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Strychnos spinosa as a potential anti-oxidants and anti-microbials natural product

Tsado Amos Ndarubu[1], Garba Rahinat[2], Abafi J Majiyebo[3], Ibrahim Ndayaman Julius[4], Abdulrazaq O Moshood[5], Ariyeloye S Damola[6], Berinyuy Bonghan Eustace[7]

[1] Department of Biological Sciences, Niger State Polytechnic Zungeru, P. M. B 001, Zungeru, Nigeria
[2, 5] Department of Biochemistry, Federal University of Technology, Minna, Niger State
[3] Department of Chemical Sciences, Biochemistry Unit, Federal Polytechnic P.M.B. 55, Bida, Niger State, Nigeria
[4] Department of Biological Sciences, Federal Polytechnic Bidda, Niger State Nigeria
[6] Department of Biochemistry, Federal University of Technology Akure, Ondo State Nigeria
[7] Faculty of Medicine and Biomedical Science, University of Yaounde 1, Yaounde, Cameroon

Abstract
Plants have been used for medicinal purpose for thousands of years. Phytochemical, antibacterial and antioxidant activities of methanol leaf extract of *Strychnos spinosa* was evaluated. Antibacterial activity of the extract was evaluated against *Micrococcus luteus, Streptococcus mutans, Streptococcus pneumoniae, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Streptococcus pyogenes*. Antioxidant activities was determined using DPPH free radical- scavenging assay. Quantitative phytochemical analysis of the extract revealed the presence of phenol, flavonoid, tannin, saponin and alkaloids at a concentration of 233.24±24.73, 183.46±2.35, 27.09±0.61, 559.40±31.40 and 15.17±0.32 mg/100g respectively. The extract shows dose dependent antimicrobial activities with diameter zone of inhibition range between 10.00±1.00 to 26.50±1.00 mm. The extract was more sensitive against *S. mutans, K. pneumonia, P. aeruginosa* and *S. pyogenes* but less active against *S. pneumonia*. MIC of the extract ranged between 1.25 to 5 mg/mL while the MBC ranged between 2.5 to 20 mg/mL. The extract shows dose dependent antioxidants activity with the highest radical inhibition of 43.47±2.50 % at 100 µg/mL compared to 90.12±1.61 % of the standard (Ascorbic acid). The results obtained support the use of *S. spinosa* in traditional medicine for the treatment of infectious diseases.

Keywords: *Strychnos spinosa,* anti-oxidants, anti-microbials, phytochemicals

Introduction

oxidative stress condition occurs with the steady increase of free radicals in cells that causes oxidize blood vessel walls, protein molecules, lipids, and DNA which can result in creating cancerous cells and different diseases (Hashemi *et al*., 2015; Lawal *et al*., 2015a) [10, 15]. Researchers have reported that these harmful effects can be reduced by regular consumption of fruits and vegetables which exhibit antioxidant activity (Farhoosh *et al*., 2016; Lawal *et al*., 2016) [3, 19]. These antioxidants can provide beneficial effects like antimutagenic, anticarcinogenic, and cardioprotective activities (Asnaashari *et al*., 2015) [3].

Current problems associated with the use of conventional drugs, increased prevalence of multiple-drug resistant (MDR) strains of a number of pathogenic bacteria such as methicillin resistant *Staphylococcus aureus, Helicobacter pylori*, and MDR *Klebsiela pneumonia* has revived the interest in plants with antimicrobial properties (Onukogu *et al*., 2019; Yusuf *et al*., 2018a) [28, 37]. Moreover, the use of plant extracts, with known antimicrobial and antioxidants properties, can be of great significance in therapeutic treatments (Tsado *et al*., 2016a; Ibrahim *et al*., 2017; Yusuf *et al*., 2018b) [12, 38].

Plants have been used for medicinal purpose for thousands of years (Bashir *et al*., 2015) [4]. Folk medicine both ancient and modern have been a source of useful chemotherapy. Nearly all cultures of the world, both ancient and the recent have heavily relied on plants as a therapeutic agent used in various forms (Mustapha *et al.*, 2012; Osuagwu and Eme, 2013; Lawal *et al*., 2015b) [23, 19, 20]. It has been reported that despite the popularity of orthodox drugs, herbal medicine in Africa and the rest of the world, continued to be practiced due to richness of certain plants in varieties of secondary metabolites such as alkaloids, flavonoids, tannins and terpenoids (Adekunle & Adekunle, 2009; Nneoma *et al.*, 2016).

Strychnos spinosa Lam. is deciduous shrub or small tree up to 10 m tall, with a trunk sometimes fluted, up to 25 cm in diameter [Neuwinger, 1996] [24]. The plant species is used in traditional medicine for treating several diseases (Neuwinger, 1996) [24]. Several secondary metabolites including flavonoids, sterols, triterpenoids, essential oils, secoiridoids, alkaloids, and monoterpenes have been isolated from *Strychnos spinosa* (Hoet *et al*., 2007; Itoh *et al*., 2005) [11]. Many pharmacological properties including antiplasmodial [Bero *et al*., 2009] [5], antioxidant (Nhukarume *et al*., 2010) [25], antitrypanosomal (Hoet *et al*., 2007) [11] and anthelmintic (Waterman *et al*., 2010) [36] activities have been reported from *S. spinosa*. However, the extracts of the stem bark of *S. spinosa* had no activity against

bacteria or fungi (Kubmarawa *et al*., 2007) [18]. Despite the fact that the leaves of the plant are used in folk medicine in the treatment of several infectious diseases, there is paucity of scientific evidence of the antimicrobial and antioxidant activities of its leaf extract. The present study therefore, evaluated the phytochemical, antioxidant and antimicrobial activity of methanol extract of *Strychnos spinosa* leaf

2. Materials and Methods

2.1 Sample collection and preparation

Fresh leaves of *Strychnos spinosa* were obtained from the biological garden of Federal University of Technology, Minna, Niger State and was authenticated at the department of Biological Science of Federal University of Technology, Minna. The leaves were washed and air dried at the Science laboratory of Niger State polytechnic, Zinger. The dried sample was blended with kitchen blender into fine powder and stored in a dried polyethene bag for future use.

2.2 Sample Extraction

One hundred grams (100 g) of the plant powder was extracted with absolute methanol using reflux method at a temperature of 45°C for 2 hours and the extract was filtered using muslin cloth followed by further filtration using whattman No 1 filter paper with pore size of 0.7 µm to obtain a fine filtrate. The filtrate was then concentrated using water bath at 45°C into fine paste and kept in the refrigerator for further analysis.

2.3 Quantitative determination of Phytochemicals

Quantitative estimation of phytochemicals including alkaloids and saponins was carried out according to Oloyede (2005), total phenolic content using Singleton *et al*. (1999) and flavonoids using Aluminum Chloride colorimetric method, described by Chang *et al*. (2005) [6].

2.4 Evaluation of Antimicrobial activity

Anti-microbial effect of the methanol extract of *Azanza garckeana* at varying concentrations (20–40 mg/mL) were evaluated against *Micrococcus luteus, Streptococcus mutans, Streptococcus pneumoniae, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Streptococcus pyogenes*. The bacterial isolates were obtained from Vaccine Laboratory of Centre for Genetic Engineering and Biotechnology, Federal university of Technology, Minna, Niger State. Antibacterial activity of the extract was carried out using agar-well diffusion method as described by Tsado *et al*., (2016b) [12]. The zones of inhibition were measured in millimeter. The above method was carried out in triplicates and the mean of the result was taken.

2.5 Determination of Minimum Inhibitory Concentration (MIC)

The tube dilution and spectrophotometric method as described by Kabir *et al.* (2005) [16] was used to determine the minimum inhibitory concentration. The MIC was determined by subtracting the absorbance of the negative control from the absorbance of the test and comparing the result with the absorbance of the positive control using the formula:

$$\text{Absorbance of Test (T)} - \text{Absorbance of control (Co)} = Absorbane\ of\ positive\ control\ (C1)$$

The concentration/test tube where significant reduction in absorbance was observed, was recorded as the MIC (Akinyemi *et al*., 2006) [2].

2.6 Determination of Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration (MBC) was determined by subculturing the cultures with the lowest optical density beginning with the test tube containing the minimum inhibitory concentration and above onto a freshly prepared nutrient agar medium. The cultures were incubated for 24 hours at 37^0C, after incubation, the culture concentration without visible growth was regarded as the minimum bactericidal concentration (Akinyemi *et al*., 2006) [2].

2.7 Determination of Free Radical Scavenging Activity

The free radical scavenging ability of the extracts against 1,1-diphenyl-2-Picrylhydrazyl (DPPH) radical was evaluated as described by Gyamfi *et al.* (1999) [9]. Briefly, an appropriate dilution of the extract (1 ml) was mixed with 1 ml of 0.4 mM methanolic solution containing DPPH radicals. The mixture was left in the dark for 30 min and the absorbance was measured at 517 nm. The DPPH free radical scavenging ability was subsequently calculated with respect to the reference (which contains all the reagents without the test sample) using the formula below:

$$\%\ Inhibition = \frac{ADPPH - AExtract}{ADPPH}\ X\ 100$$

Where ADPPH = absorbance of DPPH radical solution at 517nm and AExtract = absorbance of Extract at 517 nm.

2.8 Statistical Analysis

Data obtained in this study were analysed using the IBM Statistical Package for Social Science (SPSS) 20.0, 2011 version (SPSS Inc., Chicago, Illinois, USA) and Microsoft Excel 2013 Version. Numerical data were presented as mean ± standard error of mean (SEM) of the triplicate.

3. Results

3.1 Phytochemical composition of *Strychnos spinosa*

The extract contains high amount of saponins (559.40±31.40 mg/100g), phenol (233.24±24.73 mg/100g) and flavonoids (183.46±2.35 mg/100g) while tannins and alkaloids were found in traces at a concentration of 27.09±0.61 and 15.17±0.32 mg/100g respectively.

Table 1: Quantitative phytochemical composition of methanol extract of *Strychnos spinosa* leaf.

Phytochemicals	Amount (mg/100 g)
Phenols	233.24±24.73
Flavonoids	183.46±2.35
Tannins	27.09±0.61
Saponins	559.40±31.40
Alkaloids	15.17±0.32

Values are expressed in mean ± standard error of mean of triplicate determination

3.2 Antibacterial activity of methanol extract of *Strychnos spinosa* leaf

The antibacterial activity of methanol extract of *Strychnos spinosa* leaf extract shows dose dependent antimicrobial activities with diameter zone of inhibition range between 10.00±1.00 to 26.50±1.00 mm. The extract was more sensitive against *S. mutans*, *K. pneumonia*, *P. aeruginosa* and *S. pyogenes* but less active against *S. pneumonia*. The standard antibiotics Amoxicillin and Ampliclox had higher inhibitory activities against the isolates than the extract (Table 2). The MIC values ranges between 1.25 mg/mL in *S. mutans*, *K. pneumoniae* and *S. pyogenes* to 5 mg/mL in *S. pneumoniae* while the MBC values ranges between 2.5 mg/mL in *K. pneumoniae* to 20 mg/mL in *S. pneumoniae* (figure 1).

Table 2: Antibacterial activity of methanol extract of *Strychnos spinosa* leaf against bacteria isolates

Bacterial Isolates	20mg/mL	30mg/mL	40mg/mL	Amoxicilin 5mg/mL	Ampiclox 5mg/mL
M. luteus	13.50±0.50^{a}	17.50±1.50^{b}	18.50±0.50^{c}	26.50±0.50^{e}	22.50±0.50^{d}
P. aeruginosa	16.50±0.50^{b}	20.00±1.00^{c}	21.00±1.00^{c}	13.50±0.50^{a}	20.50±0.50^{c}
S. mutans	17.50±0.50^{a}	18.50±0.50^{a}	20.00±1.00^{b}	25.50±0.50^{c}	28.50±1.50^{c}
K. pneumonia	14.50±0.50^{a}	22.00±2.00^{c}	26.50±2.50^{d}	18.50±1.50^{b}	24.50±0.50^{c}
S. pneumonia	10.00±1.00^{a}	12.00±1.00^{b}	15.50±1.50^{c}	15.50±0.50^{c}	24.00±1.00^{d}
S. pyogenes	14.50±0.50^{a}	18.00±1.00^{b}	21.00±1.00^{c}	25.50±0.50^{d}	27.50±0.50^{d}

Values are expressed in mean ± standard error of mean, values with the same superscript on the same row have no significance difference (p>0.05), n=3

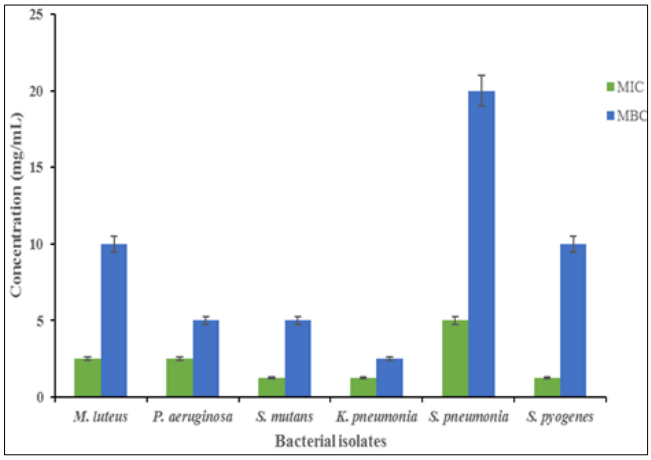

Fig 1: MIC and MBC of methanol extract of *Strychnos spinosa* leaf

3.3 Antioxidant activity of methanol extract of *Strychnos spinosa* leaf

DPPH scavenging activity of *S. spinosa* shows the highest inhibition of the extract at 100 µg/mL to be 43.47 % which is significantly different with the standard (Ascorbic acid) with an inhibition of 90.12% (Table 4.3).

Table 3: DPPH Scavenging activity of methanol extract of *Strychnos spinosa* leaf

Concentration (µg/mL)	% Inhibition	Ascorbic acid
20	21.55±0.67	48.57±1.50
40	33.95±0.71	72.39±1.67
60	36.73±1.21	89.69±1.40
80	41.36±1.33	90.07±1.00
100	43.47±2.50	90.12±1.61

Values are expressed in mean ± standard error of mean of duplicate determination.

4. Discussion

The potency of medicinal plant against any target disease majorly depends on the presence and amount of the phytochemicals contained in the plant (Lawal *et al*., 2005) [21]. Quantitative phytochemical analysis of *Strychnos spinosa* methanol leaf extract revealed the presence of saponins (559.40±31.40 mg/100g), phenol (233.24±24.73 mg/100g) and flavonoids (183.46±2.35 mg/100g), tannins (27.09±0.61 mg/100g) and alkaloids (15.17±0.32 mg/100g) (Table 1). Alkaloids and flavonoids have been reported for several pharmacological activities including; antioxidants, antimicrobial, anti-inflammatory and analgesic activities (Jigam *et al*., 2017; Umar *et al*., 2019a; Umar *et al*., 2019b; Adesina *et al.*, 2013) [15, 19, 1].

Mechanism of action of tannins involve the conversion of protein to water soluble compounds which result in the inactivation of bacterial as a result of damage on their cell membrane (Elmarie *et al.*, 2001) [7]. Saponins have been used by plants as antimicrobial, to protect against insect attack and have been included in a large group of protective molecules found in plants named phytoanticipins or phytoprotectants (Mamta *et al.*, 2013) [22].

The strength of the antimicrobial substance in agar diffusion methods is determined by measuring the diameter in comparison with a standard. The present study revealed a linear relationship between the inhibition zone and the active ingredient concentration in plant extracts (Table 2). Also, the inhibition area depends on the ability of the antibacterial compounds to diffuse uniformly through the agar. This phenomenon was noted in many reports (Rauha *et al*., 2000) [30]. Furthermore, the results of the disk and well diffusion assays for the extract against the organism were relatively similar, and *K. pneumonia* as Gram- negative bacteria indicated the largest zone of growth inhibition (26.50±2.50 mm) compared with others. Antimicrobial activity of plant extracts is said to be significant if the MIC ≤ 0.1 mg/ml moderate if 0.1 < MIC ≤ 0.625 mg/ml and weak if MIC > 0.625 mg/ml (Isa *et al.*, 2014). Based on this classification, the extract of *Strychnos spinosa* maybe said to have moderate antibacterial activity. The activity of the extract maybe attributed to the presence of the phytochemicals present in the extract at varying concentrations. (Oladunni *et al.*, 2017) [26].

Antioxidant activities of the extracts was determined using free radical- scavenging DPPH activity (Table 3). In all the concentration, the antioxidant activity was much lower than that of the positive controls (Ascorbic acid). However, the antioxidant activity of the methanol fruits extract of *S. spinosa* was reported

and the free-radical depletion was attributed not only to phenolic contents but also to the presence of traces of vitamin C in the extract (Nhukarume *et al.,* 2010) [25]. The antioxidant activity activity of the extract maybe attributed the phenolic and flavonoid content present. Many studies have shown a correlation between the total phenol contents of plants and their antioxidant abilities (Karou *et al.,* 2005) [17].

5. Conclusion

Strychnos spinosa demonstrated significant antioxidants and antimicrobial activities, the results obtained therefore supported the use of *S. spinosa* in traditional medicine for the treatment of infectious diseases.

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