INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

Volume: 2: Issue-3: July-Sept -2011 IJABPT *ISSN 0976-4550*

ANTIBACTERIAL AND PHYTOCHEMICAL SCREENING OF THE ETHANOLIC LEAF EXTRACT OF *AZADIRACHTA INDICA* (NEEM) (MELIACEAE)

Timothy, S.Y.[1*], Goji, S.Y.[1], Abdussalam.B[1], Mava, Y.[2], and Galadima, I.H.[1]

[1]Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Maiduguri, P.M.B. 1069, Maiduguri, Nigeria

[2]Department of Paediatrics, University of Maiduguri Teaching Hospital, Maiduguri, Nigeria

E-mail: satiye2002@gmail.com satiye2002@yahoo.com

ABSTRACT : *Azadirachta indica* is a very useful traditional medicinal plant in the sub-continent and each part of the tree has some medicinal properties. The plant is native to Asia, but has now naturalized in West Africa and is widely cultivated in Nigeria as an ornamental as well as medicinal plant. This study aimed at screening the active components and the antibacterial effects of the ethanolic leaf extract of *Azadirachta indica*. Fresh leaves of the plant were collected, dried, homogenized and extracted using 95% Ethanol. The extract was used for the phytochemical screening and bacterial susceptibility testing using cup-plate method.The result obtained from this study showed the presence of tannins, saponins, flavonoids, alkaloids, glycosides, reducing sugars and terpenes in the ethanolic leaf extract. The antibacterial effect produced by the extract was dose-dependent at the tested doses (6.25 mg, 12.5 mg, 25 mg, 50 mg and 100 mg) on *Escherichia coli (E. coli)*, *Staphylococcus aureus (S. aureus)* and *Salmonella typhi (S. typhi)*. The minimum inhibitory concentration (MIC) for *E. coli, S. aureus* and *S. typhi* were 2.39 mg, 3.31 mg and 4.79 mg respectively. The effect produced by the ethanolic leaf extract at 100mg (18 mm) was statistically significantly higher than that of 1.5 mg ciprofloxacin (14 mm) on *S. typhi* ($p<0.05$). *Azadirachta indica* ethanolic leaf extract contains pharmacologically active constituents that may be responsible for its activity against *S. aureus, E. coli and S. typhi*. Therefore, the use of Neem plant in our community for treating diverse medical ailments especially infectious diseases is highly justified.

Keywords: Antibacterial, Phytochemistry, Ethanol, *Azadirachta indica,* Nigeria

INTRODUCTION

Medicinal plants have been extensively studied as an alternative treatment for diseases in order to overcome the problem of antibiotic resistance by pathogenic organisms (Timothy *et al*., 2008; Phillip *et al*., 2005). Numerous plants or their phytochemical constituents have been proven by rigorous science or approved by regulatory agencies such as the United State Food and Drug Administration or European Food Safety Authority to have medicinal value of which *Azadirachta indica* is inclusive. Many plants of medicinal value remain untapped, thus, a need to encourage researchers to evaluate those medicinal plants for the betterment of mankind. *Azadirachta indica* is a very useful traditional medicinal plant in the African sub-continent and each part of the tree has some medicinal properties (Abdullah *et al*., 2011). The plant is native to Asia, but has now naturalized in West Africa and is widely cultivated in Nigeria as an ornamental and medicinal plant. It is used extensively in Nigeria for the traditional treatment of malaria and other associated conditions in form of decoction, in which unspecified quantities are usually consumed without due regards to toxicological and other adverse effects (Bokhari and Aslam, 1985; Khattak *et al*., 1985; Katsaya1 *et al*., 2008). Based on this traditional and other uses of *Azadirachta indica*, this study was conducted to ascertain it's potentially pharmacologically active components and antibacterial activities.

MATERIALS AND METHODS

Source of the plan material, collection and authentication

The leaves of *Azadirachta indica* were collected in the month of March, 2011 from the tree growing inside the University of Maiduguri, Nigeria. The leaves of the plant were identified by Prof. Sanusi, S. of the Herbarium unit, Department of Biological Sciences, University of Maiduguri, where voucher specimen (No. 965182) was deposited.

Preparation of the plant leaf extract

The fresh leaves were allowed to dry under shade and grounded into powder using wooden pestle and mortar. The powdered material was weighed using metler weighing balance until a constant weight was obtained. Two hundred and fifty grams of the powder was placed in a container and was defatted using petroleum ether, following which it was subjected to maceration using 300 ml of 95% ethanol so as to obtain the ethanolic extract of the plant. The mixture was stirred and kept for 24 hours. The mixture was filtered at which another 300 ml of the ethanol was added to the residue and kept for another 24 hours before filtration. This procedure was repeated 3 times and the combined filtrate was subjected to rotory vapour machine to obtain the dried extract. The weight of the plant extract obtained was 29.3 g and thus, the percentage yield was 11.7%.

Source of the microorganisms

Clinical isolates of *Staphylococcus aureus, Salmonella typhi and Escherichia coli* were obtained from the Department of Microbiology, University of Maiduguri Teaching Hospital, Maiduguri, Nigeria.

Sterilization of the equipments and disinfection

All the equipments were disinfected with cotton wool soaked in methylated spirit so as to maintain sterility throughout the process. Wire loop, conical flasks and beaker were sterilized by hot air oven at 160^{o}C for 45 minutes, whereas moisture insensitive materials were sterilized by autoclaving at 121^{o}C for 15 minutes.

Phytochemical analysis of the plant extracts.

Phytochemical screening of the plant extracts was conducted following the standard procedure described by Harbone (1973), Sofowora (1978) and Trease and Evans (1989).

Preparation of the media and cup hole

The nutrient agar media (Biochemika Fluka) was used. The composition of the nutrient agar (gm/litre) were: Meat extract 1 g, yeast extract 2 g, peptone 5 g, NaCl 5 g and agar 15 g. Twenty-eight grams of nutrient agar was weighed and dissolved in 1000 ml of distilled water and adjusted to pH of 7.4 ± 0.2 at 37^{o}C. This was sterilized by autoclaving at 121^{o}C for 15 minutes at 15 psi pressure and was used for sensitivity tests. The doubled layer preparative nutrient agar was used for the susceptibility testing. Each of the layers consists of 30 ml making 60 ml of the nutrient agar for each plate. A sterilized improvised cork borer of 6 mm in diameter was used to bore holes on the plates and each media contained seven holes of 6 mm in diameter in which 100 mg, 50 mg, 25 mg, 12.5 mg and 6.25 mg of the reconstituted extract were administered into the holes. In addition distilled water and ciprofloxacin (30mg/ml) was also administered into the remaining 2 holes serving as the negative control and positive control respectively.

Antibacterial activity assay of the plant extracts

An overnight broth culture was diluted with peptone water to match turbidity of McFarland standard number 3 which was used as inoculums for the microorganism.

One ml of the bacterial inoculums was transferred into pure agar plates with the aid of a new sterile syringe, and the agar plates were slanted to ensure the spread of the inoculums on the entire surface of the agar plates. The plant extract was administered into 5 holes on each plate with a varying strength of 100 mg, 50 mg, 25 mg, 12.5 mg and 6.25 mg using sterile syringe. Likewise, distilled water and ciprofloxacin (30 mg/ml) were administered into two additional holes serving as the negative and positive controls respectively. Five plates of each organism were replicated (15 plates for the three organisms). One hour was allowed for diffusion before incubating the plates at 37^{o}C for 24 hours. The clear zones of inhibition (mm) were measured using meter rule.

Determination of the minimum inhibitory concentration (MIC)

The minimum inhibitory concentration values of the plant extract were obtained by extrapolation from the plot of the log strength (per hole) of the extract against the clear zones of inhibition in millimeter.

Statistical analysis

Results were expressed as mean ± standard error of the mean. A student t-test was used to determine the level of significance of the various zones of bacterial inhibition where P-values less than 0.05 were considered significant.

RESULT AND DISCUSSION

The result of this study showed that the ethanolic leaf extract of *Azadirachta indica* contains saponins, tannins, glycosides, alkaloids, terpenes, flavonoids and reducing sugars. Anthraquinones were not detected from the extract (Table 1). This is in agreement with the report of Biu *et al* (2009) in which the aqueous extract of *Azadirachta indica* leaf contains pentosis and carbohydrates in addition to those chemical constituents detected. Anthraquinones, ketones and monosaccharides were also not detected. Quantitatively, the plant leaf extract also revealed a greater proportion of saponins, moderate concentrations of tannins and glycosides, while flavonoids, alkaloids, reducing sugars and terpenes were in low concentrations (Table 2). The presence of these phytochemical components may be responsible for the observed antimicrobial activity of the plant leaf extract. This findings conforms to the report of Anyanwu and Dawet (2005) in which similar constituents was found to exhibits antiprotozoal and antibacterial activities. Flavonoid has also been reported to have greater potential benefit to human Health (Jouad *et al*., 2001). Another phytochemical analysis of the leaf extract of *Azadirachta indica* was reported by Imran *et al* (2010) in which petroleum ether, chloroform and methanolic extracts was found to contain only glycosides, triterpenes and fatty acids in relatively higher quantities. This could explain why the choice of solvent for the extraction of a particular constituent is vital.

Table 1: Qualitative determination of phytochemical groups of ethanolic extract of *Azadirachta indica* leaf

Phytochemical groups	Results
Saponins	+
Flavonoids	+
Tannins	+
Alkaloids	+
Anthraquinones	-
Glycosides	+
Reducing sugars	+
Terpenes	+

+ = Present
- = Absent

Table 2: Quantitative phytochemical screening of the ethanolic extract of *Azadirachta indica* leaf

Phytochemical components	Results
Tannins	++
Saponins	+++
Flavonoids	+
Alkaloids	+
Glycosides	++
Reducing sugars	+
Terpenes	+
Anthraquinones	-

- = Not detected
+ = Low concentration
++ = Moderate concentration
+++ = High concentration

The result obtained from this study showed dose dependent activity of *Azadirachta indica* leaf extract at the tested doses (6.25 mg, 12.5 mg, 25 mg, 50 mg and 100 mg) on the test organisms (*E. coli*, *S. typhi* and *S. aureus*) (Table 3). However, on *E. coli* the 50 mg of the plant leaf extract produced better antibacterial activity (14 mm) when compared to the 100 mg dose (10.7 mm) (Table 3). The diffusion the extract suffers through the media could be responsible for the observed effects. The results of this finding strongly agrees with several literature reports (Umar *et al*., 2002; Makeri *et al*., 2007; Imran *et al*., 2010; Joshi *et al*., 2011) in which *Azadirachta indica* was found to possess significant antimicrobial activities against several pathogens. However, the result of this study contradicts the findings of Joshi *et al* (2011) where the plant extract was found to be ineffective against *E. coli*. The minimum inhibitory concentration of the ethanolic leaf extract of the plant against *S. aureus, S. typhi and E. coli* were 3.31 mg, 4.79 mg and 2.39 mg respectively (Table 4).

Table 3: A**ntibacterial activity of the ethanolic extract of *Azadirachta indica* leaf**

Strength of the plant extract (mg)	Log strength (mg)	Mean zones of inhibition (mm)		
		E. coli	S. aureus	S. typhi
100.00	2.00	10.70	22.30c	18.00e
50.00	1.70	14.00a	17.00	14.40
25.00	1.40	13.70	13.00	10.20
12.50	1.10	8.00	8.60	6.00
6.25	0.80	4.30	5.00	3.20
Controls				
Ciprofloxacin (1.5 mg)		22.00b	32.00d	14.00f
Distilled water		0.00	0.00	0.00

ab = $p<0.05$ (t_{test}), cd =$p<0.05$(t_{test}) ,ef=$p<0.05$(t_{test}), N = 5

Table 4: Minimum inhibitory concentration (MIC) of the ethanolic extract of *Azadirachta indica* leaf determined graphically

Test organisms	Log strength (mg)	Log-1 strength [MIC (mg)]
E. coli	0.38*	2.39
S. aureus	0.52*	3.31
S. typhi	0.68*	4.79

* = Means obtained by extrapolation of the Y-axis, log strength (mg).

It is evident from the result of this study that *S. typhi* was more susceptible to the plant extract compare to *S. aureus* and *E. coli*. The effect produced by the standard (ciprofloxacin, 1.5 mg) on *S. aureus and E. coli* was statistically significantly higher than the leaf extract tested ($p<0.05$). However, the activity of 100 mg ethanolic leaf extract on *S. typhi* was significantly higher than that of 1.5 mg ciprofloxacin ($p<0.05$) (Table 3). The antibacterial effect produced by 100 mg of the ethanolic leaf extract against *S. typhi* compared with that of ciprofloxacin to the best of our knowledge has never been reported elsewhere and this could be attributable to the varying concentrations of secondary metabolites such as saponins and flavonoids contained in the ethanolic leaf extract.

Conclusion

From this study it can be concluded that *Azadirachta indica* ethanolic leaf extract contains saponins, tannins, glycosides, flavonoids, alkaloids, reducing sugars and terpenes and these secondary metabolites could be responsible for its activities against *Staphylococcus aureus, Escherichia coli* and *Salmonella typhi*. The wide use of Neem plant in our community is attributable to the presence of these bioactive compounds which may explain its diverse traditional usage against various ailments. Therefore, further pharmacological as well as toxicological studies are hereby encouraged with the view to isolate and characterize the specific active constituents of the plant responsible for its therapeutic effects.

Acknowledgement

Authors are sincerely thankful to Maspalma Ibrahim Dauda of Department of Pharmacology, Faculty of Pharmacy and Staff of the Department of Chemical Pathology, University of Maiduguri Teaching Hospital for their moral and technical support.

REFERENCES

Abdullah, A., Shahed, S.A., Farzana, A., Sajal, K., Sreedam, C.D. and Sitesh, C.B. (2011). Evaluation of brine shrimp lethality and antimicrobial activity of *Azadirachta indica* leaf extract on some drug resistance bacteria in Bangladesh. *Pharmacognosy Journal*, 20(3): 66-71.

Anyanwu, G. I. and Dawet, A. (2005). Pharmacological and phytochemical screening of *Hyptis suaveolens* poit (Lamiaceae) for bioactivity in rodents. *Nigerian Journal of Botany*, 18: 190-196.

Biu, A.A., Yusuf, S.D. and Rabo, J.S. (2009). Phytochemical screening of *Azadiracta indica* (neem) (meliaceae) in Maiduguri, Nigeria. *Biosci Res. Comin*. 21(6): 281-283.

Bokhari, M.H. and Aslam, K.M. (1985). Neem (*Melia Azadirachta* A. Juss). A useful tree in Northern Nigeria. *Annals of Borno* 2: 83-86.

Harbone, J.B. (1973). Phytochemical methods. A guide to modern techniques of plant analysis. Chapman and Hall, London. Page 279.

Imran, K., Rao, S., Srikakolupu, A., Darsipudi, S., Gotteti, S.D. and Amaranadh, H.C. (2010). Phytochemical studies and screening of leaf extract of *Azadirachta indica* for its antimicrobial activity against dental pathogens. *Applied Science Research*, 2(2): 246-250.

Joshi, B., Prasad, G.V., Buddha, B.B., Bhatt, M.J., Dinita, S.K., Janardhan, S.P. and Rajani, M. (2011). *Journal of Microbiology and Antimicrobials*, 3(1): 1-7.

Jouad, H., Lacalle-Duboi, M.A., Lyoussi B. and Eddouks M. (2001). Effect of the flavonoids extracted from *Spergularia purpurea* pers on arterial blood pressure and renal function in normal and hypertensive rats. *J. Ethnopharmacol,* 76(2): 159-163.

Katsayal, U.A., Nadabo, Y.A. and Isiorho, V.J. (2008). Effects of Methanol extract of *Azadirachta indica* leaves on the histology of liver and kidney of wister rats. *Nig. Journ. Pharm. Sci*., 7(1): 9-14.

Khattak, S.G., Gilani, S.N. and Ikram M. (1985). Antipyretic studies on some indigeneous Pakistani medicinal plants. *J. Ethopharmacol,* 14: 45-51.

Makeri, H.K., Maikai, V.A. and Nok, J.A. (2007. Effect of topical application of neem seed (*Azadirachta indica*) extract on sheep infested with *Amblyomma variegatum*. *African Journal of Biotechnology*, 6(20): 2324-2327.

Philip, A.I., Jones, O.M. and Herbert, A.O. (2005). Phytochemical and antimicrobial screening of three Nigerian medicinal plants used to treat infectious diseases traditionally. *J. Pharm. Biores*. 2(2): 116-119.

Sofowora, A. (1978). The state of medicinal plants research in Nigeria. University press, Ibadan, Nigeria, pp 86.

Timothy, S.Y., Adamu, S., Nyandaiti, W.Y., Sugun, M.Y., Bukbuk, D.N., Wazis, C.H. and Gamaniel, K.S. (2008). Phytochemical and Antimicrobial Activity of Aqueous leaf extract of *Senna siamea* (Lam.) on Enterobacteriaceae. *Nig. J. of Exp. and Applied Biol*, 9(2): 159-163.

Trease, G.E. and Evans, W.C. (1989). Pharmacognosy. Bailliere Tindals, London, pp 687-689.

Umar, A., Abdulrahman, H.T. and Kokori, M. (2002). Preliminary studies of the efficacies of the aqueous extracts of leaf and seed kernel of Neem (*Azadirachta indica* A. Juss) for the control of cowpea bruchid Callobruchus maculates (Coleoptera:Bruchidae). *Research Journal of Science* 8(1&2): 25-30.