Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Asian Journal of Plant Science and Research, 2014, 4(2):64-67

ISSN : 2249-7412
CODEN (USA): AJPSKY

Antibacterial effect of *Azadirachta indica (CN: Neem or Dongo Yaro)* parts on some urinary tract bacterial isolates

[*1]Orhue P. O., [2]Momoh A. R. M., [1]Igumbor E. O. and [1]Esumeh F. I.

[1]Department of Microbiology, Faculty of Natural Sciences, Ambrose Alli University, Ekpoma-Nigeria
[2]Medical Microbiology, Faculty of Clinical Sciences, College of Medicine, Ambrose Alli University, Ekpoma-Nigeria

---

ABSTRACT

Several scientific findings have attached various biological activities of clinical significance on several wonder plants that are indigenous to Africa. Hence, this study investigates the antimicrobial activity of leaves and back extracts of Azadirachta indica (Dongo yaro) on human urinary tract bacteria isolates. Following standard laboratory procedures, the leaves and back extracts of Azadirachta indica were studied on Gram positive bacteria (Staphylococcus aureus) and two strains of Gram-negative bacteria (Escherichia coli and Pseudomonas aeruginosa). The results showed that the degree of antibiotic potentials varies with the extraction solvents (water, ethanol, 1% HCl, acetone and petroleum ether). Comparatively, petroleum ether extract of leave and bark of Azadirachta indica were more potent against the urinary tract bacterial isolates, however, there was no different in the parts of Azadirachta indica used expect for the water extract where the bark extract was more potent than the leave. The differences in MIC between extraction solvents and that of the standard drugs (perflacine; 4.0 mg/ml, and cefuroxime; 6.0 mg/ml) were significant differences ($p<0.05$) from those of Azadirachta indica leave and bark extracts. Judging by the findings of this study, leave and bark extracts of Azadirachta indica demonstrated antibacterial potential against gram positive and negative bacteria and as such could be a source of antibiotic.

Key words: Herbs, *Azadirachta indica*, Antimicrobial, infections.

INTRODUCTION

Many of the existing synthetic drugs are known to cause various side effects. In this regards, Srivastava *et al*. (2000), suggested drug development from plant based compounds are now being employed to be useful in meeting this demand for newer drugs with minimal side effects. By implication, herbal medicine is now forming an alternative therapy that has become the mainstream throughout the world due to the growing resistance of pathogens to conventional antibiotics (De Smet, 2002). On the other hand, in Nigeria, until recently, the practices of the use of herbs is characterised with secrecy and shrouded in dreaded magical incantations, rituals and sacrifices (Orhue et al., 2014). Interestingly, therapeutically properties of medicinal plants have long been reported to be very useful in healing various diseases and the advantage of these medicinal plants are 100% natural (Serrentino, 1983), man is now being dedicated to herbal medicine as many academics are now into ethnomedicine.

Of interest is the evergreen and fast growing tree in the mahogany family Meliaceae; *Azadirachta indica* (Neem), which normally reach a height of 15-20 metres and whose twigs is used as chewing stick (Almas and Ansal -Lafi, 1995). It is native to Indian subcontinent and grows in tropical and semitropical regions and commonly known by different names such as Nim (in Bengali), Tamar (in Burmese), Grossblacttigerzedrach (in German), Muarubaini (in Swahili; meaning the tree of the 40, as it is used to treat 40 different diseases), Dongoyaro (in northern Nigeria; literarily; “tall boy”).

Azadirachta indica is a wonder plant with valuable economic and health significance attached to all its parts. In fact, it is a well know versatile medicinal plants with wide spectrum of biological activities (Siddique et al., 2004). For example, its leaf, back, roots, fruit coat, seed and flowers (Atawodi and Atawodi, 2009) have been demonstrated to exhibit immunomodulatory (Haque et al., 2006), anti-inflammatory (Akihisa et al., 2011), anti-hyperglycaemic and antidiabetic (Bhat et al., 2011; Sudha et al., 2011), antiulcer (Chattopadhyay et al., 2004), antimalarial (Isah et al., 2003), antifungal (Natarajan et al., 2003), antibacterial (Thakurta et al., 2007), antiviral (Parida et al., 2002) anticarcinogenic (Kumar et al., 2006) and spermicidal (Khillare and Shrivastav, 2003) properties and antifertility agent (Gbotolorun *et al*., 2008).

Considering these ranges of biological potentials, this present study is aim at investigating the antimicrobial activity of *Azadirachta indica* leaves and back acting separately on human urinary tract bacteria isolates.

MATERIALS AND METHODS

Processing of plant samples: *Azadirachta indica* (leave and back) were collected from in and around Ekpoma, Edo State, Nigeria and authenticated by a Botanist in the Department of Botany, Ambrose Alli University, Ekpoma. The leave and back were separately washed in tap water, rinsed in sterile distilled water and dried for 5 days and 10 days respectively at 60^0 C in Lab 1 of the Department of Microbiology, Ambrose Alli University, Ekpoma. The dried leave and back were then separately blended to powder with a clean kitchen blender (Sonik, Japan) and stored in air tight glass containers kept in laboratory cupboard, until required for preparation.

Preparation of extracts: 5grams each of leave and back of *Azadirachta indica* were weighed into 100ml reagent bottle and 95ml of extraction solvent (water, ethanol, 1% HCl, acetone and petroleum ether) was added and left to extract on a mechanical shaker overnight at room temperature. This was done using all the five extraction solvents on the different studied parts of *Azadirachta indica*.

The extract solutions were filtered aseptically into another 100ml reagent bottle using a watt-man No 1 filter paper. All the filtrate were screened for purity by inoculation unto MacConkey agar and nutrient agar plates and incubated at 37^0C for 48 hours following the methods outlined in Orhue (2004). Filtrates yielding growth of any organism was re-filtered and rescreened for purity until a sterile extract solution was obtained.

Micro organism preparation/growth: The test organisms used were all human pathogenic organisms of clinical origin and were isolated from urinary tract of infected patients attending the University of Benin Teaching Hospital, Benin City, Nigeria. They include one strain of Gram positive bacteria (*Staphylococcus aureus*) and two strains of Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). They were stored in the Department of Microbiology, Faculty of Natural Sciences, Ambrose Alli University, Ekpoma-Nigeria, where they were kept as stock cultures at 4°C. Biochemical analysis was carried out on each of the test organisms for confirmation.

Determination of Minimum Inhibitory Concentration (MIC): Using a 50ml specific gravity bottle, the density of the extract solution was determined. In a similar manner, the density of the plain solvent was also determined. To determine the concentration of the extract, the density of the plain was subtracted from that of the extract solution. This was done for all 5 extraction solvents on the leave and back of *Azadirachta indica*. With the known extract concentrations and the three clinical isolates of *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, the MIC of the extract solutions of *Azadirachta indica* leave and back and standard drugs (Peflacine and Cefuroxine) were determined. The experiments were performed in 3 repetitions for each of the extraction solvents of *Azadirachta indica* leave and back and the average calculated.

Data analysis: Data were keyed into SPSS (version 16) and the average of each determined MIC was then presented in suitable table for simple descriptive statistics. The MICs of the different *Azadirachta indica* leave and back in the different extraction solutions were compared with the values of the standard antibiotic drugs.

RESULTS

As shown in table 1 and 2, there were difference in the potency of *Azadirachta indica* extracts and this is shown by the MICs recorded for the different solvents. Although the degree of antibiotic potentials varies with the extraction solvents, there was no different in the parts of *Azadirachta indica* used expect for the water extract where the bark extract was more potent than the leave. These differences in MIC between extraction solvents were significant differences ($p<0.05$) and that of the standard drugs (perflacine; 4.0 mg/ml, and cefuroxime; 6.0 mg/ml) were significantly different ($p<0.05$) from those of *Azadirachta indica* leave and bark extracts. Comparatively, petroleum

ether extract of leave and bark of *Azadirachta indica* were more potent against the urinary tract bacterial isolates compared to the other solvents and this was statistically significant (P<0.05).

Although all the extraction solvent show antibacterial potentials against all the three bacterial isolates, but the water, ethanol, 1%HCl and acetone leave and bark extracts were not antibiotic again *P. aeruginosa*. Only the petroleum ether of *Azadirachta indica* leave and bark extracts was sensitive to *P. aeruginosa* with MIC of 20.0mg/ml.

However, extracts in water, ethanol, 1% HCl, acetone and petroleum ether were bactericidal against *S. aureus* and *E. coli* (table 1 and 2).

Table 1: MIC of extracts from leaves of *Azadirachta indica* compared with standard antibiotics

Organisms Isolated	Standard anti-biotic drugs		Extraction Solutions from leaves of *Azadirachta indica (CN: Neem or dongo yaro)*				
	Perflacine	Cefuroxime	Water	Ethanol	1%HCl	Acetone	Petroleum ether
S. aureus,	4.0*	6.0*	2,500.0	200.0	2,500.0	700.0	20.0*
E.coli,	4.0*	6.0*	2,500.0	100.0**	2500.0	700.0	20.0*
P. aeruginosa	4.0*	6.0*	0.0	0.0	0.0	0.0	20.0*

** signifies statistical significant in antimicrobial activities between standard drugs and leaves of Azadirachta indica extraction solutions. ** signifies statistical significant between the different extraction solutions.*

Table 2: MIC of extracts from bark of *Azadirachta indica* compared with standard antibiotics

Organisms Isolated	Standard anti-biotic drugs		Extraction Solutions from bark of *Azadirachta indica (CN: Neem or dongo yaro)*				
	Perflacine	Cefuroxime	Water	Ethanol	1%HCl	Acetone	Petroleum ether
S. aureus,	4.0*	6.0*	2000.0	200.0	2,500.0	700.0	20.0**
E.coli,	4.0*	6.0*	2000.0	100.0**	2500.0	700.0	20.0**
P. aeruginosa	4.0*	6.0*	0	0.0	0.0	0.0	20.0**

** signifies statistical significant in antimicrobial activities between standard drugs and bark of Azadirachta indica extraction solutions. ** signifies statistical significant between the different extraction solutions.*

DISCUSSION

The results of the antibacterial sensitivity test showed that the antimicrobial potential of the extracts in petroleum was more effective than other extraction solutions herein studied. Furthermore, the different plant parts herein studied show similar antibacterial potentials, indicating comparative constituents. Although several studies have indicated that the ethanolic extracts of plants parts were more inhibitory than the aqueous extracts, which is also in line with this study considering the differences in antibacterial potential between aqueous and ethanolic extracts; thus suggests that ethanol may be a better extracting solvent (Ke-Qiang et al., 2001). However, in this study, petroleum ether was more potent, suggesting petroleum ethet to be a better extracting solvent than ethanol. Indeed, different solvents have various degrees of solubility for different phyto-constituents (Majorie, 1999).

The antibacterial properties of *Azadirachta indica* leaves and back reported in this study is in line with the report by Faiza aslam *et al. (*2009). In accordance with the present findings, Kapur et al. (1995), had reported *Azadirachta indica* in the treatment of vaginal infections. Similarly, in a 2-week double-blind, placebo-controlled clinical trial of 55 women with abnormal vaginal discharge due to bacterial vaginosis, treatment with *Azadirachta indica* oil was reported to cure the symptoms of the infection (Chinnasamy et al., 1993). Also, *Azadirachta indica* leaves has been reported to possessed good anti bacterial activity and this lead Saradhajyothi and Subbarao (2011) to conclude it confirmation as a great potential of bioactive compounds and is useful for rationalizing the use of this plant in primary health care.

This antimicrobial potential of *Azadirachta indica* leaves and back extract may be due to it constituents. Indeed, the phyto-constituents alkaloids, glycosides, flavanoids and saponins which are importance components of *Azadirachta indica* contain antibiotic principles of plants. These antibiotic principles are actually the defensive mechanism of the plants against different pathogens (Hafiza, 2000).

Conclusively, *Azadirachta indica* leaves and back extracts demonstrated bactericidal potential against both gram-positive and gram-negative bacteria and as such an indication that the plant can be a source antibacterial drug. Hence, extracts of *Azadirachta indica* used as medicine, could be useful in the inhibition of human biotic bacterium.

REFERENCES

[1] Akihisa T, Takahashi A, Kikuchi T, Takagi M, Watanabe K, Fukatsu M, Fujita Y, Banno N, Tokuda H, Yasukawa K. *Journal of Oleo Sciences*. **2011**; 80: 53-9.
[2] Almas, K. Ansal lafi, T.R. (**1995**) The natural toothbrush. *World health Forum* 16: 206-210.
[3] Atawodi SE, Atawodi JC *Phytochemical Review*. **2009**; 8:601-20.
[4] Bhat M, Kothiwale SK, Tirmale AR, Bhargava SY, Joshi BN. Antidiabetic properties of Azardiracta indica and Bougainvillea spectabilis: *Azadirachta indica* studies in murine diabetes model. Evidence Based Complementary and Alternative Medicine. **2011**; **2011**: ID 561625. http://www.ncbi.nlm.nih.gov/pmc/articles/ PMC3136679/pdf/ECAM2011-561625. pdf [Access date 22 August **2011**].
[5] Chattopadhyay I, Nandi B, Chatterjee R, Biswas K, Bandyopadhyay U, Banerjee RK. *Inflammopharmacology*. **2004**; 12: 153-76.
[6] Chinnasamy N, Harishankar N, Kumar PU. *Food Chem Toxicol*. **1993**;31(4):297–301.
[7] De Smet, P.A.G. (**2002**). *New Engl. L. Med*. 347: 2046 – 2056.
[8] Gbotolorun S.C. Osinubi A.A., Noronha C.C., Okanlawon A.O (**2008**) *African Health Science* 8(3): 168-173.
[9] Hafiza, R.E. (**2000**) *Lancet* 349: 418-422.
[10] Haque E, Mandal I, Pal S, Baral R. *Immunopharmacology and Immunotoxicology*. **2006**; 28:33-50.
[11] Isah AB, Ibrahim YKE, Iwalewa EO. *Phytotherapy Research*. **2003**; 7: 807-10.
[12] Kapur S, Garg S, Pharma M, Upadhyay SN, Suri S, et al. et al. *Aust N Z J Obstet Gynecol*. **1995**;35(2):190–191.
[13] Ke-Qiang CAO, Ariena HC van Bruggen (**2001**). Inhibitory efficacy of several plant extracts and plant products on *Phytophthora infestans.* Journal of Agricultural University of Hebei pp.1-9.
[14] Khillare, B. and Shrivastav, T.G. *Contraception*. **2003**; 68: 225-9.
[15] Kumar S, Suresh PK, Vijayababu MR ,Arunkumar A, Arunakaran J. *Journal of Ethnopharmacology*. **2006**; 105: 246–50.
[16] Natarajan V, Venugopal PV, Menon T. *Indian Journal of Medical Microbiology*. **2003**; 21: 98-101.
[17] Orhue P.O., Edomwande E.C., Igbinosa E., Momoh A.R.M. and Asekomhe O. (**2014**). *Inter. J. Herbs* Pharmaco. Res., 3(1): 24 – 29
[18] Parida MM, Upadhyay C, Pandya G, Jana AM. *Journal of Ethnopharmacology*. **2002**; 79: 273-8.
[19] Saradhajyothi Koona, Subbarao Budida (**2011**) *Nat Sci Biol*, 3(1) 65-69.
[20] Siddiqui, B.S., Rasheed, M., Ilyas, F., Gulzar, T., Tariq, R.M. and Naqvi, S.N. (**2004**). *Z. Naturforsch* C, 59: 104-112.
[21] Sudha P, Zinjarde SS. Bhargava SY. And Kumar AR. (**2011**). Potent a-amylase inhibitory activity of Indian Ayurvedic medicinal plants. BMC Complementary Altrn. Med.
[22] Thakurta P, Bhowmika P, Mukherjee S, Hajra TK, Patra A, Baga PK. *Journal of Ethnopharmacology*. **2007**; 111: 607-12.