near the peduncle, in the upper part of the inflorescence, and their nectar is attractive to bats that pollinate the flowers. The flowers begin to open at dusk, close and wilt at dawn, lasting only a single night (Akintobi *et al*., 2016). The fruit is a linear, glabrous and smooth, indehiscent pod that becomes brown at maturity. It is 12-30 (-35) cm long x 1.5-2.5 cm wide and contains up to 23 seeds embedded in a yellowish mealy pulp. The seeds are globose-ovoid, 5-15 mm, smooth and glossy dark in color. There are about 2800-6700 seeds/kg. The seeds are hard-coated and can remain viable up to 8 years (Anthony *et al*., 2019).

2.4.2 Economic importance of *Parkia biglobosa*

African locust bean is a multipurpose tree. The seeds, pods, fruit pulp and leaves are edible and used as cooking or drinking ingredients. The tree is particularly valued for its fermentable seeds. They are fermented to prepare a condiment that is called (soumbala), (dawadawa), (netetu) or (afinti) that is a strongly pungent as French cheese. This condiment used for sauce and soup seasoning is one of the most important commercial products traded in western Africa. Where the tree is grown, crushing and fermenting of these seeds constitutes an important economic activity. Various parts of the locust beans tree are used for medicinal and food purposes. Its health benefits range from anti-hypertention to wound healing ability, antimaleria, anti diarrhoea (Oladeinde *et al*., 2018; Builders *et al*., 2019).

Ground with moringa leaves, the seeds are ingredients for sauces and doughnuts. They can be roasted to make a coffee substitute known as "Sudan coffee". In the mature pod, seeds are surrounded by a quantitatively important mucilaginous pulp which is separated from the seeds when they are collected. This mealy pulp is traditionally consumed as fresh food by local African populations. It makes valuable baby food and is used to make a refreshing drink. The leaves can be boiled, mixed with cereal flour and eaten as vegetable. Flower buds are edible and added to salads (Anthony *et al*., 2019; Verma *et al*., 2019).

Fruit pulp, foliage and seeds of the African locust bean can be used to feed livestock and poultry. The fruit pulp and the seeds, once processed to remove anti-nutritional factors, can be included in livestock feed. The leaves provide useful though not very palatable fodder. Their usefulness is increased by the fact that they can be harvested during the dry season when feed is scarce. However, it should be mixed with other feed because their mineral content is too low. The

flowers are attractive to bees and a good source of nectar. The African locust bean trees are suitable for beehives (Murphy, 2017).

The wood is used in light constructions, poles, mortars, and many kinds of furniture and utensils. It is valuable firewood and provides pulp to make paper. The bark has many traditional uses in ethnomedicine. A root decoction is reported to treat coccidiosis in poultry. Green pods are used as fish poison to catch fish in rivers. African locust bean trees are used as ornamental. They are useful soil improvers and their leaves provide green manure (Verma *et al*., 2019).

2.4.3 Phytochemical components and toxicity of *Parkia biglobosa*

Different parts of *Parkia biglobosa* have been validated as good sources of phenolic compounds, saponins, terpenoids, steroids, tannins, fatty acids, and glycosides. Various phytochemicals are found in the stem barks, leaves, seeds, and pods of these plants. The stem bark of *P. biglobosa* is reported to contain phenols, flavonoids, sugars, tannins, terpenoids, steroids, saponins, alkaloid, and glycosides, while the leaves contain glycosides, tannins, and alkaloids in trace amount, in addition to flavonoids, phenols, and anthraquinones. Phytochemical screening of the seeds shows the presence of saponins, alkaloids, flavonoids, polyphenols, terpenoids, glycosides and tannins. Fermentation or roasting of *P. biglobosa* seeds results in the alteration of the bioactive components (Mohammed *et al*., 2021).

Acute Toxicity Studies on *Parkia biglobosa* showed a LD_{50} greater than 5000mg/kgbw. Rubbing of nose and mouth on the floor of the cage and restlessness were the only behavioral signs of toxicity shown by the animals, which disappeared within 24 hours of extracts administration (Builders *et al*., 2019).

2.4.4 Distribution of *Parkia biglobosa*

African locust bean is native to tropical Africa. It occurs in open savannah woodlands, in bush fallow and wooded farmland where cultivation is semi-permanent, in areas ranging from tropical forests with high and well-distributed rainfall, to arid zones (Anthony *et al*., 2019; Verma *et al*., 2019). It was introduced as a food plant into the West Indies and is now naturalized in Haiti. It was also introduced into Sao Tomé by the Portuguese (Hopkins, 1983).

Locust bean is found between 5°N and 15°N, from the Atlantic coast in Senegal to southern Sudan and northern Uganda in Africa. It grows from sea level up to an altitude of 1350 m and prefers regions where mean annual temperature range is about 26-28°C (Verma *et al*., 2019). African locust bean grows in areas corresponding to a wide range of annual rainfall with a marked dry season of 5-7 months. In Guinea Bissau, Sierra Leone and Guinea, it grows where average annual rainfall range is between 2200 and 4500 mm. African locust bean can withstand arid zones with less than 400 mm rainfall (Anthony *et al*., 2019). It is very tolerant of poor soil conditions though it prefers deep well-drained and fertile soils (Verma *et al*., 2019). It can grow on rocky slopes, stony ridges or sandstone hills as well as on shallow lateritic soils. African locust bean trees are deeply taprooted and have the ability to restrict transpiration, which makes them able to withstand drought conditions. African locust bean is a fire-resistant heliophyte (Anthony *et al*., 2019).

In West Africa, the fermented seeds of African locust bean are commercially traded within the region and their use has been described since the 14th century. In northern Nigeria the annual production of African locust bean seeds is estimated at 200,000 t (Anthony *et al*., 2019).

2.4.5 Forage management of *Parkia biglobosa*

African locust bean is mainly propagated by seeds. As the seeds are hardcoated, they should be treated with concentrated sulphuric acid and/or soaked in water during 24h prior to sowing. The seeds are generally sown in pots or in seedbeds as they require much attention: watering twice a day and weeding every two weeks. Direct sowing is possible but success rate is low as the strong smell of the seeds is attractive to rodents and other pests. Once the seedlings have reached 20-25 cm high, they can be planted out at 10 m x 10 m intervals. Vegetative propagation is possible. Grafting, cuttings taken from seedlings, and marcotting of 11–25-year-old trees showed good results in Burkina Faso and Nigeria (Verma *et al*., 2019).

In the first stages of establishment African locust bean is sensitive to browsing and needs to be protected from livestock (Anthony *et al*., 2019). African locust bean is a long-lived perennial that starts flowering 5-7 years after planting. Flowering occurs during the dry season in the drier places. The tree reaches its maximum height between 30 and 50 years and may live up to 100 years. Unlike other trees, African locust bean retains its leaves during the dry season and is thus much valued by farmers who can lop the branches and supplement the low-quality forage available to their livestock (Akintobi *et al*., 2016).

2.4.6 Environmental impact of *Parkia biglobosa*

Many crops can benefit from being planted under African locust bean canopy: maize, cassava, yams, sorghum and millet (Anthony *et al*., 2019). African locust bean trees provide shade for forage grasses and livestock. They protect the soil from heat and wind and are potent nutrient cyclers from the deep soil layers. They bind soil particles and therefore have a potential merit in silvopastoral systems. Leafshed provides litter and hence organic matter to the soil. As livestock stands under the trees, they fertilize the soil with their dung (Akintobi *et al*., 2016).

2.5 *Nelsonia canescens*

Nelsonia canescens is commonly called "Bluepussy Leaf" and locally called *Tamiyan biri* in Hausa. They are annual, creeping decumbent herb whose height ranged from 10-20cm. It belongs to the family Acanthaceae, with a substantial number of similar plant specimens now identified as synonyms. They include; Nelsonia villosa Oerst, Nelsonia vestita Schult, Nelsonia tomentosa A. Dietr, Nelsonia senegalensis Oerst, Nelsonia rotundifolia R. Br., Nelsonia pohlii Nees, Nelsonia origanoides Roem. & Schult, Nelsonia nummulariifolia Roem. & Schult.e.t.c (Yoshida *et al.,* 2020). Figure 2.4 is the picture of *Nelsonia canescens*.

Figure 2.4: *Nelsonia canescens,* Retrieved from "https://uforest.org/Species/N/Nelsonia_canescens.php."

2.5.1 Morphology of *Nelsonia canescens*

Nelsonia canescens is a perennial herb with several trailing stems from a central taproot or rootstock; stems not (or rarely) rooting at lower nodes, up to 60 cm long, pilose or densely so with thin hairs up to 4 mm long, and with subsessile capitate glands (Mohaddesi *et al*., 2015). Leaves with petiole 0.2–2(–2.5) cm long; lamina ovate to elliptic, not (or very rarely) abruptly narrowed below middle, largest 1.5–7(–10) × 1–4(–5) cm, apex acute to rounded, base attenuate to truncate; beneath sparsely to densely pubescent to pilose with thin hairs (rarely with thick hairs), densest on veins but lamina always uniformly hairy, above with similar or sparser indumentum (Mohaddesi *et al*., 2015).

Spikes are 1–12 cm long, axis pilose; peduncle 0.2–4.5 cm long, indumentum as stems; bracts ovate-elliptic or broadly so to orbicular, 5–9 × 3.5–6 mm (lowermost pair sometimes slightly leafy and larger), narrowing gradually or concave (with distinct ""shoulders"") below the acute tip, pilose and with stalked capitate glands; pedicel ± 0.5 mm long, with dense pilose hairs. Sepals ± equal in length, 4.5–7 mm long, acute; dorsal elliptic; ventral elliptic, divided ± 1/3 down; lateral lanceolate; usually with long pilose hairs up to 3 mm long all over (very rarely towards base only) and with stalked capitate glands (Eloff *et al*., 2018).

Corolla in pale clear blue to bright deep blue or bluish purple, subactinomorphic, with hairs in throat; tube (3–)4–6 mm long, widened into a campanulate throat; lobes 2.5–5 mm long, ± spreading, slightly emarginate to rounded. Stamens included to slightly exserted; filaments 0.5–2 mm long; anthers ± 1 mm long, thecae diverging, basal flanges conspicuous. Capsule 4–7 mm

long, sparsely glandular near apex. Seeds numerous (but usually less than 20), ± 0.5 mm long (Mahat *et al*., 2018).

2.5.2 Economic importance of *Nelsonia canescens*

Nelsonia canescens is used traditionally to treat the following diseases; diarrhoea, dysentery, vermifuges, cutaneous and subcutaneous parasitic infection, small pox, chicken pox, measles and others. The sap of the leaves is applied topically to guinea worm sores in Ivory Coast-Upper Volta to kill the the causative parasite. Nupe of Northern Nigeria prepare a brew of the plant for treating small pox, and in Tanganyika, the sap is taken for diarrhea and the root in decoction for schistosomiasis. *Nelsonia canescens* is used in African and Asian traditional medicine (Burkill, 1985). In Africa, it is used to reduce fever and as an analgesic in a wide range of conditions including colds, flu, and viral infections. In India, it is called "Bada Rasna" and it is used in traditional medicine to treat pain and inflammation (Mohaddesi *et al*., 2015; Mahat *et al*., 2018). *Nelsonia canescens* is also reported to be used as a cover crop to suppress the growth of weeds in banana plantations. Here, this species can invade large areas of the plantation with no visible adverse effects on the banana crop but limiting the possibility of other weeds to invade the land (Fongang *et al*., 2020).

2.5.3 Phytochemical components and toxicity of *Nelsonia canescens*

Results of qualitative phytochemical screening of the aqueous leaves extract of *N. canescens* showed the presence of alkaloids, phenols, tannins, flavonoids and saponins. The acute toxicity test of the extract *N. canescens* revealed an oral $LD_{50} > 2000$ mg/kg body weight in mice. The presence of some of the phytochemicals and the values of the LD_{50} explains why the plant is being used traditionally for the treatment of a wide range of illnesses such as fever, pain,

chicken pox, measles, constipation and gastric ulcer without reports of unwanted effects (Haruna and Elinge, 2019).

2.5.4 Distribution of *Nelsonia canescens*

Although the genus *Nelsonia* appears to be native to Africa, Asia, and Australia (Thomas and Lucinda 2014), the origin of the species *N. canescens* is uncertain. It is not known with confidence whether this species is native or introduced to the New World. *Nelsonia canescens* can be found growing in secondary wet evergreen forests, savannah woodlands, grassy places, and open and disturbed habitats, especially in moist areas along roadsides, trails, and streambeds. It also grows as a weed in agricultural lands (Daniel *et al*., 2017). Currently *N. canescens* occurs in tropical Africa, Madagascar, southern Asia, Australia, southern North America, Central America, tropical South America, and Puerto Rico. Some authors consider that until additional data is presented on the taxonomy and distribution of this species, it should be treated as native in the American tropics (Gaber *et al*., 2020).

2.6. *Staphylococcus aureus*

Staphylococcus aureus is facultative anaerobic Gram-positive cocci. It is frequently part of the skin flora but can also be found in the nose (Liu *et al*., 2015). About 20% of the human population is long term carriers of *S. aureus*. It is the most common species of staphylococci that causes staphylococcal infection. *Staphylococcus aureus* can cause a range of illnesses from minor skin infections such as pimples, impetigo, boils, cellulitis, folliculitis, carbuncles, scalded skin syndrome (SSS), and abscesses to life threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS) and sepsis (Cosgrove *et al*. 2015). It is

still one of the five most common causes of nosocomial infections, often causing post surgical wound infection (Baba *et al*., 2016).

Combination therapy with gentamycin may be used to treat serious infections, such as endocarditis, but its use is controversial because of high risk of damage to kidney. Worse even is the evolution of many strains of *S. aureus* which are resistant to contemporary antibiotics (Aniagu *et al*., 2016). Antibiotics are the main line of treatment for most staph infections. The choice of antibiotic depends in part on how serious the infection is and what type of staph that is involved. Some minor skin infections will heal on their own with no drug treatment. In addition to antibiotics, sometimes surgery is also needed to drain a wound, abscess, or boil. Invasive staph infections usually require hospitalization for IV antibiotic treatment to fight the infection and other supportive treatment to help the healing (Johnson *et al*., 2022). Antibiotics commonly used to treat staph infections include; Nafcillin, Oxacillin, Vancomycin, Daptomycin, Telavancin, Linezolid (Kansal *et al*., 2020).

2.7. *Streptococcus pyogenes*

Group A streptococci (GAS or *Streptococcus pyogenes*) are strictly human pathogens that normally colonize the throat or skin without causing disease. Members of this species are differentiated into >100 types on the basis of immunogenic differences in their surface M proteins and polymorphisms in the emm gene (Kotb *et al*., 2020). The range of GAS diseases is broad and includes both localized and systemic infections that can cause acute or chronic illnesses. In most cases, these bacteria cause pharyngitis (sore throat), tonsillitis, or skin infections such as impetigo/pyoderma (Spellerberg and Brandt, 2016). At times, however, the bacteria gain access to normally sterile sites and cause invasive disease. Depending on complex

host-pathogen interactions, invasive GAS infections can cause either severe shock and multiple organ failure or non-severe systemic disease, e.g., mild bacteremia and cellulitis (Johnson *et al*., 2022).

The drug of choice for treatment of bacterial pharyngitis is oral penicillin for 10 days or IM benzathine penicillin. This treatment is cost-effective and has a narrow spectrum of activity.

In patients with penicillin allergy, macrolides and first-generation cephalosporins can be used. However, some strains of *S. pyogenes* have developed resistance to macrolides and macrolides are used as third-line of treatment for Streptococcal throat infection (Kansal *et al*., 2020).

Severe invasive *S. pyogenes* infections can be treated with vancomycin or clindamycin. Intravenous antibiotic therapy and surgery for the removal of necrotic tissue are recommended in the case of soft tissue skin infection by *S. pyogenes* (Johnson *et al*., 2022).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area: The study was carried out in Minna, Niger State. It is a city in middle belt Nigeria and lies on latitude 9.5836^0 N and longitude 6.5463^0 E (Musa and Muhammad, 2022). Figure 3.1 is a picture of the map of Minna where this thesis was carried out.

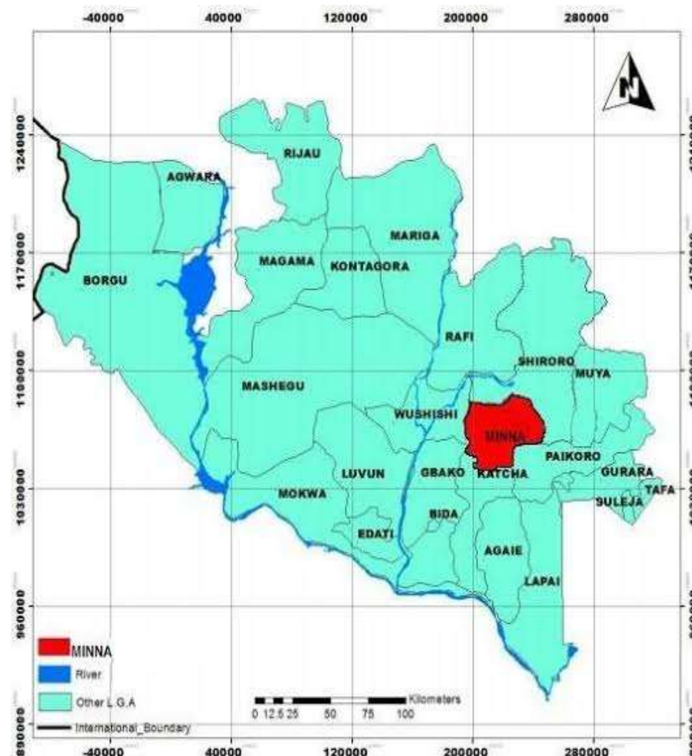

Figure 3.1: Map of Niger State showing Minna (Department of Logistic and Transport Technology, FUT Minna).

3.2 Collection of Plant Materials

The leaves of *Parkia biglobosa* were collected from within Federal University of Technology Minna, Gidan Kwano campus, *Syzygium aromaticum* was bought from Kasuwan Gwari market in Minna, while *Nelsonia canesens* were collected from Talba Estate, along Minna-Bida Road, Niger State.

3.3 Plant Identification

The plant materials that were collected above were taken to Plant Biology Department, Federal University of Technology Minna for authentication by ethnobotanist. Voucher specimens were deposited in the Department for future reference.

3.4 Plant Processing and Extraction

The plants were processed according to the methods described by Nayan and shukla (2011). Each plant parts were rinsed in distilled water and air-dried for 2-3 weeks. After which they were pulverized into powder and stored separately in three clean airtight-containers. Cold extraction method as described by Okigbo and Omodamiro (2006) and Okigbo and Mmeka (2008) was adopted. Two solvents, viz; Ethanol and distilled water were utilized for the extraction. Exactly

100g and 40 g of *N. canescens* were soaked into 700 ml ethanol and 500 ml distilled water respectively for 72 hours. While 100 g and 50 g of *P. biglobosa* were soaked into 500 ml ethanol and 300 ml distilled water respectively for 72 hours. Lastly, 100 g and 50 g of *S. aromaticum* were soaked into 500 ml and 300 ml of ethanol and distilled water respectively for 72 hours. The resultant mixtures were shaken daily and sieved at the end of the 72 hours and collected into well labeled and clean separate beakers and conical flask for concentration. They were evaporated to dryness using rotary evaporator at 50 ^{0}C. After complete evaporation, the crude extracts were scrapped separately into clean containers, labelled and stored at 4 ^{0}C until required for further use.

3.5 Source of Media, Solvents and Reagents

The media, solvents and reagents that were used for this study were obtained from Tech Trics Innovators Laboratory, located at Bosso, opposite Federal University of Technology, Minna.

3.6 Preparation of Media

Nutrient agar, nutrient broth and typtic soy agar were used for this study and they were prepared according to the manufacturer's instructions.

3.7 Qualitative Phytochemical Screening

3.7.1 Test for flavonoids

One milliliter of the plant sample in each case was heated with 10 ml of ethyl acetate in a test tube over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was

homogenized with 1 ml of dilute ammonia solution. Yellow coloration was observed thus indicating the presence of flavonoids (Sofowora, 2013).

3.7.2 Test for tannins

Exactly 1ml of the plant sample was boiled in 20 ml distilled water in a test tube and filtered. Specifically, 0.1% ferric chloride ($FeCl_3$) solution was added to the filterate. The appearance of brownish green or a blue-black coloration indicated the presence of tannins in the test sample (Sofowora, 2013).

3.7.3 Test for saponins

A measured quantity of 1ml of the plant sample was boiled in 20ml of distilled water in a test tube in boiling water bath and filtered. About 10 ml of the filtrate was mixed with 5 ml of distilled water and was shaken vigorously to the formation of stable persistent froth. The frothing was mixed with drop of olive oil and shaken vigorously for the formation of emulsion which is a characteristic of saponins (Sofowora, 2013).

3.7.4 Test for alkaloids

Exactly 0.5 ml of the plant extract was stirred with $5cm^3$ of 1% aqueous HCI on a steam bath. Few drops of picric acid solution were added to $2cm^3$ of the extract. The formation of a reddish-brown precipitate was taken as preliminary evidence for the presence of alkaloids (Trease and Evans, 2019).

3.7.5 Test for steroids

Specifically, 0.5ml of fraction of each plant extract was mixed with 2 ml of acetic anhydride followed by 2 ml of sulphuric acid. The colour changed from violet to blue or green beneath the browning in some samples indicating the presence of steroids, while in the acetic acid, layer; a greenish ring may also form just gradually throughout the thin layer (Sofowora, 2013).

3.7.6 Test for terpenoids

The extract was mixed with 2 ml of chlorofoam and concentrated tetraoxosulphate (iv) acid (3 ml) is carefully added to form a layer. A reddish-brown coloration of the interface was formed to show the presence of terpenoids (Sheell *et al*., 2010).

3.7.7 Test for reducing sugar (Benedict test)

Zero-point five milliliter (0.5ml) of the plant extract was mixed thoroughly with $3cm^3$ distilled water and filtered, 3 drops of the filtrate was added to $3cm^3$ Benedict reagents and placed in a boiling water bath for 5minutes. The formation of a brick red precipitate indicated the presence of reducing sugar (Ejikeme *et al*., 2016).

3.7.8 Test for phenols

One milliliter (1ml) of the plant sample was mixed with few drops of 1% $FeCl_3$. The resulting solution was thoroughly mixed. The appearance of a blue or violet colour is a confirmatory test for the presence of phenol (Ejikeme *et al*., 2016).

3.8 Quantitative Phytochemical Screening of the Extracts

Qualitative Screening of the extracts was done according to the method reported by Ajuru *et al*. (2017).

3.8.1 Test for tannins

Analytical method for quantitative determination of tannins was done by dissolving 50 g of sodium tungstate (Na_2WO_4) in 37 cm^3 of distilled water, Folin-Denis reagent was made. To the reagent prepared above, 10g of phosphomolybdic acid ($H_3PMo_{12}O_{40}$) and 25 cm^3 of orthophosphoric acid (H_3PO_4) were added. Two-hour reflux of the mixture was carried out, cooled, and diluted to 500 cm^3 with distilled water. One gram of each powdered plant (sample) in a conical flask was added to 100 cm^3 of distilled water. This was boiled gently for 1hour on an electric hot plate and filtered using number 42 (125 mm) Whatman filter paper in a 100 cm^3 volumetric flask. Addition of 5.0 cm^3 Folin-Denis reagent and 10cm^3 of saturated Na_2CO_3 solution into 50 cm^3 of distilled water (aliquot volume) was carried out after being pipetted into a 100 cm3 conical flask for color development. The solution was allowed to stand for 30 minutes in a water bath set at a temperature of 25 ^{0}C after thorough agitation. With the aid of a Spectrum Lab 23 A spectrophotometer, optical density was measured at 700 nm and compared on a standard tannic acid curve. Dissolution of 0.20 g of tannic acid in distilled water and dilution to 200 cm^3 mark (1mg/cm^3) was used to obtain tannic standard curve. Varying concentrations (0.2–1.0mg/cm^3) of the standard tannic acid solution was pipetted into five different test tubes to which Folin-Denis reagent (5cm^3) and saturated Na_2CO_3 (10cm^3) solution were added and made up to the 100 cm^3 mark with distilled water. The solution was left to stand for 30 minutes in a

water bath at 25 ^{0}C. Optical density was ascertained at 700 nm with the aid of a Spectrum Lab 23 Aspectrophotometer. A graph of optical density (absorbance) versus tannic acid concentration was plotted. The amount of tannin was calculated using Equation 3.1.

$$Tannic\ acid = C \times extract\ volume \times 100\ Aliquot\ volume \times weight\ of\ sample \qquad (3.1)$$

$$Where\ C\ is\ concentration\ of\ \tan nic\ acid\ read\ off\ the\ graph$$

3.8.2 Test for alkaloids

Exactly 200 cm^3 of 10%acetic acid in ethanol was added to each powdered plant sample in a 250 cm^3 beaker and allowed to stand for 4hours. The extracts were concentrated on a water bath to one-quarter of the original volume followed by addition of 15 drops of concentrated ammonium hydroxide drop-wisely to the extract until the precipitation was complete immediately after filtration. After 3 hours of mixture sedimentation, the supernatant was discarded and the precipitate was washed with 20 cm^3 of 0.1 M of ammonium hydroxide and then filtered using Gemfilter paper (12.5 cm). Using an electronic weighing balance, the residue was dried in an oven and the percentage of alkaloid was calculated using Equation 3.2.

$$\%Alkaloid = \frac{Weight\ ofalkaloid}{Weight\ of\ sample} \times 100 \qquad (3.2)$$

3.8.3 Test for flavonoids

Exactly 50 cm^3 of 80% aqueous methanol was added to 2.50 g of each plant powdered sample in a 250 cm^3 beaker, covered, and allowed to stand for 24 hours at room temperature (25±2^0C). After discarding the supernatant, the residue was re-extracted (three times) with the same volume of ethanol. Whatman filter paper 42 (125 mm) was used to filter whole solution of each extract. Each extract's filtrate was later transferred into a crucible and evaporated to dryness over a water bath. The content in the crucible was cooled in a desiccator and weighed until constant weight was obtained. The percentage of flavonoid was calculated using Equation 3.3.

$$\%Flavonoid = \frac{Weight\ of flavonoid}{Weight\ of\ sample} \times 100 \qquad (3.3)$$

3.8.4 Test saponins

Exactly 100 cm^3 of 20% aqueous ethanol was added to 5 grams of each powdered plant part in a 250 cm^3 conical flask. The mixture was heated over a hot water bath for 4 hours with continuous stirring at 55 ^{0}C. The residue of the mixture was re-extracted with another 100 cm^3 of 20% aqueous at a constant temperature of 55 ^{0}C with constant stirring. The combined extracts were evaporated to 40 cm^3 over water bath at 90 ^{0}C. Specifically, 20 cm^3 of diethylether was added to the concentrate in a 250 cm^3 beaker and the aqueous layer was recovered while the ether layer was discarded. This purification process was repeated twice. Exactly 60 cm^3 of n-butanol was added and extracted twice with 10cm^3 of 5% sodium chloride. After discarding the sodium chloride layer the remaining solution was heated in a water bath for 30 minutes, after which the solution was transferred into a crucible and dried in an oven to a constant weight. The saponin content was calculated using Equation 3.4.

$$\%Saponin = \frac{Weight\ of saponin}{Weight\ of\ sample} \times 100 \qquad (3.4)$$

3.8.5 Test for phenols

Phenol quantitative determination was done according to the method reported by Ezeonu and Ejikeme, 2016. Each plant sample was boiled with 50 ml of ether for 15 minutes. Five milliliter (5 ml) of the extract was pipetted into a 50 ml flask, and then 10 ml of distilled water was added, 2 ml of ammonium hydroxide solution and 5 ml of the extract were pipetted into a 50 ml flask, and then 10 ml of distilled water was added, 2 ml of ammonium hydroxide solution and 5 ml of concentration amyl alcohol were also added. The samples were left to react for 30 minutes for colour development. The absorbance of the solution was read using a spectrophotometer at 505 nm.

3.8.6 Test for reducing sugar content (RSC)

The reducing sugar content (RSC) was determined using the 3,5-dinitrosalicylic acid (DNSA) method. The measurement was performed according to the procedure of Krivorotova and Sereikaite (2014) with slight modification. The DNSA reagent was prepared by dissolving 1 g of DNSA and 30 g of sodium-potassium tartaric acid in 80 mL of 0.5 N NaOH at 45 °C. After dissolution, the solution was cooled down to room temperature (25±2 ^{0}C) and diluted to 100 mL with distilled water. For the measurement, 2 mL of DNSA reagent was pipetted into a test tube containing 1 mL of plant extract (1 mg/mL) and kept at 95 °C for 5 min. After cooling, 7 mL of distilled water was added to the solution and the absorbance of the resulting solution was

measured at 540 nm using a UV-VIS spectrophotometer (Shimadzu UV-1800). The reducing sugar content was calculated from the calibration curve of standard D-glucose (200-1000 mg/L), and the results were expressed as mg D-glucose equivalent (GE) per gram dry extract weight.

3.9 Source and Confirmation of Test Microorganisms

Culture of *Staphylococcus aureus* was collected from the Department of Microbiology, Federal University of Technology Minna. Certain Biochemical tests were carried out to confirm the identity of *S. aureus* such as coagulase, catalase, indole production, methyl red, Voges-proskauer reaction, urease production, citrate utilization, sugar fermentation tests and gram staining of the isolates were undertaken.

Culture of *S. pyogenes* was obtained from the Microbiology laboratory of General Hospital Minna. They were transported to Microbiology Department of Federal University of Technology, Minna for confirmation. The isolate was sub cultured on Tryptic Soy Agar before inoculating it on a blood agar.

3.10 Reconstitution of the Extracts

The extracts were reconstituted to 60, 80, 100 and 120 mg/ml in accordance with the method used by Tibuhwa (2016) using DMSO (Dimethylsulfoxide) for ethanolic extracts and distilled water for aqueous extracts.

3.11 Standardization of Test Organisms

Suspension of each of the test organisms were made by collecting a loopful of colony from each plate and were incubated overnight at 37°C in Nutrient broth. The overnight broth cultures of organisms were diluted in nutrient broth to an inoculum load of approximately $1x10^6$ cfu/ml. It

was then standardized according to National Committee for Clinical Laboratory Standards by gradually adding normal saline and comparing its turbidity to McFarland standard of 0.5 which is approximately 1.0×10^6 cfu/ml.

Sterile swab sticks were used to inoculate the organisms on solidified Mueller Hinton agar plates ensuring that the plates were completely covered for uniform growth.

3.12 Susceptibility Test of Crude Extracts

3.12.1 Determination of zone of inhibition: Agar well diffusion technique as described by Saikumari *et al*. (2016) was adopted for the study. Five petri-dishes of Nutrient Agar were inoculated with 0.5 Mcfarland standard of *Staphylococcus aureus by* diping a sterile swab stick into the test organism solution and using it to rub the entire surface of the medium (Cheesbrough, 2006). Quadruplet wells were bored using 8mm diameter sterile cork-borer on the five petri-dishes. Specifically, 0.4ml of the prepared extract of *Parkia biglobosa* in varying concentrations (60mg/ml, 80mg/ml, 100mg/ml and 120mg/ml) were pipetted into the four wells of three (3) Petri-dishes. The remaining two (2) Petri-dishes were used as positive and negative controls. Ampiclox (60mg/ml) was used as the positive control. This same procedure was followed using *Syzygium aromaticum and Nelsonia canescens* as the antimicrobial agents. And the overall processes were repeated using *Streptococcus pyogenes.* The plates were incubated at 37ºC for 18-24hours. Zones of Inhibition were measured in millimeter (mm) and the average values was calculated and recorded.

3.12.2 Determination of the minimum inhibitory concentration (MIC)

The MIC was determined based on the method described by Taiwo *et al.* (2018). It was carried out on the extract against the test isolates due to the susceptibility of the test organisms to the extracts. About 100 ml of nutrient broth was prepared and 5ml each was dispensed into 18 test-tubes. They were sterilized at 121°C for 15 minutes and allowed to cool to 40-45°C. Subsequently, 0.5ml of 0.5 Mc farland standard of *Staphylococcus aureus* were introduced into 5 different test tubes while 5 ml of each extract concentrations (7.5, 15, 30, 60, and 120 mg/ml) were introduced into 5 of the test-tubes containing the sterilized broth and the test organisms. Plant extracts wasn't added to one of the 6 test-tubes, this was used as control. All the 6 test tubes were labelled and incubated at 37°C for 24 hours. The whole processes were repeated using *Streptococcus pyogenes* as the test organisms. The lowest concentration of extracts which was able to inhibit the growth of the test organisms were taken to be the Minimum Inhibitory Concentration (MIC).

3.12.3 Determination of the minimum bactericidal concentration (MBC) value

The Minimum Bactericidal Concentration (MBC) values were determined by spot inoculation method. A sample was taken from the test-tubes without bacterial growth, the MIC tubes and spotted on nutrient agar plate by means of sterile cotton swabs. The plates were incubated for 24 hours at 37°C. The MBC value was considered as the lowest concentration of plant extract that inhibited the growth of the organisms after incubation (Usman *et al*., 2009).

3.13 Fractionation of Crude Extracts

Parkia biglobosa crude extract was chosen to be fractionated due to its high antimicrobial activity against the test organisms. *The* fractionation was done according to the method described by Yamin *et al*. (2011). Every 1 g of crude extract was dissolved in 20 ml of distilled water with

solid to solvent ratio of 1:20 (w/v) and poured into the separating funnel then 20 ml of 100% hexane was added. Ratio of 20:20 (v/v) of water and hexane was used for separating the non-polar compounds. The separating funnel was shaken for 2 minutes before being allowed to settle down for 2 hours at room temperature (25±2 ºC). The lower phase which was the water layer was withdrawn from the funnel's stopcock while the hexane layer on the upper part was collected into separate clean beaker. The procedure was repeated 5 times when the n-hexane layer turned colorless and the total hexane layer was collected and concentrated by air drying. The aqueous layer was returned back into the separating funnel and 20 mL of ethyl acetate was added. The funnel was shaken for 2 minutes and left at room temperature (25±2 ºC) for 2 hours to settle down. The aqueous layer settled at the bottom layer and was withdrawn from the stopcock while the ethyl acetate layer settled on the upper layer was collected into a separate clean beaker. The procedure was repeated 6 times when the ethyl acetate layer turned colorless and total ethyl acetate layer was collected. The eluted ethyl acetate layer was concentrated by air drying. The aqueous layer was freeze-dried. All fractions were kept inside sterile sample bottles and refrigerated at -4 ºC until further use.

3.14 Antibacterial Activity of the Fractions

Agar diffusion was used to perform the antibacterial activity of the fractions of *P. biglobosa* leaf extracts that were obtained through the process of fractionation. The fractions were applied against the test organisms using standardized culture. Zero point six (0.6 g) of each fractions ware weighed and dissolved in 5 ml of water to give 120 mg/ml. This was done for all the fractions and was used against *Streptococcus pyogenes* and *Staphylococcus aureus.* Sterile nutrient agar plates were prepared and standard culture was streaked on the surface of the agar plates. An eight (8mm) sterile cork borer was used to bore holes on the surface of the agar plates.

Zero point two (0.2) milliliters of the reconstituted extracts were dispensed into the holes and incubated for 24 hours after which the zones of inhibition around the holes were measured and recorded.

3.15 Identification of Bioactive Compounds Using GC-MS (Gas Chromatography-Mass Spectrometry)

The GC-MS analysis of the fractions obtained from *Parkia biglobosa* plant was performed according to the method described by Hossain *et al*. (2014). The analysis was done at Shimadzu Science and Technology, Lagos by using a PerkinElmer Clarus 600 GC system equipped with a fused silica gel column (30 m×0.25mm ID, film thickness 0.25 μm) coupled with a PerkinElmer Clarus 600C MS. The detection of data or spectra was done using an electron ionization system with ionization energy of 70 eV. Inert helium gas (99.999%) was used as a carrier gas at a constant flow rate of ±1 mL/min. Mass transfer line and injector temperatures was set at 220 and 290 °C, respectively. The temperature programmed for oven was from 60 °C (hold 2 min) to 270 °C at 4 °C/min, this was held isothermally for 20 min and finally raised to 300 °C at 10 °C/min. The samples were diluted with methanol (1/100, v/v, in methanol). The tested samples were then filtered with 0.45 μm Millex membrane filter paper (Millipore, France) to remove any dust particles. One microliter filtered test sample was injected in the split mode. The split ratio was 120:1. The percentage (%) of the bio active constituents from the fractions were expressed as percentage by peak area. The whole process was carried out carefully from the light and heat.

The bioactive compounds were identified in the different fractions based on GC retention time on Rtx®-5MS fused silica capillary column. The mass spectra were compared to the standards as seen in computer (NIST 2005 v.2.0 and Wiley Access Pak v.7, 2003 of GC-MS systems).

3.16 Toxicity Test of Extracts Using Laboratory Rats

Acute and sub-acute toxicity test of the extracts was carried out using forty-eight (48) laboratory adult wistar rats weighing between 100-150 g obtained and kept at Animal House, Federal University of Technology, Minna. They were maintained under standard conditions of humidity, temperature and 12 h light/ darkness cycle. The animals were acclimatized to their new environment for one week before the commencement of the study. They were fed vital feed and had free access to water during this period. This toxicology test was carried out based on the guidelines of The Organization of Economic Co-operation and Development (OECD, 2008).

3.16.1 Extracts reconstitution for acute and sub-acute toxicity test

The most active extract was utilized to carry out the toxicity study. The evaporated extract was reconstituted in distilled water at 30% (w/v). The extract was reconstituted to 200, 350, 500, 600, 800 and 1000 mg/kgbw. The reconstituted extracts were labelled crude aqueous extract and was afterwards stored in small-capped plastic containers at 4 °C until required. These were used for both the acute and sub-acute toxicity study

3.16.2 Sub-acute toxicity study of rats

Twenty-Four (24) rats were selected by randomization from the total number of rats (48) and then divided into four groups of six rats each. The first group served as control and was administered distilled water while the remaining three groups were given 200, 350 and 500

mg/kgbw of *P. biglobosa* aqueous leaf crude extract single oral dose per day. The extracts were administered for 28 days. This was carried out according to the methods of Orisakwe *et al*. (2016).

3.16.3 Acute toxicity study of rats

The method employed was that reported by Saidu *et al*. (2017). The remaining twenty-four (24) rats were selected randomly and then divided into four groups of six rats each. The first group served as control and was administered distilled water while the remaining three groups were given 600, 800 and 1000 mg/kg of *P. biglobosa* aqueous leaf crude extract single oral dose per day. The dosage was administered for 14 days. The first day of dosing was taken as D_0 whereas the day of sacrifice was designated as D_{14}. The number of deaths in each group within 24 h was recorded and the final LD_{50} values were calculated as the Geometric mean of the highest non-lethal dose (with no deaths) and the lowest lethal dose (where deaths occurred).

3.16.4 Weekly body weight of rats in acute and sub-acute toxicity study

For the acute toxicity test, the body weight of each rat was measured using a sensitive balance during the acclimatization period, the 7th day after commencing extracts administration and on the day of sacrifice which is the 14th day (Aniagu *et al*., 2016). For the sub-acute toxicity test, the body weights of the rats were taken during acclimatization, 7th, 14th and 28th day after the extracts administration.

3.16.5 Relative organ weight of rats in acute and sub-acute toxicity study

On day 14 of the dosing period, all the animals were anesthetized by the use of chloroform and slaughtered after which their blood samples were collected for haematology and biochemical test. Different organs namely the heart, liver and kidneys were carefully dissected out and weighed in grams (absolute organ weight) as described by (Asokkumar, 2016). The relative organ weight of each animal was then calculated using Equation 3.5.

$$Relative\ Organ\ Weight\ =\ \frac{Absolute\ organ\ weight}{Body\ weight\ of\ rat\ on\ sacrifice\ day} \times 100 \qquad (3.5)$$

3.16.6 Histopathological study of rat

Histopathological investigation of the kidneys, hearts and livers were done according to the method described by Pieme *et al.* (2016). The organ slices were fixed in 10% formalin for 24 h and washed under running water. Samples were dehydrated in an auto Technicon and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50 °C and then in a cubical block of paraffin made by the “L” moulds. They were worked on by a microtome and the slides were stained with Haematoxylin-eosin. Detailed microscopic examination was carried out in those organs of both control and treatment groups.

3.16.7 Serum biochemistry of rats

Blood sample of rats were collected into non heparinized tubes and were centrifuged at 3000 rpm for 10 min. The serum separated was analyzed to evaluate the liver enzymes [Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and Alkaline phosphatase (ALP)], using the method of Pieme *et al*. (2016). Glucose concentration and lipid level were determined by the method of Hong *et al*. (2016).

3.17 Data analysis

The data represent mean of three replicates ± standard deviation (SD). Results were subjected to multi-way analysis of variance and the mean comparisons were performed by Turkey's multiple range test using SPSS version 20.0. Differences between means were considered significant at p-value < 0.05. (Builders *et al*., 2019).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1 Qualitative phytochemical component of the extracts

The qualitative phytochemical screening of the three plants (*Parkia biglobosa, Syzygium aromaticum* and *Nelsonia canescens*) is shown in Table 4.1. The result revealed the presence of alkaloids, streroids, tannin, flavonoids, saponin, reducing sugar, phenol and terpenes at varying amount. Phytochemical screening of *S. aromaticum* revealed only the presence of alkaloids, flavonoids and saponins. It lacked steroids, tannins, reducing sugar, phenol and terpenes. *N. canescens* leaf extract on the other hand contains alkaloids, tannin, saponins, reducing sugar and phenol but lacked steroids, flavonoids and terpenes while the Phytochemical screening of *P. biglobosa* revealed the presence of eight (8) secondary metabolites which include; tannins, terpenes, flavonoids, steroids, alkaloids, saponins, phenols and reducing sugars. The result showed that *P. biglobosa* had the highest number of phytochemicals with flavonoids, saponins and phenols being the highest in concentration.

Table 4.1: Qualitative Phytochemical Components of the Extracts

Chemical compounds	*S. aromaticum* ethanolic extracts	*S. aromaticum* Aqueous extracts	*P. biglobosa* ethanolic extracts	*P. biglobosa* Aqueous extracts	*N. canescens* Ethanolic extract	*N. canescens* Aqueous extracts
Alkaloid	+	-	-	-	+	+
Steroids	-	-	+	+	-	-
Tannins	-	-	+	+	+	+
Flavonoids	+	+	+	+	-	-
Saponins	+	+	+	+	+	+
Reducing Sugars	-	-	+	+	+	+
Phenols	-	-	+	+	+	+
Terpenes	-	-	+	+	-	-

Key

- : Not present

+ : Present

4.1.2 Quantitative phytochemical component of the extracts

The Quantitative phytochemical screening of the three plants as seen in Table 4.2 revealed that the secondary metabolites that were present in *S. aromaticum* and *N. canescens* were small in

quantity varying from 3.2 to 7.0% while those in *P. biglobosa* were high ranging from 4.3 to 63.36%. The high amount of certain phytochemicals components of *P. biglobosa* explains why it is widely used traditionally as antimaleria, antidiarrhea, antifungal, antihelminthes and others. Particularly, flavonoids, saponins and phenols were found to be 62.51, 63.36 and 41.8% respectively in ethanolic extracts of *P. biglobosa* while it's aqueous extracts revealed the amount to be 48.5%, 55.6% and 40.2% respectively.

Table 4.2: Quantitative Phytochemical Components of the Extracts in Percentage (%)

Chemical compounds	*S. aromaticum* Ethanolic extracts	*S. aromaticum* Aqueous extracts	*P. biglobosa* Ethanolic extracts	*P. biglobosa* Aqueous extracts	*N. canescens* Ethanolic extract	*N. canescens* Aqueous extracts
Alkaloid	3.4	-	-	-	5.4	7.0
Steroids	-	-	6.9	9.0	-	-
Tannins	-	-	15.6	46.9	4.5	3.6
Flavonoids	4.3	4.5	62.51	48.5	4.1	3.3
Saponins	3.2	3.7	63.36	55.6	3.5	3.7
Reducing Sugars	-	-	4.3	8.6	6.3	5.0
Phenols	-	-	41.8	40.2	5.2	4.0
Terpenes	-	-	30.0	12.5	-	-

Key

- : Not detect

4.1.3 Antibacterial activities of the plant extracts against *Streptococcus pyogenes*

The results of the antimicrobial activity of the three (3) plants as shown in Table 4.3 revealed that only *P. biglobosa* was effective against the test organisms (*S. aureus* and *S. pyogenes*) as the

test organisms were sensitive to the ethanolic and aqueous extracts of *P. biglobosa* leaves. The highest mean zone of inhibition exhibited by the ethanolic and aqueous leaf extracts of *P. biglobosa* against *S. pyogenes* were 22.5±1.3 mm and 24±1.0 mm at 120 mg/ml of the extracts. The test organisms on the other hand were both resistant to the ethanolic and aqueous extracts of *S. aromaticum* and *N. canescens* at 60, 80, 100 and 120 mg/ml.

Table 4.3: Activities of Extracts against *Streptococcus pyogenes* (mm)

Concentration Extracts	60 mg/ml	80 mg/ml	100 mg/ml	120 mg/ml
AQLE of *P. biglobosa*	-	-	17.5±1.2 [a]	24±1.0 [a]
ETLE of *P. biglobosa*	8.5±1.4 [b]	20±1.2 [a]	19.5±0.9 [a]	22.5±1.3 [a]
AQLE of *S. aromaticum*	-	-	-	-
ETLE of *S. aromaticum*	-	-	-	-
AQLE of *N. canescens*	-	-	-	-
ETLE of *N. canescens*	-	-	-	-
Ampiclox (60mg/ml)		15±1.6 [a]		

Mean± SD values carrying superscripts different from the control are significantly different (P<0.05).

Key

AQLE=Aqueous Leaf Extract

ETLE=Ethanolic Leaf Extract

NOTE: Zones of inhibition greater than 14mm were considered 'sensitive,' those between 11mm and 13mm were considered 'moderately sensitive' and those below 11mm were considered 'resistant.'

4.1.4 Antibacterial activities of the plant extracts against *Staphylococcus aureus*

Table 4.4 shows the antibacterial activity of the three plants against *Staphylococcus aureus*. The highest mean zone of inhibition exhibited by the ethanolic and aqueous leave extracts of *P. biglobosa* against *S. aureus* were 19±1.4 mm and 22.5±1.3 mm. The test organisms on the other hand were both resistant to the ethanolic and aqueous extracts of *S. aromaticum* and *N. canescens* at 60, 80, 100 and 120 mg/ml.

Table 4.4: Activities of Extracts against *Staphylococcus aureus* (mm)

Concentration Extracts	60 mg/ml	80 mg/ml	100 mg/ml	120 mg/ml
AQLE of *Parkia. Biglobosa*	18±1.5 [b]	19±1.0 [a]	20.5±1.4 [a]	22.5±1.3 [a]
ETLE of *P. biglobosa*	15.5±1.2 [c]	16.5±0.8 [b]	17±1.1 [a]	19±1.4 [a]
AQLE of *Syzygium. aromaticum*	-	-	-	-
ETLE of *Z. aromaticum*	-	-	-	-
AQLE of *Nelsonia. Canescens*	-	-	-	-
ETLE of *N. canescens*	-	-	-	-
Ampiclox (60 mg/ml)	27.5±1.5 [a]			

Mean± SD values carrying superscripts different from the control are significantly different ($P<0.05$)

Key

AQLE=Aqueous Leaf Extract

ETLE=Ethanolic Leaf Extract

NOTE: Zones of inhibition greater than 14 mm were considered 'sensitive,' those between 11 mm and 13 mm were considered 'moderately sensitive' and those below 11 mm were considered 'resistant.'

4.1.5 Minimum inhibitory concentration of the plants' crude extracts against test organisms

Table 4.5 shows the minimum inhibitory concentration of the plants' crude extracts against the test organisms. Minimum Inhibitory Concentration (MIC) of the ethanolic and aqueous extracts of *P. biglobosa* was 60 mg/ml on *S. aureus*. While the MIC of the ethanolic and aqueous extracts *of P. biglobosa* on *S. pyogenes* were 60 mg/ml and 120 mg/ml respectively. The MIC of ethanolic and aqueous leave extract of *Nelsonia. canescens* and *S. aromaticum* could not be determined because the test organisms were resistant to them.

Table 4.5: Minimum Inhibitory Concentration of Plant's Crude Extracts against Test Organisms (mg/ml).

PLANT EXTRACTS	STREPTOCOCCUS PYOGENES	STAPHYLOCOCCUS AUREUS
AENC	-	-
EENC	-	-
AEPB	60	60

EEPB	120	60
AESA	-	-
EESA	-	-
Ampiclox (control)	30	15

Key

-: Resistant

AENC: Aqueous extract of *Nelsonia canescens*

EENC : Ethanolic extract of *Nelsonia canescens*

AEPB : Aqueous extract of Parkia biglobosa

EEPB : Ethanolic extract of *Parkia biglobosa*

AESA : Aqueous extract of *Syzygium aromaticum*

EESA : Ethanolic extract of *Syzygium aromaticum*

4.1.6 Minimum bactericidal concentration (MBC) of plants' crude extracts against test organisms

Table 4.6 captures the minimum bactericidal concentration (MBC) of plants' crude extracts against test organisms. The Minimum Bactericidal Concentration (MBC) for both ethanolic and aqueous leaf extract of *P. biglobosa* on *S. aureus and S. pyogenes* was 120 mg/ml. The MBC of ethanolic and aqueous leaf extract of *Nelsonia canescens* and *Syzygium aromaticum* could not be determined because the test organisms were resistant to them.

Table 4.6: Minimum Bactericidal Concentration (MBC) of Plants' Crude Extracts against Test Organisms (mg/ml)

PLANT EXTRACTS	STREPTOCOCCUS PYOGENES	STAPHYLOCOCCUS AUREUS
AENC	-	-
EENC	-	-
AEPB	120	120
EEPB	120	120
AESA	-	-
EESA	-	-
Ampiclox (control)	60	60

Key

-: Resistant

AENC: Aqueous extract of *Nelsonia canescens*

EENC : Ethanolic extract of *Nelsonia canescens*

AEPB : Aqueous extract of *Parkia biglobosa*

EEPB : Ethanolic extract of *Parkia biglobosa*

AESA : Aqueous extract of *Syzygium aromaticum*

EESA : Ethanolic extract of *Syzygium aromaticum*

4.1.7 Fractions of *Parkia biglobosa* crude extracts

The results of the fractionation of *Parkia biglobosa* is displayed in Table 4.7. Aqueous extracts of *Parkia biglobosa* was fractionated being the most active extract against the test organisms. The fractionation was done using ethyl acetate, aqueous and n-hexane solvents. The weight of the dried resultant fractions obtained range from 0.15 g to 0.28 g.

Table 4.7: Fractionation of *Parkia biglobosa* Crude Extracts

FRACTIONS	WEIGHT OF CRUDE EXTRACTS (g)	WEIGHT OF FRACTIONS OBTAINED(g)
EFAE	2.02	0.15
NFAE	2.24	0.18
AFAE	2.35	0.20
EFEE	2.50	0.25
NFEE	2.45	0.28
AFEE	2.41	0.22

Key

EFAE : Ethylacetate Fraction of the Aqueous Extract

NFAE : N-hexane Fraction of the Aqueous Ectracts

AFAE : Aqueous Fraction of the Aqueous Extract

EFEE : Ethylacetate Fraction of the Ethanolic Extract

NFEE : N-hexane Fraction of the Ethanolic Extract

AFEE : Aqueous Fraction of the Ethanolic Extract

4.1.8 Antibacterial activity of the fractions of the leaf extracts of *P. biglobosa* on *S. pyogenes* and *S. aureus* at a concentration of 120 mg/ml

The result of the antibacterial activity of the fractions of the leaf extracts of *P. biglobosa* is captured in Table 4.8. Only EFAE (Ethylacetate Fraction of the Aqueous Extract) was active against *S. pyogenes*, exhibiting a zone of inhibition of 22 mm. On the other hand, the active fractions of *Parkia biglobosa* crude leaf extract against *S. aureus* were N-hexane Fraction of the Aqueous Extracts (NFAE), Ethylacetate Fraction of the Ethanolic Extract (EFEE) and Aqueous

Fraction of the Ethanolic Extract (AFEE) which produced 18, 15 and 20 mm zones of inhibitions respectively.

Table 4.8: Activities of the Fractions of the Leaf Extracts of *P. biglobosa* Against *S. pyogenes* and *S. aureus* at 120 mg/ml (mm).

FRACTIONS	*S. pyogenes*	*S. aureus*
EFAE	-	-
NFAE	22	18
AFAE		-
EFEE		15
NFEE		-
AFEE		20

Key

- : No activity

EFAE: Ethanolic Fraction of the Aqueous Extract

NFAE: N-hexane Fraction of the Aqueous Extract

AFAE: Aqueous Fraction of the Aqueous Extract

EFEE: Ethylacetate Fraction of the Ethanolic Extract

NFEE: N-hexane Fraction of the Ethanolic Extract

AFEE: Aqueous Fraction of the Ethanolic Extract

4.1.9 Gas-chromatography mass spectrometry of *Parkia biglobosa*

The Gas Chromatography-Mass Spectrometry of *Parkia biglobosa* result is shown in Table 4.9 and 4.10. This revealed the presence of 26 different compounds in the crude extracts of *Parkia*

biglobosa. The chromatograms (Figures 4.1 – 4.6) shows various chemical compounds detected in six (6) fractions of *Parkia biglobosa* leaves extract. Each compound is numbered accordingly with their respective names, retention time and intensity as listed in the subsequent tables 4.9 and 4.10 which are the key to understanding the chromatograms.

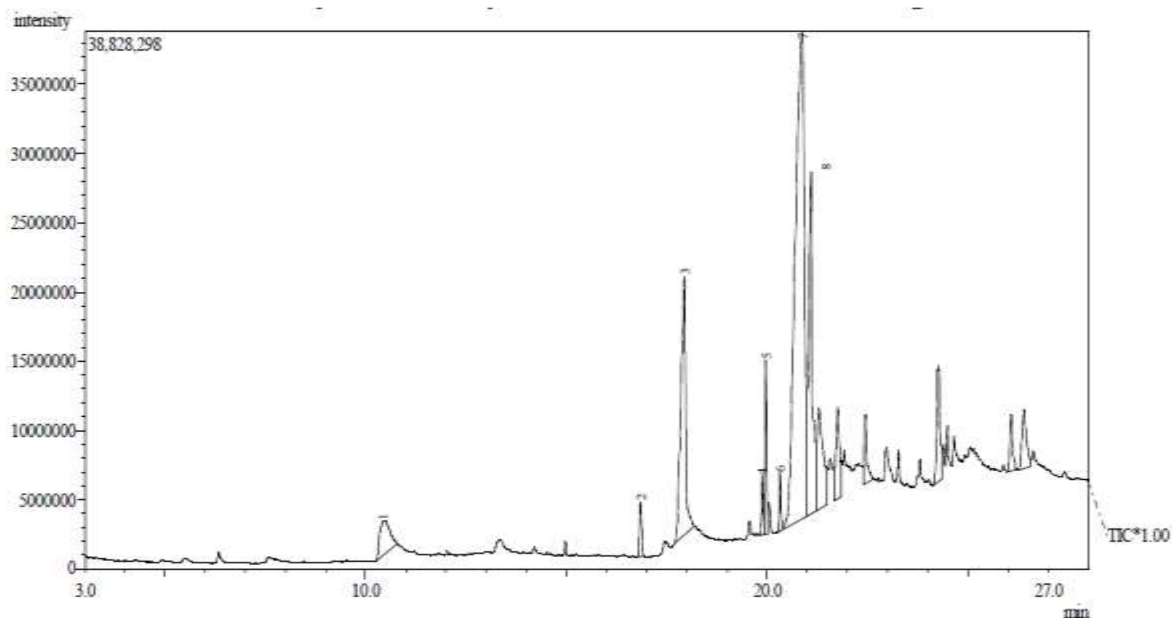

Figure 4.1: Chromatogram of ethyl acetate fraction of ethanolic extract of *P. biglobosa* leaf

Key

1. Bis(tridecyl)phthalate
2. 1,2,3-Benzenetriol
3. 9,9-dimethoxybicyclo(3.3.1)nona 2,4-dion
4. 3-hydroxy-4-dimethyl
5. Cis-ii-Eicosenamide
6. H2-Pyran2-(2-heptadecynyloxy)tetrahydro
7. 9-Octadecenamide(Z)
8. n-Hexadecanoic acid

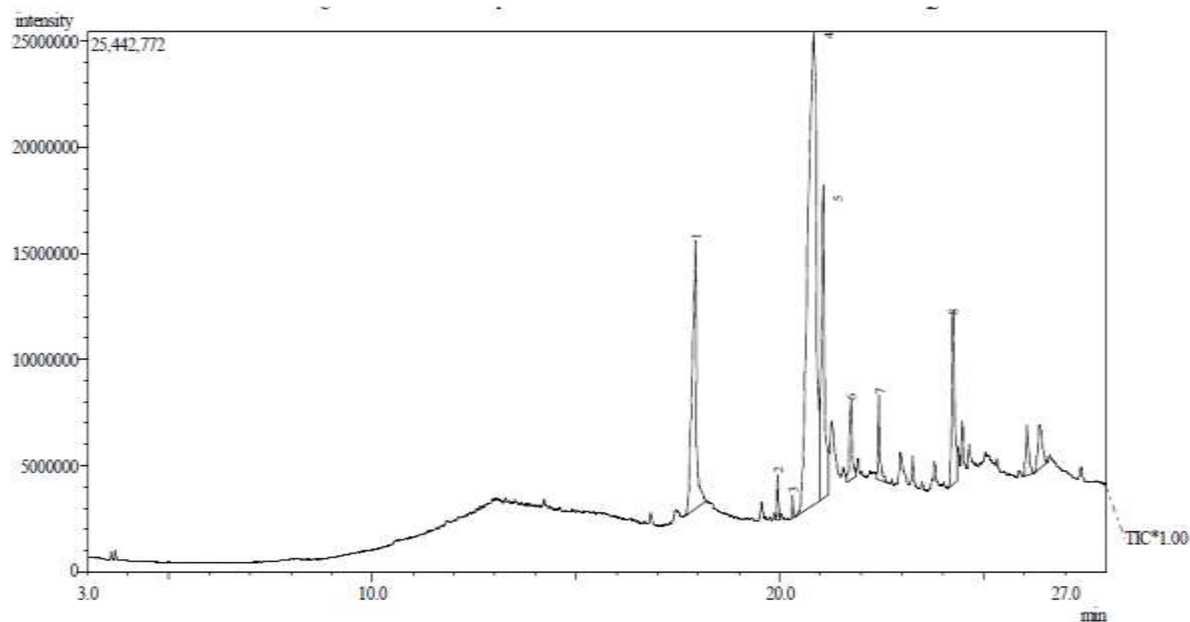

Figure 4.2: Chromatogram of N-hexane fraction of ethanolic extract of *P. biglobosa* leaf

Key

1. 9,12-Octadecadienoic acid
2. Cis-5,8,11-Eicosatrienoic acid
3. 9,10-secocholesta-5,7,10,(19)-triene-1,3-diol
4. Phenol,2,4-bis(1,1-dimethylethyl)
5. 7-Hexadecenoic acid
6. Octadecanoid acid
7. 1,2,3-Benzenetriol
8. Cis-9-hexadecenal

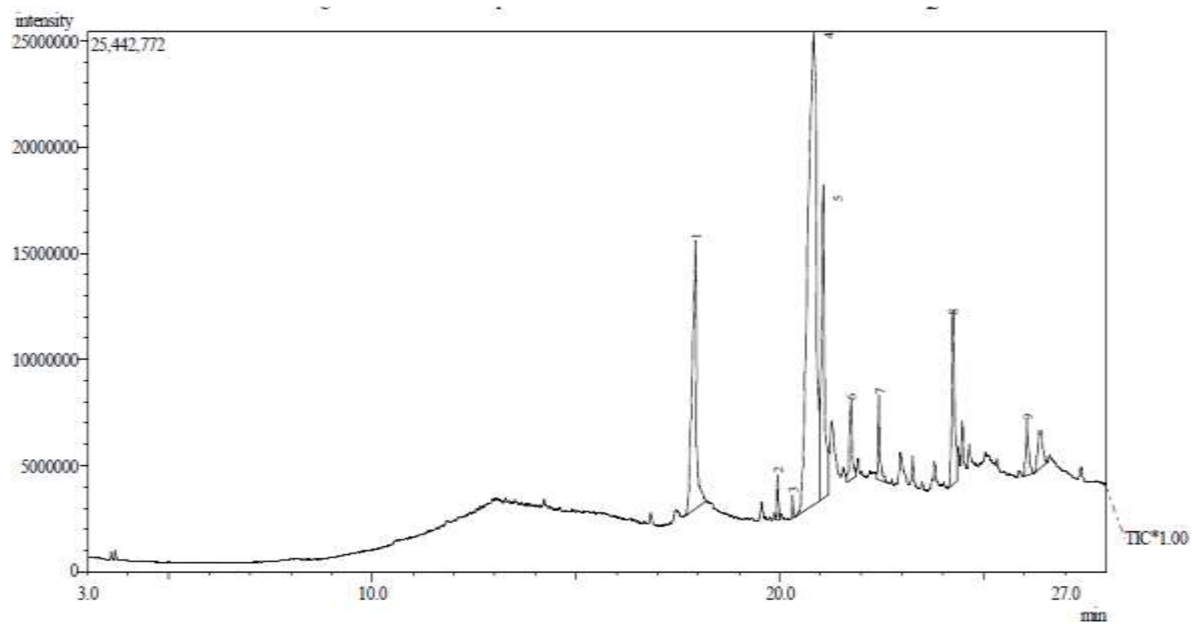

Figure 4.3: Chromatogram of aqueous fraction of ethanolic extract of *P.biglobosa* leaf

Key

1. 1,2-dihydro-acetate
2. 2-Naphthalenol
3. 1,2, cyclopentanedimethanol
4. Olean-12-ene-28-al
5. n-Hexadecanoic acid
6. Heptadecanone
7. Alpha-D-Glucopyranoside
8. 9-Octadecenamide(Z)
9. C-9-hexadecenal

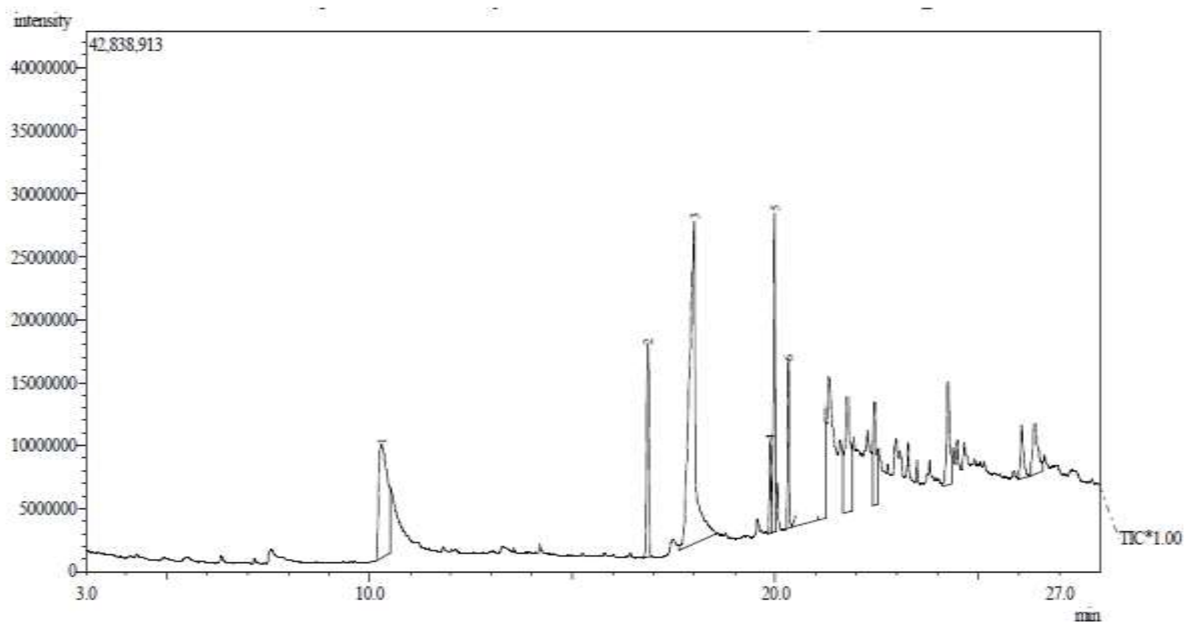

Figure 4.4: Chromatogram of ethyl acetate fraction of aqueous extract of *P.biglobosa* leaf

Key

1. 1,11,13-Octadecatriene
2. 11-Octadecanoic acid
3. 1,1,6-trimethyl-3-methyl-2-(3,6,9,13)-tetramethyl
4. 9,9-dimethoxybicyclo (3.3.1) nona-2,4-dion
5. 3-hydroxy-4-dimethyl
6. 9-Octadecenamide(Z)

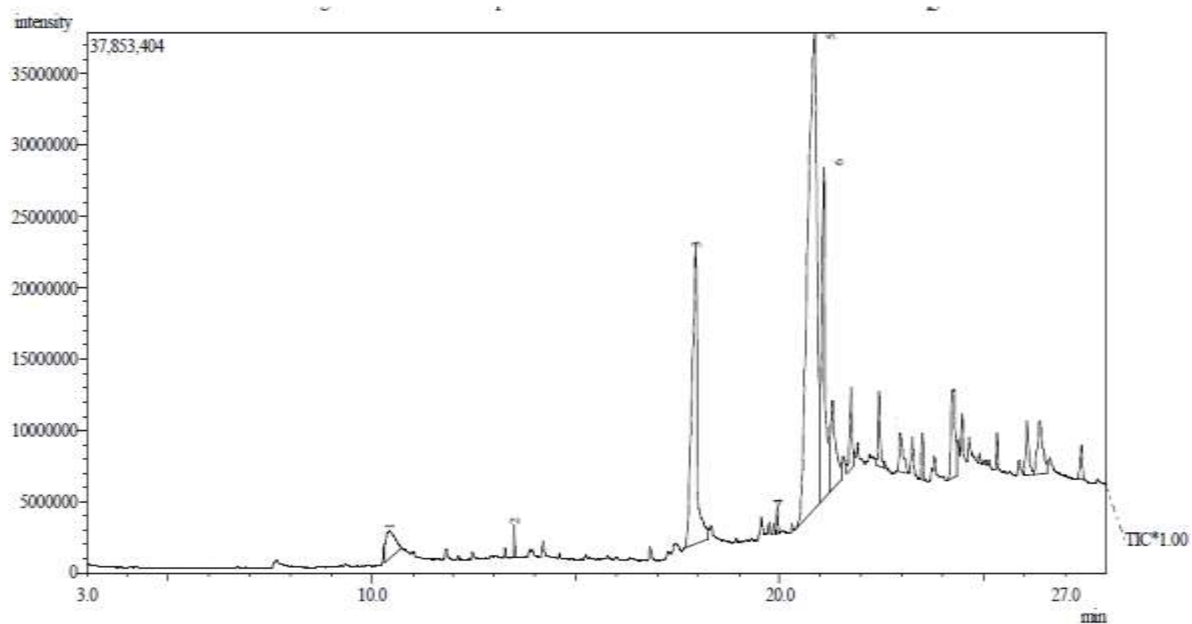

Figure 4.5: Chromatogram of N-hexane fraction of aqueous extract of *P.biglobosa* leaf

Key

1. Methyl ester
2. Phenol,2,4-bis(1,1-dimethylethyl)
3. 7-Hexadecenoic acid
4. Octadecanoid acid
5. C-9-hexadecenal
6. 1,2,3-Benzenetriol

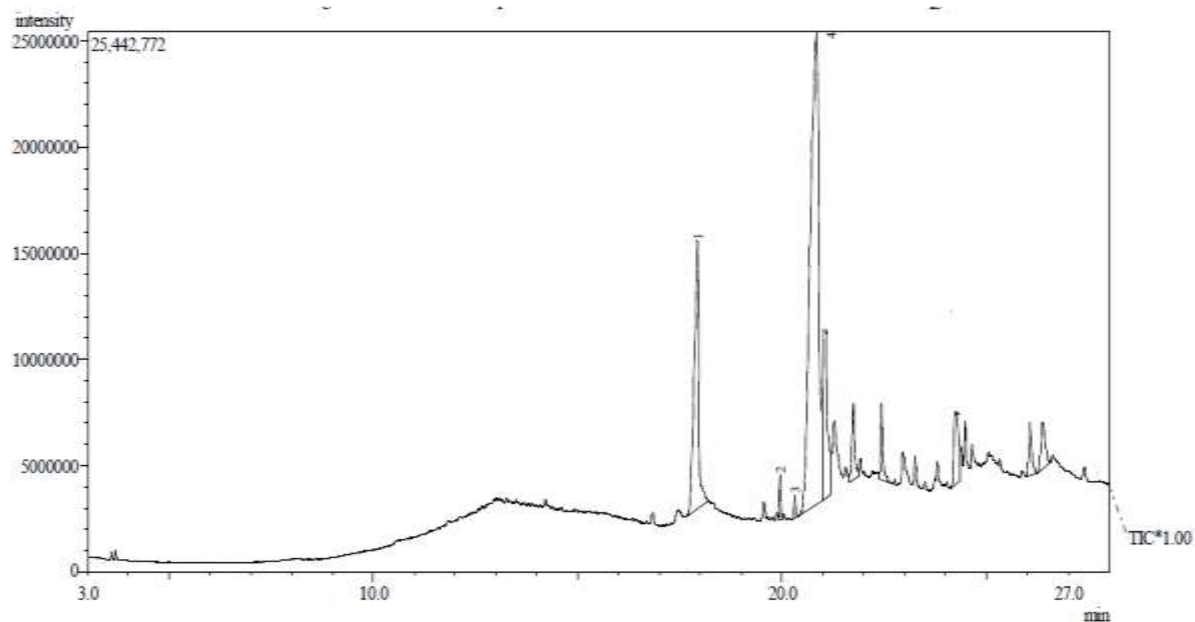

Figure 4.6: Chromatogram of aqueous fraction of aqueous extract of *P.biglobosa* leaf

Key

1. Pentadecanoic acid
2. n-Hexadecanoic acid
3. Alpha-D-Glucopyranoside
4. Cis-9-Hexadecenal

Twenty-six (26) different compounds as revealed by the GC-MS are listed in Tables 4.9 and 4.10. Each of them being separated into various fractions of *P. biglobosa* in which they are contained.

Table 4.9: Compounds in *Parkia biglobosa* Ethanolic Fractions as Revealed by GC-MS

Solvents	S/N	Compound name	Retention time (minutes)	Intensity (counts)
N-hexane Fraction	1.	9,12-Octadecadienoic acid	10.50	15,500,000
	2.	Cis-5,8,11-Eicosatrienoic acid	6.91	4,000,000
	3.	9,10-secocholesta-5,7,10 (19)-triene-1,3-diol	7.92	3,000,000
	4.	Phenol,2,4-bis(1,1-dimethylethyl)	19.80	25,000,000
	5.	7-Hexadecenoic acid	19.91	18,000,000
	6.	Octadecanoid acid	20.35	7,500,000
	7.	1,2,3-Benzenetriol	21.11	8,000,000
	8.	Cis-9-hexadecenal	20.64	12,000,000
Ethylacetate Fraction	1.	Bis(tridecyl)phthalate	10.50	3,500,000
	2.	1,2,3-Benzenetriol	16.91	5,000,000
	3.	9,9-dimethoxybicyclo (3.3.1) nona 2,4-dion	17.80	21,000,000
	4.	3-hydroxy-4-dimethyl	19.83	6,800,000
	5.	Cis-ii-Eicosenamide	19.94	15,000,000
	6	H2-Pyran2-(2-heptadecynyloxy) tetrahydro	20.21	7,000,000
	7.	9-Octadecenamide(Z)	20.65	39,0000,000
	8.	n-Hexadecanoic acid	21.20	29,000,000
	1.	1,2-dihydro-acetate	17.96	15,500,000

Aqueous Fraction	2.	2-Naphthalenol	19.95	4,500,000
	3.	1,2, cyclopentanedimethanol	20.40	3,500,000
	4.	Olean-12-ene-28-al	20.81	25,400,000
	5.	n-Hexadecanoic acid	21.13	18,000,000
	6.	Heptadecanone	21.80	8,000,000
	7.	Alpha-D-Glucopyranoside	22.37	8,200,000
	8	9-Octadecenamide(Z)	24.38	12,000,000
	9.	C-9-hexadecenal	26.12	7,000,000

Table 4.10: Compounds in *Parkia biglobosa* Aqueous Fractions as Revealed by GC-MS

Solvent	S/N	Compound name	Retention time	Intensity (counts)
N-hexane Fraction	1.	Methyl ester	10.40	3,000,000
	2.	Phenol,2,4-bis(1,1-dimethylethyl)	13.51	3,300,000
	3.	7-Hexadecenoic acid	17.98	23,000,000
	4.	Octadecanoid acid	19.99	5,000,000
	5.	C-9-hexadecenal	20.80	38,000,000
	6.	1,2,3-Benzenetriol	21.20	29,000,000
Ethylacetate Fraction	1.	1,11,13-Octadecatriene	10.35	10,000,000
	2.	11-Octadecanoic acid	16.90	18,000,000
	3.	1,1,6-trimethyl-3-methyl-2-(3,6,9,13)-tetramethyl	18.00	28,000,000
	4.	9,9-dimethoxybicyclo (3.3.1) nona-2,4-dion	19.90	10,000,000
	5.	3-hydroxy-4-dimethyl	20.01	28,500,000
	6.	9-Octadecenamide(Z)	20.37	17,000,000

Aqueous Fraction	1.	Pentadecanoic acid	17.92	15,500,000
	2.	n-Hexadecanoic acid	19.97	4,500,000
	3.	Alpha-D-Glucopyranoside	20.44	3,500,000
	4.	Cis-9-Hexadecenal	20.83	25,400,000

Initial weight of rats administered with Aqueous Extracts of *P. biglobosa* in Sub-acute and Acute Toxicity Study are displayed in Tables 4.11 and 4.12.

Table 4.11: Initial Weight of Rats Administered with Aqueous Extracts of *P. biglobosa* in Sub-acute Toxicity Study (weight in gramme)

GROUP 1 (control)	GROUP 2 (200 mg/kg)	GROUP 3 (350 mg/kg)	GROUP 4 (500 mg/kg)
103	105	116	112
112	103	109	130
109	138	124	116
139	110	114	141
110	134	130	105
111	105	124	113

Table 4.12: Initial Weight of Rats Administered with Aqueous Extracts of *P. biglobosa* in Acute Toxicity Study (weight in gramme)

GROUP 1 (control)	GROUP 2 (600 mg/kg)	GROUP 3 (800 mg/kg)	GROUP 4 (1000 mg/kg)
122	104	106	113
102	102	107	120
107	118	123	126
139	110	115	144
113	124	138	112
101	105	122	116

4.1.10 Effects of aqueous extracts of *P. biglobosa* on body weight of rats in sub-acute toxicity study

The effects of aqueous extracts of *P. biglobosa* on body weight of rats in sub-acute toxicity study is shown in Table 4.13. After the duration of the extract administration (28 days), the highest weight gain and weight loss amongst the rats was ±10.81 (mg). This was observed amongst the rats in group four (4). Rats in group two (2) had a highest weight gain and weight loss of ± 6.19 mg. There was a corresponding increase in the weights of rat administered with extracts as the extract concentration increases from 200 mg/kgbw to 500 mg/kgbw.

Table 4.13: Effect of Aqueous Extracts of *P. biglobosa* on Weight of Rats in Sub-acute Toxicity Study.

GROUPS			
1 (control)	**2**	**3**	**4**
103±3.51 [a]	105±4.29 [a]	116±5.04 [a]	112±7.27 [b]
112±2.72[a]	103±5.58 [a]	109±8.55 [b]	130±9.24 [b]
109±2.54 [a]	138±5.82 [a]	124±7.34 [b]	116±8.30 [c]
139±3.03 [a]	110±4.97 [a]	114±6.29 [b]	141±10.81 [c]
110±1.91 [a]	134±5.73 [b]	130±8.34 [c]	105±9.16 [c]
111±2.86 [a]	105±6.19 [b]	124±7.20 [b]	113±9.20 [c]

Mean± SD values carrying superscripts different from the control are significantly different ($P<0.05$)

Key

Group 1, Administered with distilled water

Group 2, Administered with 200 mg/kgbw of the extracts

Group 3, Administered with 350 mg/kgbw of the extracts

Group 4, Administered with 500 mg/kgbw of the extracts

4.1.11 Effect of aqueous extract of *Parkia biglobosa* on body weight of rats in acute toxicity study

The effects of aqueous extracts of P. biglobosa on body weight of rats in acute toxicity study is shown in Table 4.14. After the duration of the extract administration (14 days), the highest weight gain and weight loss amongst the rats was ±10.95 (mg). This was found amongst the rats in group four (4). Rats in group two (2) had a highest weight gain and weight loss of ± 6.92 mg. There was a corresponding increase in the weights of rat administered with extracts as the extract concentration increases from 600mg/kgbw to 1000mg/kgbw.

Table 4.14: Effect of Aqueous Extracts of *P. biglobosa* on Weight of Rats in Acute Toxicity Study.

GROUPS			
1 (control)	**2**	**3**	**4**
122±2.13 [a]	104±4.45 [a]	106±6.30 [b]	113±8.64 [b]
102±2.16 [a]	102±5.67 [a]	107±5.13 [a]	120±10.95 [b]
107±2.39 [a]	118±5.86 [b]	123±8.10 [b]	126±10.18 [c]
139±2.26 [a]	110±7.10 [b]	115±8.19 [b]	144±11.61 [c]
113±3.37 [a]	124±6.92 [a]	138±9.04 [c]	112±11.25 [c]
101±1.73 [a]	105±5.20 [b]	122±9.32 [b]	116±10.84 [c]

Mean± SD values carrying superscripts different from the control are significantly different (P<0.05)

Key

Group 1, Administered with distilled water

Group 2, Administered with 600 mg/kgbw of the extracts

Group 3, Administered with 800 mg/kgbw of the extracts

Group 4, Administered with 1000 mg/kgbw of the extracts

4.1.12 Effects of aqueous extract of *Parkia biglobosa* on relative organ weights of rats in sub-acute and acute toxicity study

Table 4.15 and 44.16 shows the effects of aqueous extract of *Parkia biglobosa* on relative organ weights of rats in sub-acute and acute toxicity study. The highest weight gained and weight loss in relative organs of rats was ±0.16 for sub-acute toxicity test. While that of acute toxicity test was ±0.23. There was no significant difference (P<0.05) in the relative weight of the hearts, kidneys and livers of the rats treated with the aqueous extract in relation to the control groups in sub-acute toxicity test. There was however a significant increase in relative weight of the kidneys and livers of the rats administered with 1000 mg/kgbw of the aqueous extract in the acute toxicity test.

Table 4.15: Effects of the Aqueous Extracts of *Parkia biglobosa* on Relative Organ Weights of Rats in Sub-acute Toxicity Study (%)

Treatment	Hearts	Livers	Kidneys

(mg/kgbw/day)			
Control	0.34±0.05 [a]	3.90±0.13 [a]	0.79±0.05 [a]
200	0.35±0.07 [a]	3.86±0.16 [a]	0.75±0.11 [a]
350	0.34±0.03 [a]	3.92±0.12 [a]	0.79±0.09 [a]
500	0.35±0.06 [a]	3.91±0.13 [a]	0.87±0.08 [a]

Mean± SD values carrying superscripts different from the control are significantly different (P<0.05)

Table 4.16: Effects of the Aqueous Extracts of *Parkia biglobosa* on Relative Organ Weights of Rats in Acute Toxicity Study (%)

Treatment (mg/kgbw/day)	Hearts	Livers	Kidneys
Control	0.36±0.02 [a]	3.87±0.12 [a]	0.76±0.15 [a]
600	0.37±0.09 [a]	3.88±0.14 [a]	0.79±0.12 a
800	0.33±0.04 [a]	3.91±0.15 [a]	0.80±0.13 [a]
1000	0.35±0.07 [a]	3.92±0.23 [b]	0.85±0.20 [c]

Mean± SD values carrying superscripts different from the control are significantly different (P<0.05)

4.1.13 Acute and sub-acute toxicity study

There was no mortality in animals at all doses of the *P. biglobosa* extracts administered up to 1000 mg/kgbw. The absence of death at doses up to 1000 mg/kgbw showed that LD_{50} of the extracts of *P. biglobosa* was greater than 1000 mg/kgbw. Animals which received a dosage of 1000 mg/kgbw of the ethanolic extract of *P. biglobosa* shivered after 3 hours of administration and were restless. This sign of toxicity however was resolved within 24 hours of the dosage

administration. Rats which received extract concentration of 800 mg/kgbw and below showed no sign of toxicity.

4.1.14 Histopathological results

Photomicrograph of the heart tissues of rats after toxicological studies are presented in Figure 4.7.

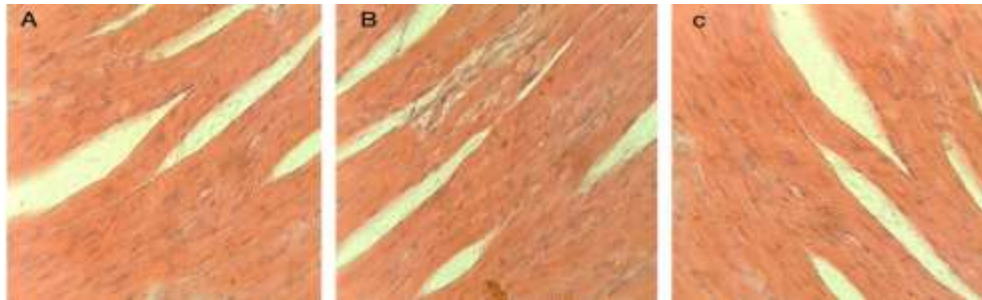

Figure 4.7: Photomicrographs of heart tissue of rat. All sections were stained with hematoxylin and eosin.

A: Section of control rat showing normal histological appearance of heart.

B: Section of rat treated with 1000mg/kgbw of aqueous extract of *P. biglobosa* showing normal architecture.

C: Section of rat treated with 500mg/kgbw of aqueous extract of *P. biglobosa* showing normal architecture. (X 40 magnification.

Photomicrograph of the liver tissues of rats after toxicological studies are presented in Figure 4.8.

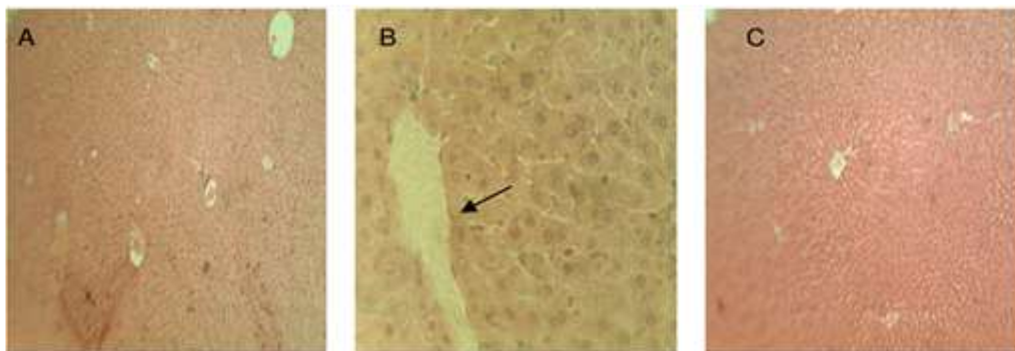

Figure 4.8: Photomicrographs of liver tissue of rat. All sections were stained with hematoxylin and eosin.

A: Section of control rat showing normal histological appearance of liver.

B: Section of rat treated with 1000mg/kg of aqueous extract of *P. biglobosa* showing fatty degeneration

(black arrow).

C: Section of rat treated with 500mg/kg of aqueous extract of *P. biglobosa* showing normal

architecture. (X40 magnifications).

Photomicrograph of the kidney tissues of rats after toxicological studies are presented in Figure 4.9.

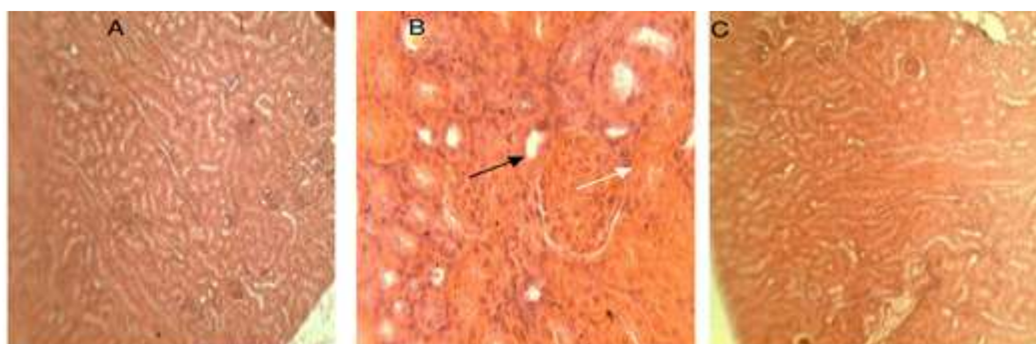

Figure 4.9: Photomicrographs of Kidney tissue of treated rat. All sections were stained with hematoxylin and eosin.

A: Section of control rat showing normal histological appearance of kidney.

B: Section of rat treated with 1000mg/kg of aqueous extract of *P. biglobosa* showing tubular degeneration

(black arrow) and dilation of glomerular capillaries (white arrow).

C: Section of rat treated with 500mg/kg of aqueous extract of *P. biglobosa* showing normal

architecture. (X40 magnifications).

4.1.15 Effects of aqueous extract of *Parkia biglobosa* on serum enzymes of rats in sub-acute toxicity study

Serum enzymes of rats treated with aqueous extract concentration of 200 to 500 mg/kgbw of *P. biglobosa* showed the same enzyme level compared with the control rats for aspartate amino trasferase, alanine amino transferase and total bilirubin. While alkaline phosphate level slightly decreased consistently as the concentration increases compared to the control. This is illustrated in Figure 4.10

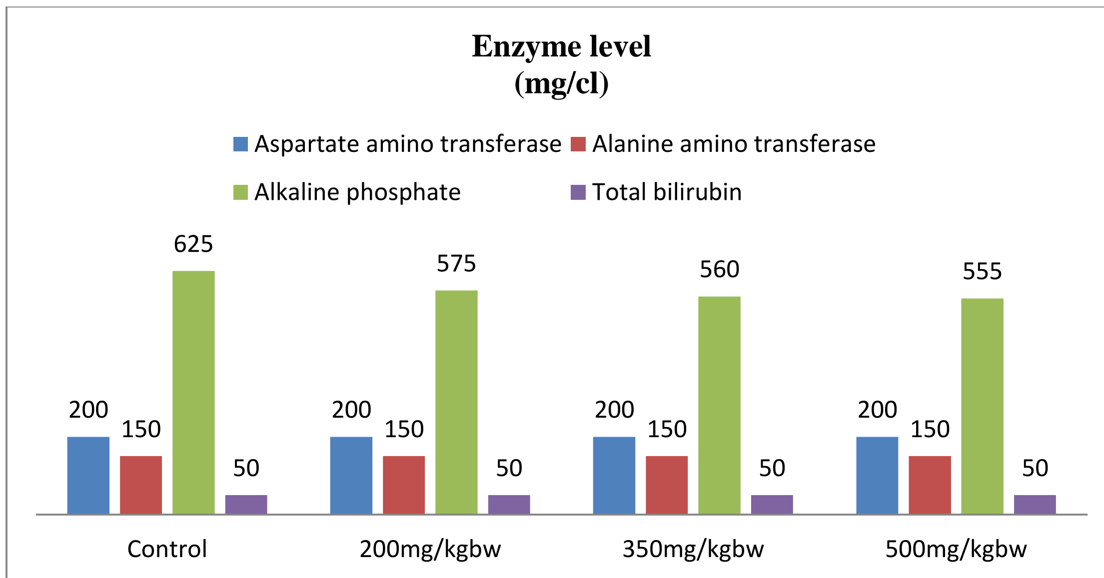

Figure 4.10: Effects of the extract of *P. biglobosa* on liver enzymes level

4.1.16 Effect of aqueous extract of *Parkia biglobosa* on plasma glucose of rats

The plasma glucose level of rats administered with aqueous extract of *P. biglobosa* slightly decreases with increasing concentration of 200 to 500 mg/kgbw as shown in Figure 4.11

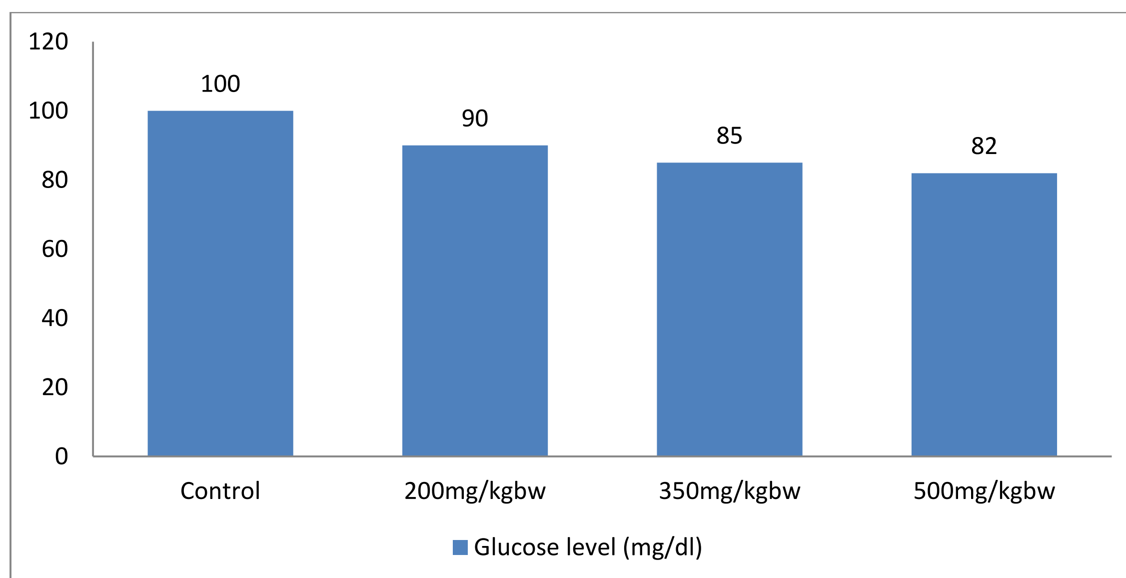

Figure 4.11: Effects of the extract of *P. biglobosa* on the plasma glucose in rats

4.1.17 Effect of aqueous extract of *Parkia biglobosa* on serum lipids of rats

The serum lipid level of rats administered with aqueous extract of *P. biglobosa* slightly decreases with increasing concentration from 200 to 500 mg/kgbw.

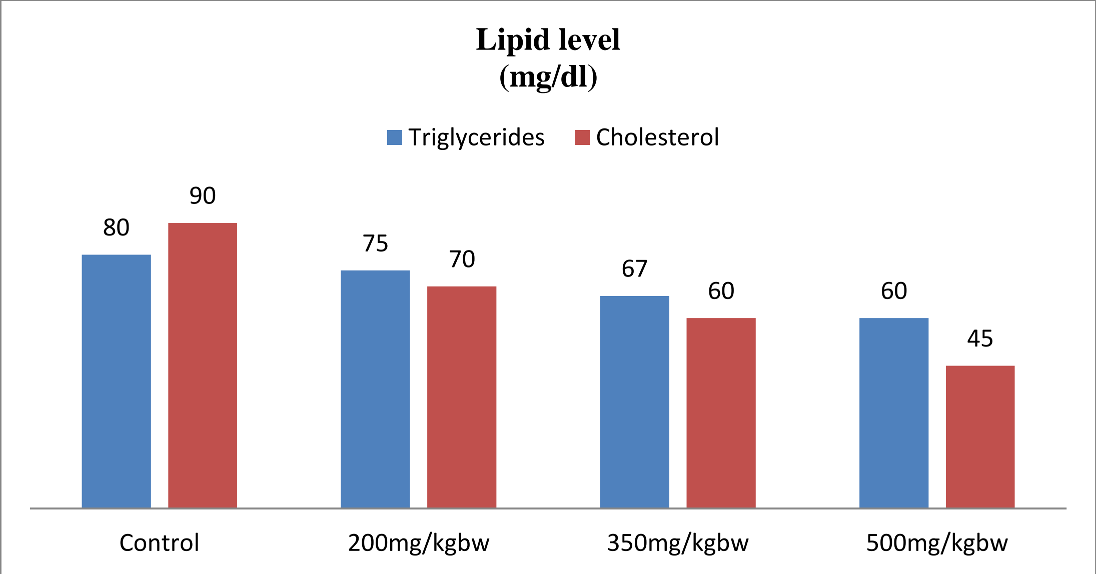

Figure 4.12: Effects of the extract of *P. biglobosa* on lipid serum profile

4.2 DISCUSSION

The plant kingdom represents an enormous reservoir of biologically active compounds with various chemical structures and protective/disease preventive properties (phytochemicals). These phytochemicals, often referred to as 'secondary metabolites' are present in smaller quantities in higher plants and they include; alkaloids, steroids, flavonoids, terpenes, tannins, and many others. The active principles of many drugs found in plants are the secondary metabolites (Yisa, 2019). Therefore, basic phytochemical investigation of these extracts for their major phyto-constituents is vital. More so, the determination of the antibacterial activity as well as the toxic effect of these extracts is important.

4.2.1 Phytochemical components of *Parkia biglobosa, Syzygium aromaticum and Nelsonia canescens.*

Syzygium aromaticum had only alkaloids, flavonoids and saponins, whereas *Nelsonia canescens* had alkaloids, tanins, saponins, reducing sugars and phenols. *Parkia biglobosa* leaf extract on the other hand had more secondary metabolites than *N. canescens* and *S. aromaticum.* It contains tannins, saponins, reducing sugar, phenol, steroids, flavonoids and terpenes except alkaloids. This was similar to the observations of Gaber *et al*. (2020).

After the quantitative phytochemical screening of the three plants, the secondary metabolites that were present in *S. aromaticum* and *N. canescens* were found to be small in quantity varying from 3.2 to 7.0 (%) while those in *P. biglobosa* were high ranging from 4.3 to 63.36 (%). The high amount of certain phytochemical components of *P. biglobosa* explains why it is widely used

traditionally as antimalaria, antidiarrhea, antifungal, antihelminthes, e.t.c. Particularly, flavonoids, saponins and phenols were found to be 62.51, 63.36 (%) and 41.8 (%) respectively in ethanolic extracts of *P. biglobosa* while it's aqueous extracts revealed the amount to be 48.5 (%), 55.6 (%) & 40.2 (%) respectively.

4.2.2 Antibacterial activities of the crude extracts

The antibacterial activities of the three (3) plants revealed that only *P. biglobosa* was effective against the test organisms (*S. aureuus and S. pyogenes*). The highest mean zones of inhibition exhibited by the ethanolic and aqueous leaf extracts of *P. biglobosa* against *S. pyogenes* were 22.5±1.3 mm and 24.0±1.0 mm at 120 mg/ml of the extracts. Those exhibited against *S. aureus* were 19.0±1.4 mm and 22.5±1.3 mm by ethanolic and aqueous leaf extracts of *P. biglobosa* leaf. These exceeded the standard zone of inhibition for ampiclox (15 mm) which was used as the control (Moklesur *et al*., 2014). This is akin to the study undertaken by Eze *et al*., (2016) that reported 21.0±2.1 mm and 23.0±1.0 mm zones of inhibition against both test organisms. The test organisms on the other hand were both resistant to the ethanolic and aqueous extracts of *S. aromaticum* and *N. canescens* at 60, 80, 100 and 120 mg/ml. This might be due to the very small quantity of flavonoids, saponins and phenols present in the two plants (*S. aromaticum* and *N. canescens*) as revealed by the quantitative phytochemical screening. The sensitivity of the test organisms to the extracts of *P. biglobosa* might be due to the high amount of flavonoids, saponins and phenols present in them as reported by Akintobi *et al*. (2016).

4.2.3 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The minimum inhibitory concentration (MIC) of the ethanolic and aqueous extracts of *P. biglobosa* was 120 mg/ml and 60 mg/ml respectively on *S. pyogenes* while the MIC of the ethanolic and aqueous extract of *P. biglobosa* on *S. aureus* was 60mg/ml on *S. aureus*. The Minimum Bactericidal Concentration (MBC) for both ethanolic and aqueous leaf extract of *P. biglobosa* on *S. aureus and S. pyogenes* was 120 mg/ml. This is similar to the report of Eze *et al.* (2016) who observed a MIC of 60 mg/ml and an MBC of 120 mg/ml against *S. aureus* and *S. pyogenes* under the same conditions. The MIC and MBC of ethanolic and aqueous leaf extract of *Nelsonia canescens* and *Syzygium aromaticum* could not be determined since the test organisms were resistant to them. This also concur to the findings of Eze *et al*. (2016) but contrary to the observation of Ayuba *et al*. (2023) that reported an MIC of 50mg/ml and an MBC of 100mg/ml on *S. aureus* and *S. pyogenes* respectively under the same conditions. This could be due to the impact of the difference in environmental conditions of the two locations of the plants from which they were obtained.

4.2.4 Antibacterial activity of the fractions of *Parkia biglobosa*

N-hexane fraction of the aqueous extract of *P. biglobosa* exhibited the highest zones of inhibitions (22 mm and 18 mm) on *S. pyogenes* and *S. aureus* respectively at 120 mg/ml followed by Ethylacetate and N-hexane fractions of the ethanolic leaf extracts of *P. biglobosa* which exhibited 15 mm and 18 mm zones of inhibition respectively against *S. aureus* at 120mg/ml. This could be as a result of the presence of certain biochemical compounds such as 1,2,3-Benzenetriol, phenol,2,4-bis(1,1-dimethylethyl) and others that were detected in the fractions of the leaf extract of *P. biglobosa* as revealed by the Gas Chromatography-Mass Spectrometry (GC-MS) analysis. A similar result was documented by Sangodare *et al*. (2017).

4.2.5 Gas chromatography-mass spectrometry (GC-MS)

The GC-MS revealed twenty-six (26) different chemical compounds. Some of these compounds are responsible for the antibacterial activity of the extract on the test organisms. Such compounds are 1,2,3-Benzenetriol with an intensity of 29,000,000 counts; phenol,2,4-bis(1,1-dimethylethyl) with an intensity of 3,300,000 counts, these were found in N-hexane fractions of *P. biglobosa* leaf extracts. The same compounds were implicated in N-hexane and Ethylacetate fraction of *P. biglobosa* ethanolic extract except Olean-12-ene-28-al with an intensity of 25,000,000 found in the aqueous fraction of the same extract (Sangodare *et al*., 2017).

4.2.6 Toxicity of crude extract on rats

The acute toxicity of *P. biglobosa* has been investigated to determine any adverse effect that may arise as a result of a short time and high dose animal exposure to the leaf aqueous extracts of *P. biglobosa* within 14 days. There was no death of any rat at oral treatment of 1000 mg/kgbw body weight of the extract, meaning the LD_{50} is greater than 1000 mgkgbw. This finding is in agreement with previous observations (Onibanjo *et al*., 2018; Onaolapo and Udobi, 2019). The results thus suggest that the extract of *P. biglobosa* has low toxicity (Builders *et al.,* 2019). The low toxicity obtained may have been responsible for its widespread use in different ethno therapeutic interventions.

Results showed that the weights of rats treated with various doses of the extract (200, 350, 500, 600, 800 and 1000 mg/kgbw) were significantly different from those of the control animals at $P<0.05$. There was an increase in body weight of rats as concentration of the extract administered increases in both acute and sub-acute toxicity study. This phenomenon is used to access the response of animal to drugs (Khetani *et al*., 2017).

Investigations revealed that there were no significant differences in the relative weights of the isolated organs of the rats treated with aqueous leaf extracts of *P. biglobosa* ($p<0.05$) in sub-acute toxicity study compared to the control. This suggests that aqueous extract of *P. biglobosa* at low concentration of 200, 350 and 500 mg/kgbw did not induce any toxic effect on any of the organs. The acute toxicity test however revealed that the weight of kidneys and livers of rats treated with 1000 mg/kgbw of aqueous leaf extract of *P. biglobosa* are significantly different from those of the control at $P<0.05$. This may be a sign of possible toxic effect of the aqueous leaf extract of *P. biglobosa* at 1000mg/kgbw (Khetani *et al*. 2017). Rats in sub-acute toxicity group showed no physical sign of toxic effect of the extracts administered to them, whereas rats in the acute toxicity group were weak and rubbed their mouth on the ground. This signs of toxicity in them were resolved 24 hours after each extract administration. Adebisi *et al*. (2021) observed a similar toxic effect of *P. biglobosa* extracts on albino wistar rats under same conditions.

4.2.7 Histopathology of rats

The histopathological examination of the kidneys of rats which received 1000 mg/kgbw of aqueous leaf extracts of *P. biglobosa* revealed tubular dilation of glomerular capillaries in the kidneys. while those which received 500mg/kgbw of the same extracts showd normal architecture. The glomerulus is the primary site of action of several chemicals and it may be injured by any toxic, metabolic and immunologic mechanism (Belmekki *et al*., 2021). The toxic irritant substances brought to the kidney by circulatory blood caused degenerative changes in the kidney (Varely, 2017).

There was fatty degeneration of the liver of rats in groups treated with 1000 mg/kgbw of aqueous extract and is in agreement with the research conducted by Adebisi *et al*. (2021), that observed same histopatological changes in the liver of rats treated with leaf of *P. biglobosa*. In the early phase of fatty degeneration, vacuoles appear in the cytoplasm around the nucleus, because their lipid content is dissolved in the course of embedding. The vacuoles appeared empty. As the damage to the cells progresses, the hepatocytes become swollen and a single large vacuole will occupy their entire cytoplasm, pushing aside the nucleus and making the hepatocyte signet ring shaped. The degenerated hepatocytes form wide trabeculae which compress and narrow the lumen of the sinusoids (Belmekki *et al*., 2021). The signs of toxicity observed in the livers and kidneys of rats treated with the aqueous leaf extract of *P. boglobosa* could be as a result of the presence of tannins in the extract. This observation tally with the findings of Aniagu *et al*. (2016)

4.2.8 Serum biochemistry of rats

Ordinarily, liver cell damage is characterized by a rise in plasma enzymes aspartate amino transferase (AST), alanine amino transferase (ALT), e.t.c. A rise in plasma alkaline phosphatase (ALP) level is usually a characteristic finding in cholestatic liver disease (Harvey, 2018). The reduction in ALP levels by the aqueous extract of *P. biglobosa* shows that no possible cholestasis occurred at the dose levels tested (200, 350 and 500 mg/kgbw). This is in consonance with the report of Orisakwe *et al*. (2016).

There was reduction in the glucose level of rats in the sub-acute toxicity group as the concentration of extracts administered increases. This suggest that aqueous extracts of *P. biglobosa* possess some hypoglycemic effects (Taga *et al*., 2018). A number of investigators

have shown that coumarin, flavonoid, terpenoid and a host of other secondary plant metabolites including arginine and glutamic acids posses' hypoglycemic effects in various experimental animal model (Nwanguma *et al*., 2019). Hypothesis stipulates that plants which contain terpenoid and/or flavonoids possess hypoglycemic activities in diabetic and normal mammal (Henry and Edward, 2016). The hypoglycemic activity of the extract could be due to the presence of terpenes in the aqueous extract. One or more of the other chemical constituents of the plant especially flavonoid is also likely to have played a crucial role in the hypoglycemic action of the plant extract (Dikko *et al*., 2019; Elita *et al*., 2021).

The rats treated with aqueous extract exhibited reduction in the triglycerides and cholesterol levels, which showed that aqueous extract of *P. biglobosa* possesses lipid lowering ability and also some beneficial effects regarding cardiovascular risk factors. This is in consonance with the observations of Lokesh and Sharma (2018). The lipid lowering effect of the aqueous extract may be as a result of abundant flavonoids in the extract. This has been shown to have numerous health benefit, one of which is lowering of tissue lipids (Louis *et al*., 2015; Jianjie *et al*., 2017; Lokesh and Sharma, 2018). Several researches conducted had indicated that many plant sterols reduce serum cholesterol absorption as well (Enwuru *et al*., 2017).

Therefore, the synergistic interaction of the polyphenol found in this extract may be responsible for its lipid lowering property (Asokkumar *et al*., 2016). The toxic effect of water extract of P. biglobosa on the kidneys and liver may be due to any one or more of the phytochemicals present in the extract. The phytochemical screening of the aqueous extract of *P. biglobosa* indicated the presence of appreciable amount of tannins. Study conducted by Aniagu *et al*. (2015) and Valencia *et al*. (2015) showed that a large intake of tannins may cause kidney and liver damage.

Conclusively, aqueous leaf extracts of *P. biglobosa* at concentration of 1000 mg/kgbw and above is not safe while its concentration of 200, 300, and 500 mg/kgbw could be safe for use as medicine.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study showed that only *Parkia biglobosa* of the three plants (*Nelsonia canescens, Syzygium aromaticum* and *Parkia biglobosa*) demonstrated antibacterial activities against the test organisms (*Staphylococcus aureus* and *Streptococcus pyogenes*). The minimum inhibitory concentration (MIC) of ethanolic and aqueous leaf extracts of *P. biglobosa* was 60 mg/ml on *S. aureus.* While the MIC of the ethanolic and aqueous leaf extracts of *P. biglobosa* on *S. pyogenes* were 120 mg/ml and 60 mg/ml respectively. The minimum bactericidal concentration (MBC) of the ethanolic and aqueous leaf extracts of *P. biglobosa on S. aureus* and *S. pyogenes* was 120 mg/ml each. The MIC and MBC of *Nelsonia canescens* and *Syzygium aromaticum* could not be determined because the test organisms were resistant to them.

Furthermore, *Parkia biglobosa* crude leaf extracts were not toxic to rats at a concentration of 200, 350 and 500 mg/kgbw. It however became toxic at a concentration of 1000 mg/kgbw and above (LD_{50} is > 1000 mg/kgbw). The antibacterial activity of *P. biglobosa* on the test organisms maybe due to the presence of certain biochemical compounds such as 1,2,3-Benzenetriol, Phenol 2,4-bis (1,1-dimethylethyl), Olean-12-ene-28-al and twenty-three others as revealed by the Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the fractions of the crude extracts of *P. biglobosa*. These biochemical compounds could be extracted and utilized to develop good, effective and safe drugs which will successfully combat the array of diseases caused by several antibiotic resistant *Staphylococcus aureus* and *Streptococcus pyogenes* strains.

5.2 Recommendations

1. Further research should be carried out on *Parkia biglobosa* using different solvents and different pathogenic bacteria in other to unveil other therapeutic potentials of the plant.
2. Other microbiological techniques should be employed to determine and ascertain the antimicrobial activities of *Nelsonia canescens* and *Syzygium aromaticum.*
3. Efforts should be made to determine which of the arrays of chemical compounds revealed by GC-MS are responsible for the antibacterial activities.

5.3 Contribution to Knowledge

The research revealed that the aqueous leaf extract of *Parkia biglobosa* had the highest antibacterial activity of 24.0±1.0 mm and 22.5±1.3 mm zones of inhibition on *Streptococcus pyogenes* and *Staphylococcus aureus* at 120mg/ml. *Nelsonia canescens* and *Syzygium aromaticum* on the other hand had no activity on the test organisms (that is *S. pyogenes* and *S. aureus*). The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal

Concentration (MBC) of *P. biglobosa* extracts on *S. aureus* and *S. pyogenes* were 60mg/ml and 120mg/ml respectively. The MIC and MBC of *S. aromaticum* and *N. canescens* could not be determined as the test organisms were resistant to them. The LD_{50} of oral administration of the aqueous extracts of *P. biglobosa* was found to be ≥ 1,000 mg/kgbw. The biochemical analysis of the serum of the rats in subacute toxicity groups revealed that aqueous leaf extracts of *P. bigobosa* has hypoglycemic effects and lipid lowering ability. The Gas Chromatography Mass Spectrometry (GC-MS) analysis of the fractions of *P. biglobosa* revealed the presence of 1, 2, 3-Benzenetriol, phenol, 2, 4-bis (1, 1-dimethyl), Olean-12-ene-28-al and 23 others. The study established that there are important bioactive compounds in the leaf extracts of *P. biglobosa* which could be very useful as lead molecules in future drug development against *staphylococcus aureus* and *streptococcus pyogenes.*

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APPENDIX

Appendix A

Pictures taken during fractionation

Appendix B

Pictures taken during toxicity test

Appendix C

Pictures taken during susceptibility test, MIC and MBC

Appendix D

Antibacterial activity *of Parkia biglobosa* on *Streptococcus pyogenes*

Plate i ii iii

Plate i: Antibacterial activity of aqueous leaf extracts on *S. pyogenes*

Plate ii: Antibacterial activity of ethanolic leaf extracts on *S. pyogenes*

Plate iii: Negative control for *S. pyogenes*

Appendix E

Antibacterial activity *of Nelsonia canescens* on *Streptococcus pyogenes*

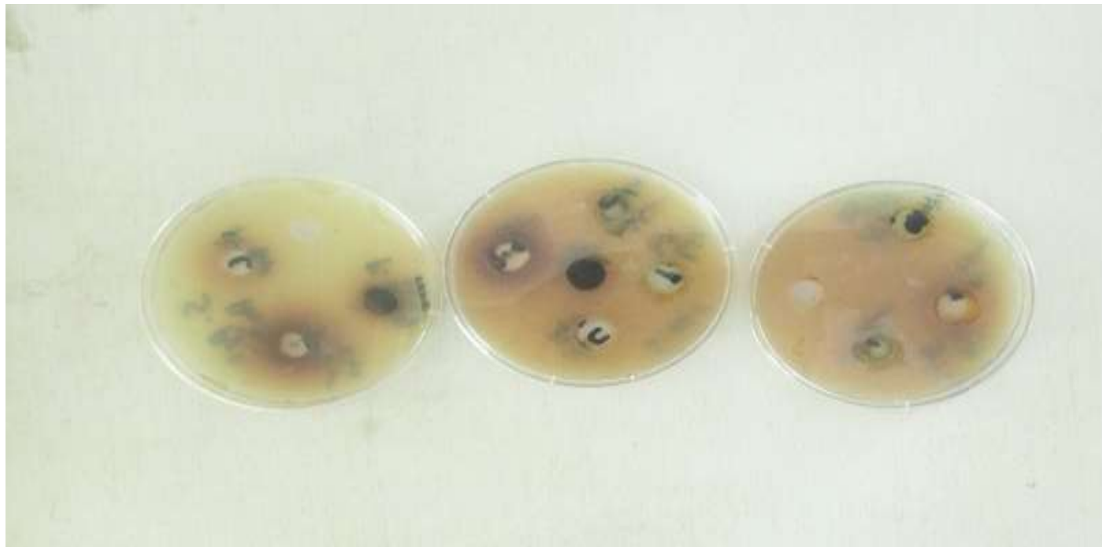


Plate iv v vi

Plate iv: Antibacterial activity of aqueous leaf extracts of *N. canescens* on *S. pyogenes*

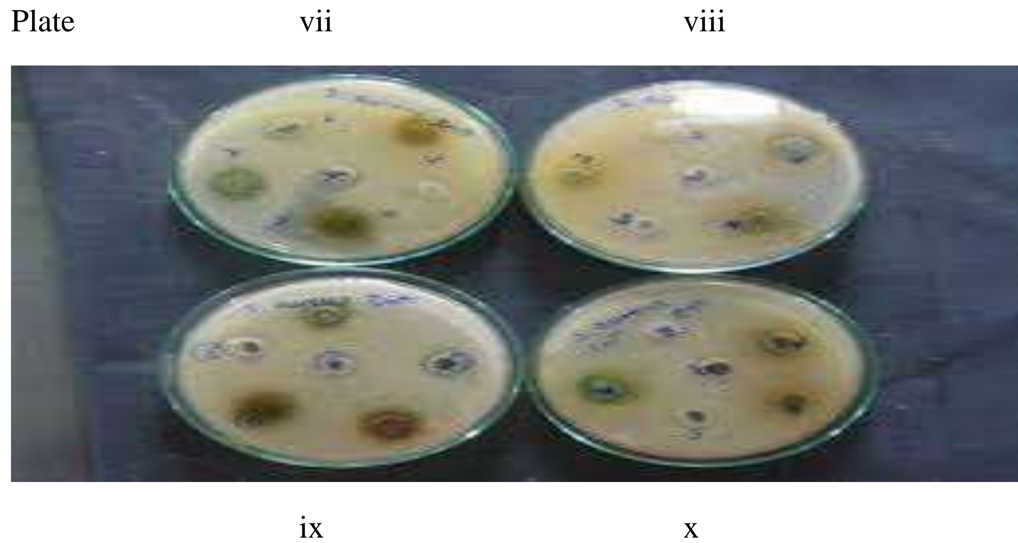
Plate v: Antibacterial activity of ethanolic leaf extracts of *N. canescens* on *S. pyogene*

Plate vi: Antibacterial activity of aqueous leaf extracts of *N. canescens* on *S. pyogenes*

Appendix F

Antibacterial activity *of Syzygium aromaticum* on *Streptococcus pyogenes*

Plate vii viii



ix x

Plates vii and ix: Antibacterial activity of ethanolic leaf extracts of *S. aromaticum* on *S. aureus.*

Plates viii and x: Antibacterial activity of aqueous leaf extracts of *S. aromaticum* on *S. aureus.*

Appendix G

Antibacterial activity *of Parkia biglobosa* on *Staphylococcus aureus*

Plate xi xii xiii

Plate xi: Antibacterial activity of ethanolic leaf extracts of *P. biglobosa* on *S. aureus.*

Plate xiii: Antibacterial activity of aqueous leaf extracts of *P. biglobosa* on *S. aureus.*

Plate xii: Duplicate of plate xiii.

Appendix H

Antibacterial activity *of Nelsonia canescens* on *Staphylococcus aureus*

Plates xiv xv xvi

Plate xiv: Antibacterial activity of aqueous leaf extracts of *N. canescens* on *S. aureu.*

Plate xvi: Antibacterial activity of ethanolic leaf extracts of *P. biglobosa* on *S. aureus.*

Plate xv: Duplicate of plate xiv.

Appendix I

Positive and negative control for *Staphylococcus aureus*

Plate xvii xviii

Plate xvii: Susceptibility of S. aureus to Ampiclox (positive control)

Plate xviii: Growth of S. aureus with no antibiotic (negative control)

Appendix J

Antibacterial activity of *Syzygium aromaticum* on *Staphylococcus aureus*

Plate xix xx xxi

Plate xix: Antibacterial activity of aqueous leaf extracts of *S. aromaticum* on *S. aureus.*

Plate xxi: Antibacterial activity of ethanolic leaf extracts of *S. aromaticum* on *S. aureus.*

Plate xx: Duplicate of plate xix.

Appendix K

Positive and negative control for *Streptococcus pyogenes*

Plate xxii xxiii

Plate xxii: Positive control for *S. pyogenes*

Plate xxiii: Negative control for *S. pyogenes*