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PHYTOCHEMICAL SCREENING, ANTIBACTERIAL AND TOXICOLOGICAL ACTIVITIES OF *ACACIA NILOTICA* EXTRACTS

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ABSTRACT
The phytochemical screening, antibacterial and toxicological activities of extracts of the leaves, stem bark and roots of Acacia nilotica were investigated. The phytochemical analyses according to standard screening tests using conventional protocols revealed the presence of tannins and sterols in the leaves stem barks and roots of the plant. Alkaloids were detected only in the leaves. Glycosides, saponins, resins and flavonoids were not detected in the plant. In-vitro agar-diffusion sensitivity tests of crude extract fractions of the plant extracts using ethanol, chloroform, methanol, petroleum ether, water and ethyl acetate were investigated on nine bacterial isolates. The extract fractions generally exhibited marked antibacterial activities on Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus vulgaris, Salmonella typhi, Shigella dysenteriae Staphylococcus aureus and Escherichia coli except on Streptococcus pneumoniae and Streptococcus pyogenes. All the leaves extract fractions of the plant exhibited weak or no antibacterial activity on the bacterial isolates tested but the stem bark and root extracts generally exhibited strong antibacterial activities on them. The minimum inhibitory concentration and minimum bactericidal concentration studies revealed that some bacterial isolates were inhibited at concentrations of about 12.5mg/ml and 50mg/ml and killed at concentrations of about 100mg/ml and 400mg/ml. Toxicity studies of the ethanol extracts revealed that they exhibited no significant toxicity (LD_{50} of 123.86µg/ml and312.55µg/ml) against Artemia salina . These results suggest that the plant may not be toxic to man and could be a potential source of novel antibacterial compound.
Keywords: Phytochemical Screening, Antibacterial Activity, Toxicological Activity Acacia nilotica, Extracts

INTRODUCTION

Acacia nilotica (bagaruwa in Hausa) has been designated and used as medicinal plants in parts of Northern Nigeria, West Africa, North Africa and other parts of the world. The plant is used to treat infections such as diarrhea, dysentery, leprosy, cancers, ulcer, and diabetes (Aliyu, 2006; http//:en.wikipedia.org/wiki.*acacia-nilotica,* 2008). Antimicrobial drug resistance is not only on the increase, it is also a serious problem to the medical profession. Moreover, the toxicity of some medicinal plants has been severally reported. For instance, *Bryophyllum calycinum* whole plant, *Annona senegalensis* root, *Hymenocardia acida* stem bark, *Erythrophleum suaveollens* leaves and *Spondiatus preussii* extracts were toxic to brine shrimps and caused chromosomal damage in rats (Sowemimo *et al;* 2007). The vast number of chemicals used industrially and pharmacologically provides an ever increasing hazard to the liver. These chemicals are thought to be responsible for one of the most common type of liver diseases, such as chemical hepatitis (Sule*,* 2006).

Brine shrimps have been used as a benchtop bioassay for the discovery and purification of bioactive natural products and they are excellent choice for elementary toxicity investigations of consumer products (Lieberman, 2008). Thus, this research is focused on the study of phytochemical screening, antibacterial activity and toxicity of the plant extracts.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

The plant materials were collected from Ungogo Local Government Area of Kano State**.** The plants were however identified at the Botany Unit of the Department of Biological Sciences by Prof. B. S. Aliyu and with the aid of botanical keys (Arber, 1972). The parts of the plants mentioned above were collected fresh, healthy and free from organic contaminants that may interfere with the substances of interest by washing them with clean water (Onoruvwe *et al.*, 1998).

Extraction and Fractionation of Plant Materials

The specimens were dried at room temperature (30^0C), and kept away from sunlight to prevent changes in the nature of the plants' constituents. The specimens were grounded to powder (fine texture) with mortar and pistol. One hundred grammes of powdered specimens were separately percolated in one liter of 96% alcohol for seven days followed by filtration.

The extracts were concentrated using a laboratory rotary vacuum evaporator at a temperature of 40^0C. The crude extracts were weighed labeled and stored in a refrigerator at a temperature of 4^0C. A fraction of each extract was partitioned between water and chloroform mixture (300:300). This was shaken for about one hour and allowed to settle for 24 hours in a separating funnel.

The water, chloroform and interface fractions were separated in glass beakers and labeled respectively. These fractions were again concentrated using a rotary vacuum evaporator, weighed, labeled and stored in a refrigerator at 4^0C respectively. Similarly, a fraction of each of the chloroform soluble extract was partitioned in a mixture of absolute methanol and petroleum ether (300:300). Again, the methanol and petroleum ether fractions were concentrated using rotary evaporator, weighed, labeled and stored as above. Finally, each of the water soluble fractions were partitioned between water and ethyl acetate (300:300). The water and ethyl acetate fractions were concentrated using a rotary vacuum evaporator, weighed, labeled and stored as above (Fatope *et al;* 1993 and Adoum *et al;* 1997).

Qualitative Phytochemical Analysis of Plant Extracts

The phytochemical analyses were carried out using standard screening tests and conventional protocols for the presence of alkaloids (Sofowora, 1993), tannins (Trease *et al.,* 1989), glycosides (Ciulei, 1994), saponins (Turner and Brain, 1975), sterol (Sofowora, 1993) and resins (Sofowora, 1993).

Extraction of Alkaloids for Quantitative Analysis

Five, (5g) of the powdered leaf extract was extracted with 50ml methanol. From the extract, 10ml was placed in 250ml separating funnel and 5ml of dilute sulphuric acid and distilled water was added. The extract was shaken twice with 10ml chloroform. The combined chloroform-extract was transferred to a second separating funnel containing 5ml of dilute sulphuric acid and 10ml of distilled water. The chloroform layer was discarded after shaking and the aqueous acidic layer was transferred to the content of the first separating funnel. The extract was basified with ammonia solution and was shaken for 30 seconds.

The alkaloids were completely extracted by successive portions of chloroform. The combined chloroform extract was shaken with 5ml of water and was run through a plug of cotton wool previously moistened with chloroform. The content was covered with a little anhydrous sodium sulphate, which was washed in 5ml of chloroform. The filtrate was then placed into 25ml conical flask after which the chloroform was distilled completely followed by the addition of 5ml of neutral alcohol, which was evaporated on a boiling water bath. The residue was further heated on a water bath for 15 minutes. The residue was dissolved in 2ml of chloroform and 20ml 0.02N sulphuric acid. The content was warmed to remove chloroform. The excess acid was titrated with 0.02N sodium hydroxide using methyl red as indicator, a colour change from pink to yellow was observed. The available content of the sample was then calculated using the formula

Alkaloid content = (mls taken of 0.02N NaOH x 0.00578/10) _= g% w/v_(El- Olemy *et al*., 1994).

Quantitative Analysis of Tannins Using Iodometric Method

From water extract of each specimen 5ml was placed into a stoppered conical flask followed by 25ml of 0 .1N iodine and 10ml of 4% NaOH. The resulting mixture was kept in the dark for 15minutes. Ten (10) ml of water was used to dilute the mixture and acidified with 10ml 4% sulphuric acid. The mixture was titrated with 0.1N sodium thiosulphate solution and starch solution was used as indicator. Titration value corresponds to the sum of tannins and pseudo tannins concentration A. Another 25ml of each water extract was placed in a stoppered conical flask followed by 15ml of gelatin. This volume was made up to 100ml with water and filtered. Aliquot of 20ml was placed in a volumetric flask, 25ml of 0.1N iodine and 10ml of 4% NaOH were added mixed and kept in the dark for 15 minutes. The mixture was diluted with 10ml of water and acidified with 10ml of 4% sulphuric acid. This was finally treated with 0.1N sodium thiosulphate using starch as indicator. The titration value that was obtained corresponds only to the pseudo tannins concentration B. The tannins and pseudo tannins content of each sample was then calculated using the formula below:

A = (Blank - Exp. A) x 0.029 x 100g % / 5 (volume taken).

B = (Blank - Exp. B) x 0.029 x 100g% / 5 (volume taken).

Where A = % of tannins and pseudo tannins, B = % of pseudo tannins only

Therefore % of true tannins = A - B g% w/v. (El-Olemy *et al.,* 1994).

Preparation Sensitivity Discs

Preparation of sensitivity discs were done in the laboratory. Whatman's No 1 filter paper were used. These were obtained by punching the filter paper with a paper punch (6mm diameter). The disc were sterilized by autoclaving at 121^0c for 15 minutes and impregnated with the prepared extracts. The impregnated discs were stored in a refrigerator for future use. Various test solutions were prepared in accordance with the dilution method used by Baker *et al.* (1993). Stock solutions of each fraction were prepared by dissolving 100mg of the extract in 10ml of dimethyl sulphur oxide (DMSO). Each stock solution thus has a concentration of 100,000µg/ml. A 1ml concentrations of 1,000µg/ml, 5,000µg/ml, 10,000µg/ml and 50,000µg/ml of each extract was prepared, which was used to impregnate 100 filter paper discs. The disc potency would therefore be 10, 50, 100 and 500µg/disc. Another 1ml of 1: 1 ratio combined forms from the above concentrations of the individual extract were used to impregnate other 100 filter paper discs (Baker *et al.,* 1993 and Mukhtar and Okafor; 2002).

Collection and Identification of Test Organisms

The organisms tested with various extracts for antibacterial activity were pure clinical isolates of *Escherichia coli, Staphylococcus aureus Streptococcus pneumoniae, Klebsiella pneumoniae, Shigella dysenteriae, Salmonella typhi, Streptococcus pyogenes. Pseudomonas aeroginosa* and *Proteus vulgaris.* The bacterial isolates were obtained from the Aminu Kano Teaching Hospital, Kano. They were subsequently transported to the laboratory in nutrient agar (NA) slant culture medial bottles. Confirmatory tests were carried on each of the isolates.

Bioassay

Nutrient agar was used to subculture *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Shigella dysenteriae, Salmonella typh, Pseudomonas aeruginosa* and *Proteus vulgaris,* while blood agar was used to subculture *Streptococcus pneumoniae* and *Streptococcus pyogenes* for 18 – 24 hours. Sensitivity tests were done using diffusion method (Baker *et al*., 1993 and Mukhtar and Tukur 2000). The organisms were inoculated by streaking method in which the surface of nutrient agar and blood agar plates was streaked with sterile swabs containing each of the standard inoculum. The filter paper discs impregnated with the above concentrations of extracts was then placed on the surface of the inoculated nutrient agar and blood agar plates with the aid of sterilized pair of forceps. Discs impregnated with DMSO only were placed at the centre of some plates to serve as negative controls while disc impregnated with perfloxacin and recophin were placed at the centre of some plates to serve as positive controls. A pre – diffusion time of 30 seconds was allowed for the extracts to diffuse from the discs into the agar medium before incubation. The plates were inverted and incubated at 37^0C for 24 hours. The degree of sensitivity of the organisms to the extracts was determined by measuring diameter of visible zones of inhibition to the nearest millimeter with respect to each isolate and extract concentration. The following keys were adopted: 0mm zone of inhibition – indicates no effects.Less than 8mm zone of inhibition – indicates low sensitivity.More than 8mm zone of inhibition – indicates high sensitivity (Mukhtar and Okafor, 2002).

Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration, (MIC) of the extracts was determined using the tube dilution method (Baker *et al.,* 1993). Dilution of the plants extracts was incorporated in nutrient broth in 1: 1 ratio Initial rough estimates of the MIC values of the plant extracts against the test organisms were estimated to determine the range of MIC values. Consequently, the following concentrations were prepared for each extract, using the dilution formula: 400, 200, 100, 50, 25, 12.5, 6.25mg/ml. In addition, 0.1ml of standard suspension of the test organisms was added to each tube. The tubes were incubated at 37^0c for 24 hours. A tube containing extract and growth medium without inoculum would be included to serve as control. The presence of growth (turbid solution) or absence of growth (clear solution) at the end of incubation period was recorded. The lowest concentration of the extract showing no growth was regarded as the minimal inhibitory concentration (MIC).

Determination of Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration, (MBC) was determined by sub culturing the last test dilution that showed visible growth (turbidity) and all others in which there was no growth on a fresh extract solid medium and incubated for further 24 hours. The highest dilution that shows no single bacterial colony was taken as the minimum bactericidal concentration (MBC) as reported by (Baker *et al*., 1993).

Brine Shrimp Lethality Bioassay

Eggs of *Artema salina* (about 50mg) were placed into a hatching chamber containing sea water (instant sea water can be prepared by dissolving 2.86g of NaCl in 75ml distilled water) and kept under a fluorescent bulb for 24hours for the eggs to hatch into shrimp larvae. In addition, 20mg of each plant fraction was weighed into sterile vials, and dissolved in 2ml absolute methanol. 500, 50 and 5µl of each these solutions was transferred into empty vials corresponding to 1000, 100 and 10µg/ml concentrations respectively. Each of these dosages for each fraction was prepared in triplicate. The vial used for the control experiment was stained with 1ml methanol. All vials containing the dosages and the control was left overnight for the methanol to vaporize, leaving only the plant extract as residue. Methanol is a poison to the shrimp larva.

To each of the vials containing the plant fraction-residue (9-vials per fraction), 2 drops of dimethyl sulphoxide (DMSO) were added to re-dissolve the dosages followed by 4ml of sea- water. Ten (10) larvae of *Artema salina* were introduced into each of the test vials using Pasteur's pipette. The volume of each vial was adjusted to 5ml with sea-water. Two drops of DMSO followed by 4ml of sea-water was added to the control vial, and 10 larvae of *Artema salina* were introduced. The volume was adjusted to 5ml with sea- water. Twenty- four hours after the inoculation, the number of surviving shrimp larvae at each dosage was counted and recorded. LC_{50} values were determined with 95% confidence intervals by analyzing the data on Kintech AT-compatible computer loaded with Finney program **(**Guerrero and Robledo, 1993; Meyer *et al.,* 1982).

RESULTS AND DISCUSSION

Tables 1 and 2 show the physical properties of *Acacia nilotica* extract fractions recovered from leaf, stem bark and root of both plants. The solvents used include ethanol, chloroform, methanol, petroleum ether, water and ethyl acetates. Twenty- one extracts were obtained from the partition method of extraction used namely ethanol, chloroform, chloroform/water interface, methanol, petroleum ether, water and ethyl acetates. All the leaf extracts were gummy in texture and dark green in colour. The other extracts were either gummy or granular in texture, dark brown or brown in colour. Most of the extracts were however granular in texture.

Table 3 shows the qualitative phytochemical screening of ethanolic extracts of *A. nilotica,* namely the leaf, stem bark and root. The results showed that glycosides saponins, resins and flavonoids were not detected in the plant. Alkaloids were detected only in the leaves of the plant. Sterols and tannins were detected in the leaves, stem bark and root of the plant. This is similar to report of Banso (2009), that ethanolic extract of *Acacia nilotica* stem contain active principles e.g. terpenoids, tannins, alkaloids, etc. Table 4 shows the percentage weight of tannins in water extracts of leaf, stem bark and root of *Acacia nilotica* were 2.64%, 6.09% and 5.26% respectively. While, the percentage weight of alkaloids in ethanol leaf extract was 7.8%. Alkaloids were not detected in the stem bark and root extracts, and were therefore not determined quantitatively. Tables 5 – 7 show the results of antibacterial activities of various leaf, stem bark and root extract fractions of *A. nilotica.* Recophin and perfloxacin were used as positive controls for the sensitivity tests based on their levels of antibacterial activities on the bacterial isolates tested. While filter paper discs soaked in the dimethyl sulphur oxide (DMSO) were used as negative control against the bacterial isolates.

Table 1: Weights of *Acacia nilotica* Extract Fractions Recovered

Plant Parts	Ethanol Extract			Chloroform Extract			Chloroform/ Water Interface Extract			Petroleum Ether Extract			Methanol Extract			Water Extract			Ethyl Acetate Extract		
	WR			WR			WR			WR			WR			WR			WR		
	Initial (g)	Final (g)	%	Initial (g)	Final (g)	%	Initial (g)	Final (g)	%	Initial (g)	Final (g)	%	Initial (g)	Final (g)	%	Initial (g)	Final (g)	%	Initial (g)	Final (g)	%
LF	100.00	34	34.00	25.00	8.10	32.40	25.00	1.50	6.00	6.00	1.30	21.70	6.00	2.30	38.33	12.40	6.00	48.39	12.40	4.50	**36.29**
SB	100.00	30.5	30.5	25.00	9.50	38.00	25.00	3.40	13.60	9.00	4.50	50.00	9.00	4.30	47.78	10.80	3.20	29.63	10.80	4.00	**37.03**
RO	100.00	27.8	27.8	22.00	7.00	31.81	22.00	5.00	22.72	7.00	3.00	42.90	7.00	2.40	34.29	8.60	3.80	44.19	8.60	2.40	**27.91**

Key: LF = leaf, SB = stem bark, RO = root

Table 2: Texture and Colour of *Acacia nilotica* Extract Fractions

Plant Parts	Ethanol Extract		Chloroform Extract		Chloroform/ Water Interface Extract		Petroleum Ether Extract		Methanol Extract		Water Extract		Ethyl Acetate Extract	
	Texture	Colour	Texture	Colour	Texture	Colour	Texture	Colour	Texture	Colour	Texture	Colour	Texture	Colour
Leaf	Gummy	Dark green	Gummy	Dark Green	Gummy	Dark Green	Gummy	Dark Green	Gummy	Dark Green	Gummy	Dark Green	Gummy	Dark Green
Stem Bark	Granular	Dark Brown	Gummy	Dark Brown	Granular	Dark Brown	Granular	Dark Brown	Granular	Dark Brown	Granular	Brown	Granular	Brown
Root	Granular	Dark Brown	Granular	Dark Brown	Granular	Dark Brown	Granular	Dark Brown	Granular	Dark Brown	Granular	Brown	Granular	Brown

Table 3: Qualitative Determination of Phytochemicals Present in the Plant

Phytochemicals	***Acacia nilotica***		
	Leaf	Stem Bark	Root
Glycosides	-	-	-
Alkaloids	+	-	-
Saponins	-	-	-
Flavonoids	-	-	-
Sterols	+	+	+
Resins	-	-	-
Tannins	+	+	+

Key: + Phytochemicals detected, - Phytochemicals not detected

Table 4: Tannins and Alkaloids in the Leaf, Stem Bark and Root Extracts *Acacia nilotica*

Plant Extracts	**Percentage of Weight Tannins/5ml of Extract**	**Percentage Weight of Alkaloids/Five Grammes of Extract**
Leaf	2.64	7.8
Stem Bark	6.09	_
Root	5.26	_

Perfloxacin was used on *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeroginosa, Proteus vulgaris* and *Salmonella typhi.* Recophin was used against *Escherischia coli, Streptococcus pneumoniae, Streptococcus pyogenes* and *Shigella dysenteriae*.

Ethanol and chloroform/water extracts of *Acacia nilotica* stem bark appear to have the highest antibacterial activities on the bacterial isolates tested, followed by methanol and ethyl acetate extracts, in that order. While all the isolates tested were generally sensitive to the various extract fractions, *S. pneumoniae* and *S. pyogenes* were resistant to them at the concentrations used. Chloroform and petroleum ether extracts however, exhibited no antibacterial activeities on any of the isolates at the concentrations used. Similarly, ethanol and chloroform/water interface extracts appears to have the highest antibacterial activities on the bacterial isolates tested at the concentrations used. This is in line with the report of Abeer and Sanaa (2007), that ethanolic bark extract of *A. nilotica* exhibited higher antibacterial activities than chloroform extract on some bacterial isolates. Moreover, this may be due to the ability of ethanol to extract a wide range of chemical constituents of the plant while the chloroform might have extracted less number of the ingredients (Abeer and Sanaa, 2007). Furthermore, ethanol extract was the first solvent used for extraction of the plant constituents before portions of the extracts were partitioned between other solvents in this present study. There is also evidence that some of the extract fractions that showed no antibacterial activity or weak antibacterial activity will show profound antibacterial activities at higher concentrations from the pilot studies conducted.

Banso (2009) reported that ethanol extract exhibited antimicrobial activity against *Streptococcus viridans, Staphylococcus aureus, Escherichia coli, Bacillus subtilis* and *Shigella sonnei.* Abeer and Sanaa (2007); reported that *A. nilotica* ethanol and chloroform fruit extracts showed varying degrees of activity against Gram- negative bacteria (*Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae* and *Pseudomonas aeruginosa)* and Gram-positive bacteria (*Staphylococcus aureus).* Olaleye (2007) reported that methanol extracts of alkaloids and saponins from *Hibiscus sabdariffa* had some pharmacologic actions on bacterial isolates like *E. coli, K. pneumoniae, S. aureus etc.* Philips (2010) reported that tannins and alkaloids are natural products that have medicinal properties. He also said that some remedial values of tannins include application on burn to heal injury and cuts to stop bleeding. Moreover, it stop infections on the skin surface, internally tannins continue to heal the wound. In the case of third degree burns using strong tannins sources will not only prevent septicemia, but also helps to save life. Alkaloids often have pharmacological effects and are used as medications. Examples are cocaine, caffeine, nicotine, morphine and quinine (Philips, 2010). Therefore, antibacterial activity showed in this present work may be due to tannins and alkaloids. The results of the study also revealed that the extracts of the stem bark and extracts of the root of the plant should be preferred for the treatment of bacterial infections as the stem bark and root extracts had better antibacterial activity on the organisms tested.

Table 5: Antibacterial Activity of Stem Bark Extracts of *Acacia nilotica*

Bacterial Isolates	Diameter of Zones of Inhibition (mm)/ Extracts Concentration (µg/disc)																											
	Ethanol				Methanol				Chloroform				Petroleum Ether				Chloroform Water Interface				Water				Ethyl Acetate				Positive Control (µg)	Negative Control (DMSO)
	10	50	100	500	10	50	100	500	10	50	100	500	10	50	100	500	10	50	100	500	10	50	100	500	10	50	100	500		
Staphylococcus aureus	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	30 (PER)	0
Escherichia coli	0	0	8	8	0	0	0	0	0	0	0	10	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	8	20 (REC)	0
Klebsiella pneumoniae	0	0	8	10	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	30 (PER)	0
Streptococcus pneumoniae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	28 (REC)	0
Streptococcus pyogenes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15 (REC)	0
Pseudomonas aeruginosa	0	0	8	10	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	27 (PER)	0
Proteus vulgaris	0	0	10	12	0	0	0	0	0	0	0	8	0	0	0	8	0	0	0	8	0	0	0	0	0	0	8	8	25 (PER)	0
Salmonella typhi	0	0	8	10	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	25 (PER)	0
Shigella dysenteriae	0	0	0	14	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	8	0	0	8	10	0	0	8	8	23(REC)	0

Key: PER = Perfloxacin, REC = Recophin, 0 = No Activity

Table 6: Antibacterial Activity of Root Extracts of *Acacia nilotica*

Bacterial Isolates	Diameter of Zones of Inhibition (mm)/ Extracts Concentration (µg/disc)																													
	Ethanol				Methanol				Chloroform				Petroleum Ether				Chloroform Water Interface				Water				Ethyl Acetate				Positive Control (µg)	Negative Control (DMSO)
	10	50	100	500	10	50	100	500	10	50	100	500	10	50	100	500	10	50	100	500	10	50	100	500	10	50	100	500		
Staphylococcus aureus	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	30 (PER)	0
Escherichia coli	0	0	8	8	0	0	0	0	0	0	0	10	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	8	20 (REC)	0
Klebsiella pneumoniae	0	0	8	10	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	30 (PER)	0
Streptococcus pneumoniae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	28 (REC)	0
Streptococcus pyogenes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15 (REC)	0
Pseudomonas aeruginosa	0	0	8	10	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	27 (PER)	0
Proteus vulgaris	0	0	10	12	0	0	0	0	0	0	0	8	0	0	0	8	0	0	0	8	0	0	0	0	0	0	8	8	25 (PER)	0
Salmonella typhi	0	0	8	10	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	25 (PER)	0
Shigella dysenteriae	0	0	0	14	0	0	0	8	0	0	0	0	0	0	0	8	0	0	0	8	0	0	8	10	0	0	8	8	23(REC)	**0**

Key: PER = Perfloxacin, REC = Recophin, 0 = No Activity

Table 7: Antibacterial Activity of Leaf Extracts of *Acacia nilotica*

Bacterial Isolates	Diameter of Zones of Inhibition (mm)/ Extracts Concentration (µg/disc)																								Positive Control (µg)	Negative Control (DMSO)
	Ethanol				Methanol				Chloroform				Petroleum Ether				Chloroform Water Interface				Water				Ethyl Acetate					
	10	50	100	500	10	50	100	500	10	50	100	500	10	50	100	500	10	50	100	500	10	50	100	500	10	50	100	500		
Staphylococcus aureus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	30 (PER)	0
Escherichia coli	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20 (REC)	0
Klebsiella pneumoniae	0	0	8	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	30 (PER)	0
Streptococcus pneumoniae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	28 (REC)	0
Streptococcus pyogenes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		15 (REC)	0
Pseudomonas aeruginosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	27 (PER)	0
Proteus vulgaris	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25 (PER0	0
Salmonella typhi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25 (PER)	0
Shigella dysenteriae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	23 (REC)	0

Key: PER = Perfloxacin, REC = Recophin, 0 = No Activity

The results of the MIC and MBC conducted (tables 8 – 10) showed that the growth of most of the bacterial isolates tested (except *S. pneumoniae and S. pyogenes*) were inhibited at concentrations ranging from 12.5mg/ml to 50mg/ml and that they were killed at concentrations ranging from 50mg/ml to 400mg/ml. This agrees with the reports of Gislene *et al.,* (2000), who investigated the antibacterial activity of extracts of guava, jambolan, pomegranate (high contents of tannins) and other plants against some antibiotic resistant bacteria. In that, study the MIC values of the plant extracts were between 10mg/ml and 400mg/ml.

Table 8: Minimum Inhibitory and Minimum Bactericidal Concentrations (mg/ml) of *Acacia nilotica* of Stem Bark Extracts

Bacterial Isolates	Ethanol		Methanol		Chloroform		Petroleum Ether		Chloroform Water Interface		Water		Ethyl Acetate	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Staphylococcus aureus	-	-	25	200	-	-	-	-	50	200	50	200	50	400
Escherichia coli	-	-	50	400	-	-	-	-	25	200	50	400	50	400
Klebsiella pneumoniae	12.5	100	50	400	-	-	-	-	25	200	-	-	50	400
Streptococcus pneumoniae	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Streptococcus pyogenes	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa	12.5	200	50	400	-	-	-	-	12.5	100	-	-	25	200
Proteus vulgaris	12.5	200	-	-	-	-	-	-	25	200	-	-	25	200
Salmonella typhi	12.5	100	-	-	-	-	-	-	12.5	100	-	-	-	-
Shigella dysenteriae	12.5	200	12.5	200	-	-	-	-	25	200	50	200	25	200

Key: - = No Activity

Table 9: Minimum Inhibitory and Minimum Bactericidal Concentrations (mg/ml) of *Acacia nilotica* Root Extracts

Bacterial Isolates	Ethanol		Methanol		Chloroform		Petroleum Ether		Chloroform Water Interface		Water		Ethyl Acetate	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Staphylococcus aureus	-				25	200		-						
Escherichia coli	50	400			25	200		-					50	400
Klebsiella pneumoniae	12.5	200			25	200		-				-		
Streptococcus pneumoniae	-			-	-			-	-			-		-
Streptococcus pyogenes	-			-	-			-	-		-			-
Pseudomonas aeruginosa	12.5	100			25	200		-			-		50	400
Proteus vulgaris	12.5	200		-	50	400	50	400	50	400			50	400
Salmonella typhi	12.5	200			25	200		-			-		50	400
Shigella dysenteriae	12.5	100	50	100	-	-		-	50	400	25	200	50	400

Key: - = No Activity

Table 10: Minimum Inhibitory and Minimum Bactericidal Concentrations (mg/ml) of *Acacia nilotica*

Bacterial Isolates	Ethanol		Methanol		Chloroform		Petroleum Ether		Chloroform Water Interface		Water		Ethyl Acetate	
	MIC		MBC		MIC		MIC		MBC		MIC		MBC	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Staphylococcus aureus	-	-	-	-	-		-		-		-		-	
Escherichia coli	-	-	-	-	-		-		-		-		-	
Klebsiella Pneumoniae	12.5	200	-	-	-		-		-		-		-	
Streptococcus Pneumoniae	-	-	-	-	-		-		-		-		-	
Streptococcus Pyogenes	-	-	-	-	-		-		-		-		-	
Pseudomonas aeruginosa	-	-	-	-	-		-		-		-		-	
Proteus Vulgaris	50	400	-	-	-		-		-		-		-	
Salmonella Typhi	-	-	-	-	-		-		-		-		-	
Shigella Dysenteriae	-	-	-	-	-		-		-		-		-	

Leaf Extracts

Key: - = No Activity

Table 11 shows the toxicity study of ethanolic extracts using brine shrimps lethality test. The LC_{50} for leaf and stem back extracts of *A. nilotica* are 253.27µg/ml, 312.55µg/ml and 123.86 respectively. However, the recommended cut off point for detecting cytotoxic activity using brine shrimp lethality test is 20µg/ml (Geran *et al*; 1972; Massele *et al.,* 1995). It therefore follows that *A. nilotia* extracts may not be toxic to humans. Brine shrimps lethality test is a general bioassay, which is indicative of cytotoxicity, antibacterial activities, pesticide effects and pharmacologic actions of plant extracts (Olaleye, 2007).

Table 11: Brine Shrimp Lethality Assay of *Acacia nilotica* Ethanolic Extracts

Plant Extracts	*Acacia nilotica*
	LC_{50} (µg/ml)
Leaf	253.27
Stem Bark	312.55
Root	123.86

CONCLUSION

From the results of the phytochemical screening, it was discovered that the leaves, stem bark and roots of *A. nilotica* contain tannins and sterols. Glycosides saponins, resins and flavonoids were not detected in the plant. Alkaloids were present only in the leaves. While *S. aureus, E. coli, K. pneumoniae, P. aeruginosa, P. vulgaris, S. typhi* and *S. dysenteriae* were generally sensitive to the extracts, *S. pneumoniae and S. pyogenes* were *resistant* to them. The study also revealed that, the stem bark and the root extracts of the plant should be preferred for the treatment of bacterial infections. The study also showed that *A. nilotica* extracts may not be toxic to humans. Therefore, the claims of literatures that *A. nilotica* has antibacterial activities is hereby verified.

REFERENCES

Abeer N. H. A. and Sanaa O. Y. (2010): Antimicrobial Activity of *Acacia Nilotica* ExtractsAgainst Some Bacteria Isolated from Clinical Specimens; http//scialert.net/fulltext/?doi=rjmp.2007.25.28&org=10 Pp. 1-3

Adoum O. A. Dabo N. T. and Fatope M. O. (1997): Bioactivities of some Savanna Plants in the Brine Shrimp Lethality Test and *in vitro* Antimicrobial Assay; *International Journal of Pharmacognosy;* **35** (4): 1-4.

Aliyu B S. (2006): *Common Ethinomedicinal Plants of the Semiarid Regions of West Africa*; Triumph Publishing Company Limited; Nigeria Pp 8, 26

http//:en.wikipedia.org/wiki.*acacia-nilotica,* 2008; *Acacia Nilotica;* Pp. 1-5

Arber (1972): *Water Plants; A Study of Aquatic Angiosperm*. Welden Wisely Limited Pp. 436

Baker F. J. Kilshaw D. and Silverton R.E. (1993): *Introduction to Medical Laboratory Technology*; 6th eds. Butterworth, London Pp. 169-218

Banso A. (2009): Phytochemical and Antimicrobial Investigation of Bark Extract of *Acacia Nilotica. Journal of Medicinal Plants Research* Vol. **3**(2) Pp. 82- 85

Ciulei I. (1994): *Methodology for the Analysis of Vegetable Drugs; Chemical Industries Branch, Division of Industrial Operations.* UNIDO, Romania; Pp. 24-67.

El- Olemy M. M. Farid J. A. and Abdel- Fattah A. A. (1994): Ethanolic Extract of *P. stratiotes; NISEB Journal* **1**(1) 51-59

Fatope M. O. Ibrahim H.and Takeda Y. (1993): Screening of Higher Plants Reputed as Herbicides Using Brine Shrimps Lethality Assay *Int. J. of Pharmacog* 31(**4)** 250

Geran R. I. Greenberg H. M. McDonald M. and Abbott B. J. (1972): Protocols for Screening Chemical Agents and Natural Products against Animal Tumors and Other Biological Systems. *Cancer Chemoth*; *Rep.* **33:** 1 – 17

Gislene G. F. N. Juliana L. Paulo C. F. and Guiliana L. S. (2000): Antibacterial Activity of Plant xtract and Phytochemicals on Antibiotic Resistant Bacteria. *Brazilian Journal of Microbiology* **31**:247-256

Guerrero R. O. and Robledo I. (1993): Endemic Plants of Puerto Rico: Brine

Lieberman M. (2008): A Brine Shrimp Bioassay for Measuring Toxicity and Remediation of Chemicals, *Journal of Chemical Education;* Vol.**76** No.12 Pp. 1-3

Massele A. Y. and Nashino C. M. (1995): Brine Shrimp Bioassay for Biological Activity of Medicinal Plants Used in Traditional Medicines in Tanzania*. East Afr.Med. J.* **72**(10) 661-663

Meyer B. N. Ferrighi N. R. Putnam J. E. Jacobson L. B. Nichols D. E. and Mclaughlin J. E. (1982): Brine Shrimp. A Convinient General Bioassay for Active Plant Constituents; *Planta Medical;* **45,** 31-34

Mukhtar M. D. and Tukur A. (2000): Antibacterial Activity of Aqueous and Ethanolic Extracts of *P. stratiotes*; *C. NISEB journal* **1:** 51-59

Mukthar M. D. and Okafor T. (2002): Antibacterial Activity of Ethanolic Extract of *Guiera senegalensis; International Journal of Pharmacology;* **56:** 213-216

Olaleye M. T. (2007): Cytotoxicity and Antibacterial Activity of Methanol Extract of *Hibiscus Sabdariffa Journal of Medicinal Plants Research* vol. **1**(1) Pg. 9 – 13

Onoruuwe O. and Olorunfemi P. O. (1998): Antimicrobial Screening and Pharmagnostical Evaluation of *Dischrostachys Cinerea* Root; *West Africa Journal of Biol. Science;* **7**: 91-99

Philips L. (2010) Online Seminars for Municipal Arborists; Medicinal Properties of Trees; http://onlineseminars.com/index.php?=1-3usingplants-as-sound-barrier; Pg. 1 – 5.

Sofowora A. (1979): *African Medicinal Plants*; University of Ife Press Ile-Ife, Nigeria Pp 14

Sofowora A.A (1993), *Medicinal Plants and Traditional Medicine in Africa*, Spectrum Book Ltd, Ibadan, Nigeria; Pp. 2-85

Sofowora, A (1984): *Medicinal Plants and Traditional Medicine Africa;* Spectrum book Ltd Ibadan; Pp135-221

Sowemimo A.A. Fakoya I.A. Awopetu I. Omobuwajo and Adesanya S.A. (2007): Toxicity of some Selected Nigerian Plants; *Journal of Ethnopharmacology*, Pp. 1-6.

Sule M.S (2006), Toxicological Studies on the Leaves of *Gueira senegalis* and *Psidium guajava* in Rats, *Biology and Environmental Science for the Tropic*; **3** (1) Pp. 81-83

Trease G.E, Evans W.C (1989): *Test Book of Pharmacology*. Battiere Tindal Ltd London; 13th ed. Pp. 194-196

Turner T.D. and Brain K R (1975). The Practical Evaluation of Phytopharmaceuticals; *Wright Scientechica, and Bristol:* 61 (6): Pp 535-539