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

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ABSTRACT
The phytochemical screening, antibacterial and toxicological activities of extracts of the stem bark of Acacia senegal were investigated. The phytochemical analyses according to standard screening tests using conventional protocols revealed the presence of tannins, saponins and sterols in the stem bark of the plant. Alkaloids, glycosides 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. Ethanol and methanol stem bark extracts exhibited antibacterial activity on Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus vulgaris, Salmonella typhi, Shigella dysenteriae and Escherichia coli except on Staphylococcus aureus, Streptococcus pneumoniae and Streptococcus pyogenes. The other extract fractions showed no activity on the bacterial isolates at the concentration used. The minimum inhibitory concentration and minimum bactericidal concentration studies revealed that some bacterial isolates were inhibited at concentrations of about 50mg/ml and killed at 400mg/ml. Toxicity studies of the ethanol extracts revealed that they exhibited no significant toxicity (LC_{50} of 100µg/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 senegal, Extracts

INTRODUCTION

Acacia senegal (Dakwara in Hausa) is used as medicinal plant in parts of Northern Nigeria, West Africa, North Africa and other parts of the world. *Acacia senegal* is used to treat infections such as bleeding, bronchitis, diarrhea, gonorrhea, leprosy, typhoid fever and upper respiratory tract infections (Aliyu, 2006; http//:en.wikipedia.org/wiki.*acacia-senegal,* 2008). Antimicrobial drug resistance is on not only the increase, but also a serious problem to the medical profession. The vast number of chemicals used industrially and pharmacologically provides an ever increasing hazard to the liver. The substances, that affect the liver adversely, will invariably cause harm to other organs and tissues in the body. The kidney for instance performs the function of getting rid of the body's waste materials that are either ingested, produced by metabolism or as a result of detoxification by the liver. This and other functions of the kidney can be disrupted by accumulation of toxic metabolites or chemicals resulting to renal disease (Arthur and John, 2000).

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.

MATERIALS AND METHODS

Collection and Identification of Plant Material

The plant material was collected from Ungogo Local Government Area of Kano State**.** The plant was 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 part of the plant 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 and Olorunfemi., 1998).

Extraction and Fractionation of Plant Material

The specimen was dried at room temperature (30^0C), and kept away from sunlight to prevent changes in the nature of the plant's constituents. The specimen was grounded to powder (fine texture) with mortal and pistol. One hundred grammes of powdered specimen was percolated in one liter of 96% alcohol for seven days followed by filtration. The extract was concentrated using a laboratory vacuum rotary evaporator at 40^0C. The crude extract was weighed labeled and stored in a refrigerator at a temperature of 4^0C. A fraction of the 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 vacuum rotary 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 vacuum 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 vacuum rotary evaporator, weighed, labeled and stored as above (Fatope *et al;* 1993 and Adoum *et al;* 1997).

Qualitative Phytochemical Analysis of Plant Extract

Standard screening tests and conventional protocols were used for the determination of tannins (Trease and Evans, 1978), alkaloids (Sofowora, 1979), saponins (Turner and Brain1975), flavonoids (Sofowora, 1993), glycoside (Ciuilei, 1994), sterols (Sofowora, 1993) and Resins (Sofowora, 1993).

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

Saponin Extraction for Quantitative Analysis

From each specimen 3.5g was placed in100ml conical flask and 21ml of 50% alcohol added. The content was refluxed for 30 minutes. The boiling alcohol extract was filtered while hot through a coarse filter paper. Two grammes (2.0g) of charcoal were added, boiled and filtered. Equal volume of acetone was added to the cool extract. The separated saponin was collected by decantation and was dissolved in 5ml of boiling 95% alcohol. The content was filtered while hot and was kept at room temperature. The supernatant liquid was decanted and the precipitated saponin was suspended in 20ml of alcohol and filtered. The filter paper was immediately transferred to a desicator containing anhydrous calcium chloride, where the saponin was dried and weighed. The supernatant was also filtered off, dried and weighed. The difference between the weight of the filter paper before and after the analysis is the saponin content (El-Olemy *et al.*, 1994).

Preparation of 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 extract. 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 and Silverton (1993). Stock solution 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 100 filter paper discs (Baker and Silverton, 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

Sensitivity tests were done using Agar diffusion method (Baker and Silverton 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 inoculums. The filter paper discs impregnated with the above concentrations of extracts were 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 and Silverton, 1993). Dilution of the plant 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 was 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 showed no single bacterial colony was taken as the minimum bactericidal concentration (MBC) as reported by (Baker and Silverton, 1993).

Brine Shrimp Lethality Bioassay

Eggs of *Artema salina* (about 50mg) were placed into a hatching chamber containing sea 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 were 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- 2 show the physical properties of *Acacia senegal* extract fractions recovered from stem bark of the plant. The solvents used include ethanol, chloroform, methanol, petroleum ether, water and ethyl acetates. The extracts were either gummy or granular in texture, dark brown or brown in colour. Most of the extracts were however granular in texture.

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

Plant Parts	Ethanol Extract			Chloroform Extract			Chloroform/ Water Interface Extract			Petroleum Ether Extract			Methanol Extract			Water Extract			Ethyl Acetate Extract		
	Weight Recovered			Weight Recovered			Weight Recovered			Weight Recovered			Weight Recovered			Weight Recovered			Weight Recovered		
	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)	%
Stem Bark	100.00	25.60	25.60	20.00	10.00	50.00	20.00	1.10	5.5	6.00	1.40	23.30	6.00	2.30	38.33	8.00	4.70	58.75	8.00	1.90	23.75

Table 2: Textures and Colours of *Acacia senegal* 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
Stem Bark	Granular	Dark Brown	Gummy	Dark Brown	Granular	Dark Brown	Granular	Dark Brown	Granular	Dark Brown	Granular	Brown	Granular	Brown

Tables 3 and 4 shows the qualitative and quantitative phytochemical screening of ethanolic extract of *A. senegal* stem bark. The results showed that glycosides, resins, alkaloids and flavonoids were not detected in the plant. Saponins, sterols and tannins were however detected the plant. This is close to the findings of Mudi and Salisu (2009), that *Acacia senegal* stem bark extracts contain steroids, tannins etc. The percentage weight of tannins and saponins are 3.08% and 0.35% respectively.

Table 3: Qualitative Determination of Phytochemicals Present in the Plant

Phytochemicals	***Acacia senegal* Stem Bark**
Glycosides	-
Alkaloids	-
Saponins	+
Flavonoids	-
Sterols	+
Resins	-
Tannins	+

Key: + Phytochemicals Detected, - Phytochemicals Not Detected

Table 4: Tannins and Saponins in *Acacia senegal* Stem Bark Extract

Plant Extracts	**Percentage of Weight Tannins/ 5ml of Extract**	**Percentage Weight of Saponins/ 3.5 Grammes Extract**
Stem Bark	3.08	0.35

Tables 5 show the results of antibacterial activities of various stem bark extract fractions of *A. senegal.* 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. Ethanol and methanol stem bark extracts exhibited antibacterial activity on *Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus vulgaris, Salmonella typhi, Shigella dysenteriae* and *Escherichia coli* except on *Staphylococcus aureus, Streptococcus pneumoniae* and *Streptococcus pyogenes.*

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. Olaleye (2007) also 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.* Mudi and Salisu (2009) also reported that tannins and saponins exhibit similar antibacterial activities. Therefore, antibacterial activity showed in this present work may be due to tannins and saponins.

Ethanol and methanol extracts exhibited antibacterial activities on the bacterial isolates tested at the concentration used. This may be due to the ability of ethanol to extract a wide range of chemical constituents of the plant while chloroform might have extracted less number of the ingredients (Abeer *et al.,* 2007). Furthermore, ethanol extract was the first solvent used for extraction of the plant constituents before portions of the extracts were partitioned.

The results of the MIC and MBC conducted (Table 6) showed that the growth of some of the bacterial isolates tested (except *S. aureus, S. pneumoniae and S. pyogenes*) were inhibited at concentration ranging from 50mg/ml and that they were killed at concentration of about 400mg/ml. This agrees to some extent with the report of Gislene *et al.,* (2000), who investigated the antibacterial activity of extracts of guava, jambolan, pomegranate (high contents of tannin) 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 7 shows the toxicity study of ethanolic extracts using brine shrimps lethality test. The LC_{50} stem back extract of *A. senegal* is 100µg/ml. 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. senegal* 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 5: Antibacterial Activity of Stem Bark Extracts of *Acacia senegal*

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		0
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	8	0	0	0	8	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	0	0	0	0	0	8	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	0	15 (REC)	0
Pseudomonas aeruginosa	0	0	0	8	0	0	8	8	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	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25 (PER)	0
Salmonella typhi	0	0	0	8	0	0	0	8	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	8	10	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

Table 6: Minimum Inhibitory and Minimum Bactericidal Concentrations (mg/ml) of *Acacia senegal* 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	-	-		-	-		-		-		-	-
Escherichia coli	50	400	50	400	-		-		-		-	-
Klebsiella pneumoniae			50	400	-		-		-		-	-
Streptococcus pneumoniae	-	-		-	-		-		-		-	-
Streptococcus Pyogenes	-	-		-	-		-		-		-	-
Pseudomonas aeruginosa	50	400	50	400	-		-		-		-	-
Proteus vulgaris	-		50	400	-		-		-		-	-
Salmonella typhi	50	400			-		-		-		-	-
Shigella dysenteriae	-		12.5	200	-		-		-		-	-

Key: - = No Activity

Table 7: Brine Shrimp Lethality Assay of *Acacia nilotica* Ethanolic Extracts

Plant Extracts	*Acacia Senegal*
	LC_{50} (µg/ml)
Stem Bark	100.00

CONCLUSION

From the results of the study antibacterial activity exhibited by *A. senegal* stem bark extracts may be due to tannins and saponins. The claims of literatures that *A. senegal* has antibacterial activities is hereby verified. These results also suggest that the plant extract may not be toxic to man and could be a potential source of novel antibacterial compound.

Recommendations

From the results of this study, the following recommendations were made:

(a) Stem bark extracts of the plant could be used to treat some bacterial infections

(b) Further studies should be conducted to ascertain the active antibacterial agents in the plant and possible production of novel antibiotics from it.

REFERENCES

Abeer N. H. A. and Sanaa O. Y. (2010): Antimicrobial Activity of *Acacia nilotica* Extracts Against 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

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

Arthur C.G and John E.H (2000): *Text Book of Medical Physiology* W.B Saunders, Philadelphia, USA, 10th eds. Pp. 279-281

http//:en.wikipedia.org/wiki.*acacia-senegal,* 2008; *Acacia senegal;* Pp. 1-3

Baker F. J. Kilshaw D. and Silverton R.E. (1985): *Introduction to Medical Laboratory Technology*; 6th eds. Butterworth, London Pp. 169-218

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): Ethanol 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 Extract 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 Shrimp Lethality Bioassay; *P. R. Health Sci* **12**(4) 259- 262

Lieberman M. (2008): 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. ed. 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 Convenient General Bioassay for Active Plant Constituents; *Planta Medical;* **45,** 31-34

Mudi S. Y. and Salisu A. (2009): Studies on Brine Shrimp Lethality and Activity of Stem Bark Extract of *Acacia Senegal* on Respiratory Tract Pathogenic Bacteria; *International Journal of Biomedical and Health Sciences* Vol. **5**. No 3 Pp. 139-143

Mukhtar M. D. and Tukur A. (2000): Antibacterial Activity of Aqueous and 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-Ifee, Nigeria; Pg 14

Sofowora 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* in *Africa;* Spectrum Book Ltd Ibadan; Pp135-221

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