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Phytochemical Screening, Antioxidant, Antimicrobial and Toxicity Activities of Polar and Non-Polar Extracts of *Albizia zygia* (DC) Stem-Bark

Ganiyat K. Oloyede[1*] and Adeniyi O. Ogunlade[1]

[1]Department of Chemistry, Natural products/Medicinal Chemistry Unit, University of Ibadan, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author GKO designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author AOO managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: To determine the secondary plant metabolites present in *Albizia zygia* (DC) stem-bark and investigate its antioxidant, antimicrobial and toxicity activities.
Study Design: Methanol (polar) and hexane (non-polar) were used to extract the dried stem bark of *A. zygia* and biological activities were carried out.
Place and Duration of Study: Natural products/Medicinal Chemistry Unit, Department of Chemistry, University of Ibadan, Nigeria, between July, 2012 and February, 2013.
Methodology: Chemical methods were used for phytochemical screening. Antioxidant screening was determined by scavenging effect on 2,2-diphenyl-1-picryl hydrazyl (DPPH) and hydroxyl radical generated from hydrogen peroxide methods, agar well diffusion method was used for antimicrobial screening while brine shrimp lethality test was used to determine the toxicity of the extracts.
Results: Alkaloid, saponin, glycoside, steroid, resin and reducing sugar were found in both the methanol and hexane extracts while flavonoid and cardio active glycoside were observed only in the hexane extract. The methanol extract had the highest % inhibition at 1.0 mg/ml (93.28%) in the DPPH method and was better than that of ascorbic acid and α-

**Corresponding author: Email: oloyedegk@gmail.com;*

tocopherol. But at 1.0 mg/ml, the n-hexane extract with % inhibition of 78.76% showed better antioxidant activity than butylatedhydroxylanisole (BHA) (40.12%) and α-tocopherol (21.69 %). The antimicrobial activities of the stem extracts of *A. zygia* against *Escherichia coli*, *Staphylococcus aereus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiellae pneumonae, Salmonellae typhi, Candida albicans, Rhizopus stolon, Aspergillus niger* and *Penicillum notatum* showed that the methanol extract was more active than n-hexane extract at 50 mg/ml. Brine shrimp lethality test showed that the methanol extract with LC_{50} of 1.70 µg/ml was more toxic than the non-polar n-hexane extract with LC_{50} of 174.19 µg/ml.

Conclusion: This study underscores the importance of application of *A. zygia* in ethno medicine and extracts from *A. zygia* could be used as antioxidant and antimicrobial agents.

Keywords: Phytochemicals; antioxidant; antimicrobial; toxicity; Leguminosea; Albizia zygia.

1. INTRODUCTION

Natural products obtained from plants; alkaloids, flavonoids, saponins, anthraquinones, tannins, glycosides, cardiac glycoside, phenolic acids, steroids which are secondary metabolites and their derivatives are used for the discovery and production of drugs. Simple standard chemical test have been devised to detect their presence in a plant extract. Also bioassay or biological standardization is conducted to measure the effect of these metabolites on a living organism using an intermediate *in vitro* tissue or cell model under controlled conditions. The common bioassays are antimicrobial and antioxidant analysis. Thus, certain plants are classified as medicinal plants because of their established pharmacological activity and they contain substances that can be used for therapeutic purposes or used as precursors for the synthesis of drugs [1-7].

Many plant families have been reported to have ethno medicinal application. The Leguminosae is one of such and forms the third largest plant family of the world, comprising about 650 genera and 1800 species. The sub-family Mimosoideae, with 3000 species are distributed worldwide and are abundant in tropical, sub-tropical and warm temperate areas. In tropical Africa, *Acacia, Albizia, Entada* and *Newtonia* are the richest in number of species [8-9]. *Albizia zygia* (DC) of the family Leguminosae is a shade-tree and timber of economic importance because of its durability and immunity to termites. The bark has a variety of medicinal applications in Africa traditional medicine; as an anti-cough, aphrodisiac and also to cure fever, expel intestinal worms and to counter female sterility. The macerate is given in draught for stomach complaints. The roots are toxic and purgative, administration as an enema in overdose can cause death by intestinal haemorrhage. The young leaf is eaten as a vegetable in soup. The foliage is readily browsed by goats and sheep and is given as an alternative fodder to cattle during the dry season [10-12]. Not much research activity has been carried out on this plant. The aims of this research work are to determine the secondary plant metabolites present in *Albizia zygia* (DC) stem-bark extracts and to investigate the antioxidant, antimicrobial and toxicity activities.

2. MATERIALS AND METHODS

2.1 Plant Collection and Identification

Albizia zygia (DC) stem-bark was collected in July, 2012 at the Botanical Garden, University of Ibadan. The identification and authentication of the freshly collected plant was done by Dr. Ayodele at Botany and Microbiology Department, University of Ibadan, Nigeria.

2.1.1 Chemicals and reagents

The following chemicals and reagents were used; hexane, ethyl acetate, methanol, ethanol, chloroform, Fehling's solution A and B, 5% Ferric chloride, concentrated tetraoxosulphate VI acid, hydrochloric acid, glacial acetic acid, ammonia solution, Dragendroff's reagent, sodium potassium tartarate, linoleic acid, ammonium thiocynate, glacial acetic acid, disodium hydrogen phosphate, dihydrogen potassium phosphate, copper acetate, Molisch's reagent, dimethyl sulphoxide (DMSO), hydrogen peroxide, copper acetate and NaOH solution. Solvents were obtained from BDH chemicals Ltd and redistilled before use. Brine shrimp larvae eggs were obtained from Ocean Star International, Inc. Company, USA. 2,2-diphenyl-1-picryl hydrazyl (DPPH), ascorbic acid, butylatedhydroxylanisole (BHA) and α-tocopherol were obtained from Sigma Chemical Co (St Louis, MO).

2.1.2 Equipment and apparatus

Mettler analytical balance H80 (UK), Steam bath (Gallenkamp), Soxhlet apparatus, Rotavapor RII0 (Buchi, England), electric pump, aspirator bottle, separating funnel, silica gel GF_{254} (precoated aluminium sheets - Merck Germany), pH meter (Jenway model), UV-Visible Beam PC scanning spectrophotometer UVD-2960 and Infrared spectrophotometer (Nicolet avatar 330 Fourier transform (FT) spectrophotometer).

2.1.3 Test organisms

Escherichia coli, *Staphylococcus aereus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiellae pneumonae, Salmonellae typhi, Candida albicans, Rhizopus stolon,, Aspergillus niger* and *Penicillum notatum* (Microorganisms were collected from the stock of the Department of Pharmaceutical Microbiology, Faculty of Pharmacy of University of Ibadan). The test organisms were maintained on nutrient agar slopes and kept in a refrigerator at 4ºC. 100 ml aliquots of nutrient broth were inoculated with the culture of test micro-organisms using a loop and then incubated at 37ºC for 24 hrs.

2.1.4 Reference standards

Standard drugs used for antimicrobial screening: gentamicin (10 mg/ml) for bacteria and tioconazole (70%) for fungi. Ascorbic acid, butylated hydroxyanisole (BHA) and α-tocopherol were used as reference standards for antioxidant activity. DMSO was used for toxicity studies.

2.1.5 Sample preparation

Fresh samples of *A. zygia* stem-bark were collected, weighed and air-dried for 5 weeks until a constant weight was achieved and then pulverized using a Mill machine at the Wood

extraction laboratory, Department of Chemistry, University of Ibadan. The pulverized samples were weighed and kept for further analysis.

2.1.6 Extraction procedure

The dried ground stem-bark of *A. zygia* (2.0 kg) was extracted with hexane (5 Lt) using a modified Soxhlet apparatus. The extract was collected and concentrated by distillation to give yellowish colored oil. Similarly, the same procedure was carried out using methanol. Thereafter, antimicrobial screening by agar well diffusion method and free radical scavenging activity test was carried out on the extracts using the following spectrophotometric experiments; scavenging effect on DPPH and scavenging effect on hydroxyl radical generated from hydrogen peroxide. Toxicity of the extracts was determined using Brine shrimp lethality assay.

2.1.7 Phytochemical screening

The crude extracts of *A. zygia* obtained above were used to test for the presence of secondary plant metabolites, alkaloid, flavonoid, steroid, saponin, phenolic acids, tannin, glycoside, reducing sugar, anthraquinone, carbohydrate, resin and cardiac glycoside.

2.1.8 Antimicrobial screening

The samples used for this study were: n-hexane extract and methanol extract of the stem bark of *A. zygia*. 0.25 g of each sample was weighed and dissolved in 5 ml of the solvent used for extraction to give 50 mg/ml. Five (5) other test tubes contained 2.5 ml of the same solvent. From the first tube that contained 50 mg/ml, 2.5 ml of the content was drawn and added into the second test tube to give 25 mg/ml and this was done serially from the first test tube to the sixth until a concentration of 1.5625 mg/ml was achieved in the sixth test tube. The seventh test tube was used to prepare negative control, which contained the solvent of dissolution only, n-hexane and methanol respectively. The eighth test tube served as the positive control and contained gentamicin for bacteria and tioconazole for fungi.

2.1.9 Agar diffusion: Pour plate for bacteria

The pour plate method was used for antibacterial screening. An overnight culture of each organism was prepared and 0.1 ml of each of the organism was taken into 9.9 ml of sterile distilled water (SDW) to give 10 ml of 1:100 (10^2) dilution. 0.2 ml was then taken into the prepared molten Nutrient Agar (NA) at 45^{o}C and this was aseptically poured into the sterile plates and allowed to set on the bench for 45 minutes. A sterile cork-borer was used to create wells (or holes) inside the set plates. Different prepared concentrations of the sample as well as the positive and negative controls were introduced using syringes into the wells. The positive control for bacteria was gentamicin at 5 mg/ml. These were allowed to stay on the bench for two hours before incubation at 37^{o}C for 24 hours. Clear zones of inhibition were observed. The diameter of the zones of inhibition was measured in millimetre (mm) using a transparent well-calibrated ruler. Average readings were calculated from triplicates analysis [13-14].

2.1.10 Agar diffusion: surface plate for fungi

Molten sterile Sabouraud Dextrose Agar (SDA) was poured aseptically into the sterile plates and allowed to cool for 45 minutes. 0.2 ml of 1: 100 dilution of the organism was spread on

the surface using a sterile spreader. Then, a sterile cork-borer was used to create wells inside the plates. The same procedure described for anti-bacterial activity above was followed from this stage. Meanwhile, positive control for the fungi was 70% tioconazole. All the plates for the fungi were incubated at 28^{o}C for 48 hours unlike that of bacteria that was incubated at 37 ^{0}C for 24 hours. The clear zones of inhibition were observed and recorded using the same method as described in the case of bacteria [14-15].

2.1.11 Antioxidant activities of extracts from *A. zygia* stem bark

2.1.11.1 Scavenging effect on 2, 2- diphenyl-1-picryl hydrazyl radical (DPPH)

Albizia zygia was screened for free radical scavenging activity using the "stable" free radical 2, 2- diphenyl-1-picryl hydrazyl radical (DPPH). DPPH (3.94 mg) was dissolved in 100 ml methanol to give a 100 µM solution. Methanol solution of DPPH (3.0 ml) was added to 0.5 ml of the methanol extract taken from the stock solution. The stock solution was prepared by dissolving 3.0 mg of the crude methanol extract into 3 ml of methanol. This was shaken well and left to stand for 10 minutes, after which the decrease in absorption at 517 nm of DPPH was measured. The actual decrease in absorption induced by the test compound was calculated by subtracting that of the control. Other concentrations of methanol extracts (0.5 mg/ml, 0.25 mg/ml, 0.125 mg/ml and 0.0625 mg/ml) were prepared from the stock solution by serial dilution and analyzed the same way. All tests and analysis were carried out in triplicates and the results obtained were averaged. N-hexane extract was analyzed same way. Butylated hydroxyl anisole (BHA), vitamin C and α-tocopherol were used as antioxidant standards. These are drugs that have proven antioxidant efficacy. The radical scavenging activity (RSA) was calculated as the percentage inhibition of DPPH discoloration using the equation below.

$$\% \text{ RSA or } \% \text{ inhibition} = (A_{DPPH} - A_s)/ A_{DPPH}\} \text{ X } 100$$

Where A_S is the absorbance of the solution when the sample extract has been added at a particular concentration to the DPPH, and A_{DPPH} is the absorbance of DPPH solution [16-18].

2.1.11.2 Scavenging effect on hydrogen peroxide

Spectrophotometric determination of n-hexane and methanol extracts of *A. zygia* stem bark to scavenge hydroxyl radical was carried out at 285 nm by the method of Oloyede *et al*., (2011) [19]. A solution of 2 mM hydrogen peroxide was prepared in phosphate-buffered saline (PBS) at pH of 7.4. Each of the extracts at the following concentration (1.0 mg/ml, 0.5 mg/ml, 0.25 mg/ml, 0.125 mg/ml and 0.0625 mg/ml) was added to the hydrogen peroxide solution. Decrease in absorbance of hydrogen peroxide (H_2O_2) at 285 nm was determined 10 minutes later against a blank solution containing the test drug in PBS without hydrogen peroxide. All tests were run in triplicate.

2.1.12 Brine shrimp toxicity test

2.1.12.1 Sample preparation and experimental procedure

The toxicity level of the extracts was conducted using the method of Falope et al. [20] and Oloyede et al. [21]. Brine shrimp eggs obtained from Artemia Incorporated, USA were hatched in a shallow rectangular plastic container. About 70 mg of shrimp eggs were sprinkled into the container which contained 250 ml of sea water. The plastic container was

placed beside a window for ray of light to penetrate. After 48 hours, the brine shrimp larvae were collected by a dropping pipette from the lightened side, having been separated by the divider from the shell. The stock solution was prepared by dissolving 20 mg of extract in 0.2 ml of analar grade dimethyl sulphoxide, $(CH_3)_2SO$, DMSO). 1.8 ml of distilled water and DMSO was added to give a concentration of 1000 ppm from where 100 ppm and 10 ppm were prepared by serial dilution. Dissolved extract (0.2 ml) was removed from the stock solution (1000 ppm) using a graduated syringe and made up to 2 ml by addition of 1.8 ml of distilled water to prepare 100 ppm concentration. The 10 ppm was also prepared from 100 ppm using the same procedure. 0.5ml each of 1000 ppm, 100 ppm and 10 ppm was transferred into three different test tubes respectively and each was made into triplicate to make experiment A, B and C. Sea water (4 ml) was added to each of the test tube. After the addition of sea water, 10 larvae of the brine shrimp was introduced into each of the test tubes with the use of a dropping pipette and the number of larvae that survived after 24 hours was taken. The control experiment was prepared by mixing 0.2 ml of DMSO, 4 ml of sea water and 10 larvae and observed for 24 hours after which the number of the larvae that survived was noted. The LC_{50} of each of the extracts was calculated using Finney Computer Programme.

3. RESULTS AND DISCUSSION

Phytochemical screening revealed the presence of reducing sugar, steroid, flavonoid, cardiac glycoside, glycosides and resin in the hexane extract while alkaloids, saponins, tannins and phenol were beyond detectable limit. Alkaloids, saponins, reducing sugar, steroid, glycosides and resin were found in the methanol extract while tannins, flavonoid, cardiac glycoside and phenol were absent.

3.1 Toxicity Results of *A. zygia* Stem Extracts

The two extracts (hexane and methanol) were toxic to brine shrimp larvae at varied degree with LC_{50} value of 174.19 µg/ml and 1.70 µg/ml respectively (Table 1). This result supports the fact that medicinally active compounds are present in *A. zygia* and that toxic chemical compounds are beneficial in the therapy of some ailments involving cell or tumour growth but their usage at high dose should be properly monitored.

Table 1. Brine shrimp lethality test of *A. zygia* stem extracts*

Conc.	1000ppm		100ppm		10ppm		control		
Sample	**Survivor**	**Dead**	**Survivor**	**Dead**	**Survivor**	**Dead**	**survivor**	**Dead**	**LC_{50} µm/ml**
HAZ	2	28	24	6	27	3	10	0	174.1910
MAZ	0	30	0	30	0	30	10	0	1.7014412

LC_{50} < 1000 µg/ml = Toxic, LC_{50} > 1000 µg/ml = Not Toxic HAZ – Hexane extract of A. zygia MAZ – Methanol extract of A. zygia

3.2 Antioxidant Analysis

3.2.1 Scavenging effect on DPPH

Hexane and methanol extracts of *A. zygia* stem bark decreased absorption values at 517 nm indicating that the fractions had hydrogen donating ability or can scavenge free radical. The activity was significant when compared to the standards used; butylatedhydroxylanisole

(BHA), ascorbic acid and α –tocopherol (Table 2). DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [22]. The extracts gave %inhibition of 90 - 98% at 1.0 – 0.0625 mg/ml (Fig. 1). The activity of the methanol extract was however better than that of all the standards.

Table 2. Absorbance of hexane and methanol extracts of *A. zygia* (HAZ and MAZ) at 517 nm*

CONC (mg/ml)	HAZ	MAZ	Ascorbic Acid	BHA	α - tocopherol
1.0	0.097±0.002	0.063±0.006	0.0843±0.010	0.0370±0.006	0.6800±0.029
0.5	0.093±0.001	0.083±0.004	0.2893±0.128	0.0460±0.006	0.7040±0.003
0.25	0.101±0.001	0.065±0.006	0.2977±0.124	0.0483±0.002	0.7047±0.007
0.125	0.108±0.006	0.072±0.001	0.3200±0.082	0.0490±0.004	0.7070±0.007
0.0625	0.098±0.002	0.085±0.001	0.5147±0.015	0.0650±0.003	0.7207±0.012

**HAZ – Hexane extract of A. zygia, MAZ – Methanol extract of A. zygia*

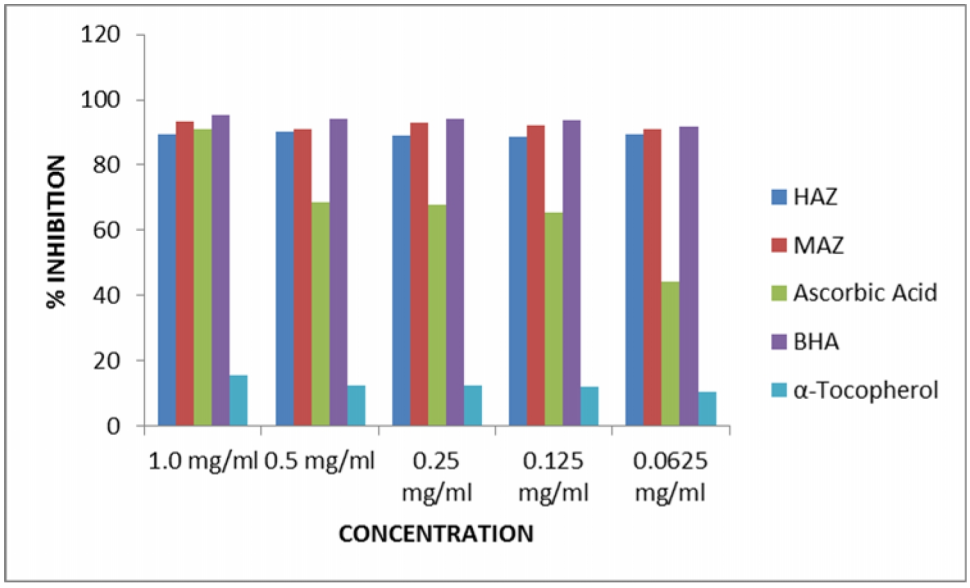

Fig. 1. DPPH free radical scavenging activity of hexane (HAZ) and methanol (MAZ) extracts of *A. zygia* stem-bark

3.2.2 Scavenging effect on H_2O_2

Absorbance measurement of scavenging effects on H_2O_2 by hexane and methanol extracts of *A. zygia* stem bark at 285 nm in triplicates after 10 min of incubation at 285 nm is presented in Table 3. H_2O_2 through the Fenton reaction is an active - oxygen specie and has the potential to produce the highly reactive hydroxyl radical which is often involved in free radical chain reactions. The extracts scavenged hydroxyl radical in a concentration dependent manner, indicating their ability to reduce or stop free radical chain reactions.

Table 3. Absorbance of hexane and methanol extracts of *A. zygia* stem-bark at 285 nm*

CONC (mg/ml)	HAZ	MAZ	Ascorbic Acid	BHA	Alpha Tocopherol
1.0	0.914±0.001	0.594±0.009	0.689±0.002	2.257±0.026	2.951±0.041
0.5	0.647±0.003	0.312±0.007	0.356±0.003	1.975±0.003	2.874±0.064
0.25	0.277±0.209	0.120±0.004	0.138±0.001	1.770±0.017	2.251±0.022
0.125	0.063±0.002	0.066±0.006	0.191±0.001	1.731±0.008	1.781±0.002
0.0625	0.021±0.015	0.011±0.004	0.113±0.002	1.699±0.030	0.935±0.002

**HAZ – Hexane extract of A. zygia, MAZ – Methanol extract of A. zygia*

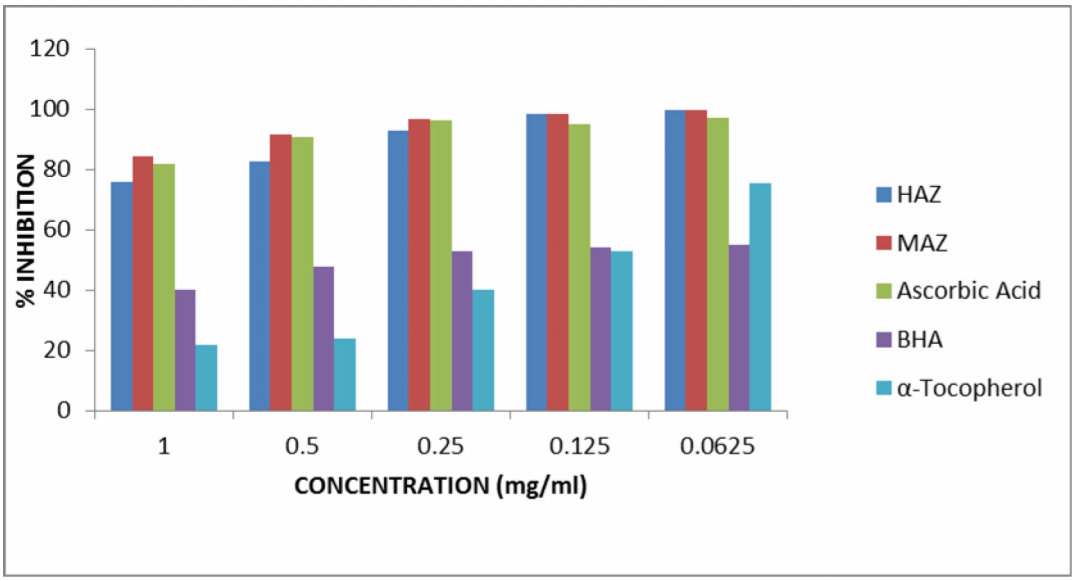

Fig. 2. H_2O_2 free radical scavenging activity of hexane and methanol extracts (HAZ and MAZ) of *A. zygia* stem-bark

3.3 Antimicrobial Activity of Hexane and Methanol Extracts of *A. zygia*

The zones of inhibition measured during the antimicrobial test by Agar well diffusion method are presented in Tables 4 and 5. It was observed that all the tested samples possessed broad spectrum antimicrobial activities on both gram positive and gram negative bacteria and the fungi used.

Table 4. Antimicrobial activity of Hexane extract of *A. zygia*

	Zones of inhibition (mm)									
Conc (mg/ml)	***S.a***	***E. c`oli***	***B.sub***	***Ps.a***	***S. typhi***	***Kleb***	***C.a***	***A.n***	***Rhiz***	***Pen***
1	19	14	17	14	13	18	20	18	14	14
2	15	10	14	12	10	14	18	16	12	12
3	13	-	10	10	-	10	14	12	10	10
4	10	-	-	-	-	-	12	10	-	-
5	-	-	-	-	-	-	10	-	-	-
6	-	-	-	-	-	-	-	-	-	-
-ve	-	-	-	-	-	-	-	-	-	-
+ve	38	36	38	38	36	38	28	28	26	28

Integer 1-6 represents n-hexane extract of the bark of Albizia zygia at various concentrations (mg/ml) viz: (1) 50 (2) 25, (3) 12.5, (4) 6.25, (5) 3.125, (6) 1.5625, –ve = negative control (DMSO), +ve = positive control : [Gentamicin at 10 µg/ml for bacteria or Tioconazole (70%) for fungi], - = no inhibition, S.a- Staphylococcus aureus, E.coli- Escherichia coli, B.sub- Bacillus subtilis, Ps.a- Pseudomonas aeruginosa, Kleb – Klebsiellae pneumonae, S.typhi – Salmonellae typhi, C.a – Candida albicanas, A.n – Aspergillus niger, Rhiz – Rhizopus stolon, Pen - Penicillus notatum.

Table 5. Antimicrobial activity of Methanol extract of *A. zygia*

	Zones of inhibition (mm)									
Conc (mg/ml)	***S.a***	***E. coli***	***B. subo***	***Ps.a***	***S. typhi***	***Kleb***	***C.a***	***A.n***	***Rhiz***	***Pen***
1	15	14	19	30	14	19	20	18	14	18
2	13	12	14	24	12	14	18	16	12	14
3	10	10	12	20	10	10	16	13	10	12
4	-	-	10	18	-	-	12	10	-	10
5	-	-	-	14	-	-	10	-	-	-
6	-	-	-	10	-	-	-	-	-	-
-ve	-	-	-	-	-	-	-	-	-	-
+ve	38	36	38	38	36	38	28	28	26	28

Integer 1-6 represents methanol extract of the bark of Albizia zygia at various concentrations (mg/ml) viz: (1) 50 (2) 25, (3) 12.5, (4) 6.25, (5) 3.125, (6) 1.5625, –ve = negative control (DMSO), +ve = positive control : [Gentamicin at 10 µg/ml for bacteria or Tioconazole (70%) for fungi], - = no inhibition, S.a- Staphylococcus aureus, E.coli- Escherichia coli, B.sub- Bacillus subtilis, Ps.a- Pseudomonas aeruginosa, Kleb – Klebsiellae pneumonae, S.typhi – Salmonellae typhi, C.a – Candida albicanas, A.n – Aspergillus niger, Rhiz – Rhizopus stolon, Pen - Penicillus notatum

3.4 Discussion

The result of the phytochemical screening of *A. zygia* stem-bark revealed the presence of alkaloids, saponins, flavonoids, cardio active glycosides, glycosides, steroids, resins and reducing sugars in the hexane extract while phenols, tannins and anthraquinones were absent. Alkaloids, saponins, reducing sugar, steroid, glycosides and resin were found in the methanol extract while tannins, flavonoid, cardiac glycoside and phenol were absent. Cardiac glycosides detected in the extract have been useful in the treatment of asthma. The presence of these secondary metabolites in *Albizia species* confirms their antibiotic properties and usefulness by the traditional medicine practitioners. Flavonoids are also known to have a wide array of therapeutic activities as antihypertensive, anti-rheumatism, antimicrobial, diuretic and antioxidants [11,23].

Brine shrimp lethality test showed that the methanol extract with LC_{50} of 1.7014412 µg/ml was more toxic than the non-polar n-hexane extract with LC_{50} of 174.1910 µg/ml. Also, the antioxidant screening methods used namely DPPH free radical scavenging effect and scavenging effect on hydrogen peroxide showed that the methanol extract had the highest % inhibition at 1.0 mg/ml (93.28%). This was better than that of ascorbic acid and α-tocopherol even at the lowest concentration but lower than that of BHA. The n-hexane extract on the other hand though lower in activity than the methanol extract, showed better activity than ascorbic acid (68.7%) and α-tocopherol (12.4%) at 0.5 mg/ml. The H_2O_2 free radical scavenging method showed that the methanol extract had better antioxidant activity than the n-hexane extract. However, at 1.0 mg/ml, the n-hexane extract with % inhibition of 78.76% showed better antioxidant activity than BHA (98.31%) and α-tocopherol (21.69 %) but ascorbic acid (81.71 %) showed better activity than n-haxane extract (Figs. 1 and 2). At 0.125 and 0.0625 mg/ml with % inhibition of 98.32 and 99.44, n-hexane extract showed better antioxidant activity than ascorbic acid (94.93% and 96.99%), BHA (54.06% and 54.93%) and α-tocopherol (52.74% and 75.19%).

The antimicrobial activities of the stem extracts of *A. zygia* against 10 microorganisms (6 bacteria and 4 fungi) by agar well diffusion method showed that methanol extract was more active than n-hexane extract at 50 mg/ml, the inhibition capacities of the extracts were considerably close to the inhibition ability of the positive controls. The n-hexane extract (Table 4) had pronounced activity on *Staphylococcus aureus* and *Candida albicans* with diameter of inhibition of 19 mm and 20 mm at 50 mg/ml respectively. Moderate activity was observed for the other entire organism when compared with standards. On the other hand, the methanol extract of *A. zygia* (Table 5) was more active on *Pseudomonas aeruginosa*, *Candida albicans* and *Bacillus subtilis* with diameters of inhibition of 30 mm, 20 mm and 19 mm at 50 mg/ml respectively while the other microbes were moderately inhibited by the extract.

4. CONCLUSION

Alkaloids, saponins, reducing sugar, steroid, glycosides, cardiac glycosides, flavonoid and resin were the secondary metabolites present in stem extracts of *A. zygia*. The polar methanol extract was more toxic than the non-polar hexane extract. This supported the observed antimicrobial activity. The methanol extract and n-hexane extracts was more active than ascorbic acid and α-tocopherol in the antioxidant screening tests. This study has shown that the extracts of *A. zygia* have *in-vitro* antioxidant and antimicrobial activities and has also given information which could lead to further research in the area of isolation and characterization of active chemical compounds present in the plant.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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