Antibacterial Activity of *Azadiracta indica* Stem Bark Extract against Bacterial Isolates from the Oral Cavity

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ABSTRACT

The Antibacterial activity of Azadirachta indica stem bark (ethanol and aqueous) extracts against bacterial isolates (Staphylococcus aureus, Streptococcus mutan and Streptococcus oralis) from the oral cavity of patients attending Dental Unit of Federal Medical Centre Birnin Kebbi was carried out using disc diffusion method. The extracts exhibited high degree of activities against the bacterial isolates. The ethanol extract recorded high activity of 21.0, 18.0, 18.0mm at the highest concentration of 150 used against S. aureus, S. mutan and S. oralis respectively. The aqueous extract also exhibited varying and high degrees of activity of 16.0, 18.0, and 16.0mm at 150mg/ml of the concentrations used against S. aureus, S. mutan and S. oralis respectively. The Minimum inhibitory concentration (MIC) and Minimum bacteriocidal concentration (MBC) of both extracts studied showed complete inhibitory and bacteriocidal effects against the bacterial isolates. The phytochemical screening carried out on this ethanol and aqueous extracts of Azadirachta indica stem bark revealed the presence of Alkaloids, Tannins and Flavonoids which may be responsible for the high activity of these extracts while Anthraquinones was absent. The research work has provided clues that Azadirachta indica stem bark can be used to formulate medicinal preparations against bacterial infections of the oral cavity.

Keywords: *Azadirachta Indica, exhibited, degree, phytochemical, revealed, responsible.*

Introduction

All over the world especially in developing countries, diseases caused by microorganism account for a significant cause in morbidity and mortality which is imposing great burden on health care system. The use of anti-infection drug in reducing and controlling these infection and disease caused by microbe has been in practice (Andreargachew *et al.,* 2004). It is important to search for an alternative, cheap and safer remedy to address the problem of this disease. In the past the use of traditional medicine in the treatment of infectious disease has being practiced though it was the available remedy then, currently, due to the absence of sufficient modern health care delivery system particularly in rural areas, people consult traditional healers and herbal medicine. The integration of traditional and modern medicine is gaining increased recognition globally (Andargachew *et al.,* 2004). The use of medicinal plants as herbal remedies to prevent and cure several ailments differs from community to community (kubmarawa *et al.,* 2007). The advent of science into the search for antibiotics largely depends on some of these medicinal plants as raw materials. For many years, medicine had depended exclusively on leaves, flowers and barks of plants; only recently have synthetic drugs come into use and in many instances, these are carbon copies of chemicals identified in plants. According to WHO (2001), a medicinal plant is any plant which in one or more of its organs, contains substance that can be used for therapeutic purpose or which are precursors for the synthetics of useful drugs are derived directly or indirectly from plants and in home-opathic or ayurvedic medicines, medicinal plants, their parts and extracts dominate the scene (Sharif and Banik, 2006).

Azadirachta indica otherwise known as neem is a tree in the mahogany family Maliaceae. It is one of the most used plants in the preparation of traditional medicines and serves as a source of many therapeutic agents in the Indian cultures and grows well in the tropical and semi-tropical countries. Its twigs are used as tooth brush and are widely used in the Indian sub-continent. Earlier studies of neem tree have showed that it contains active substances in almost every part of the seeds, leaves, roots, bark, trunk and branches with multiple medicinal properties. It is now considered as a valuable source of unique natural products for development of medicine against various diseases and also for the development of industrial products. *Azadirachta indica* is used for the treatment of diabetes, like neem leaf extract has a good therapeutic potential role of anti-diabetic activity. Aqueous extract of neem biscuits and shows the potential role of anti-diabetic activity. Aqueous extract of neem leaf extract has a good therapeutic potential as anti hyperglyeaemic agent. Anti-inflammatory effect of neem extract is less than that produced by dexamethasone. Neem leaves have antibacterial properties and could be used for controlling airborne bacterial contamination in the residential premise. Neem seeds are used in traditional medicine to treat infectious conditions especially those involving the eye and ear. Administration of alcoholic extract of neem flower disrupts the estrous cycle in Sprague Dawley rats and causes a partial block in ovulation and has the potential of an ideal antifertility agent. Neem aqueous extract has powerful chemotherapeutic and viral agent (Biswas et al., 2002).

Medicinal Properties of Neem

For thousands of years the beneficial properties of Neem *(Azadirachta indica A. Juss)* have been recognized in the Indian tradition. Each part of the neem tree has some medicinal properties (Biswas, *et al.*, 2002).

Leaf: Leprosy, skin problems, skin ulcers, intestine worms, anorexia, eye problems, epistaxis biliousness.

Bark: Analgesic, curative of fever.

Flower: Elimination of intestine worms, phlegm, bile suppression.

Fruit: Diabetes, eye problem, piles, intestine worms, urinary disorder, wounds, leprosy, epistaxis.

Twig: Asthma, cough, piles, intestine worms, obstinate urinary disorder, phantom tumor, spermatorrhoes.

Gum: Scabie, wounds, ulcer, skin disease.

Seed: Intestine worms and leprosy.

Oil: Intestine worms, skin diseases and leprosy

Root: Refrigerant, diuretics.
(Biswas et al., 2002)

Significance of the Study

Since prehistoric time, humans have used indigenous plants to treat infectious diseases and to maintain oral hygiene (Okemos *et al.*, 2001). While a number of synthetic and natural antibacterial agent are available for controlling bacterial infections, increased resistance cells for new antibacterial drugs, one source of which are traditional medicinal plants (Owour *et al.*, 2002). Many medicinal plants around the world contain many compounds with antibacterial activity (Marjoric, 2006). Moreover, to many communities in the developing countries, antibacterial drugs and pharmaceuticals are not accessible to the majority of the people who need them. It is thus important to test for the efficacy of stem bark extract of this plant against bacterial isolates from the oral cavity in order to ascertain the potency of the plant extracts in maintaining oral hygiene (Majorie, 2006).

Materials and Methods

Sample Collection

The Stem bark of *Azadirachta indica* used in this research work was obtained from Birnin Kebbi Central Market and transported to Microbiology Laboratory of Waziri Umaru Federal Polytechnic, Birnin Kebbi, Kebbi State.

Preparation of Plant Material

The freshly collected Stem bark of *Azadirachta indica*were was washed thoroughly with running tap water, pealed and then dried under shade at room temperature for a period of seven days until they were completely dry. The small pieces were pounded into powdered using wooden mortar and pestle (Elliot et al., 2007).

Extraction of plant materials and formation of concentrations

The extraction of the Stem bark of *Azadirachta Indica* sample was done in accordance to the method proposed by El-kamali and Mahjoub (2009). 100g of the powdered extract of Stem bark of *Azadirachta indica* was weighed, soaked in 250ml of ethanol for 72hours and the mixture was shaken after some interval of time, the mixture was filtered using whattman No.1 filter paper and the filtrate was concentrated using water bath 40^0C to obtained the crude ethanol extract.

The Stem bark of *Azadirachta Indica* was extracted using warm water; 100g of powdered sample was soaked in 250ml of distilled water and was allowed to stay for 24hours and was sieved and evaporated to obtain the crude aqueous extract.

In this way the ethanol extract and the aqueous extract obtained were used for antibacterial sensitivity test. Exactly 1g of the prepared ethanol extract of *Azadirachta indica* was measured using weighing balance and poured into 10ml of distilled water. The same procedure was repeated for 5g, 10g, and 15g, to obtain various concentrations of 10mg/ml, 50mg/ml, 100mg/ml and 150mg/ml respectively. The same procedure was repeated for the aqueous.

Preparation of Media

The media used in this research work were prepared in accordance to the manufacturer's instructions. The media used are nutrient broth, nutrient agar, Mueller Hington Agar and mannitol Salt Agar.

Nutrient Broth

Nutrient Broth was prepared according to the manufacturer's instruction. 2.6g of nutrient broth was weighed and dissolved in 200ml of distilled water. It was then sterilized in an autoclave at 121^0C for 15 minutes at 15 Pascal.

Nutrient Agar

Nutrient Agar (28g) was weighed according to the manufacturer's instruction and taken into a conical flask containing 100cm^3 of distilled water. This mixture was sterilized in an autoclave at 121^{0}C for 15 minutes at 15 Pascal (Moellering, 2010).

Mannitol Salt Agar

31.3mg of the agar was weighed and dissolved in 125ml of distilled water and was sterilized using autoclave at 121^{0}C for 15 minutes at 15 Pascal.

Mueller Hington Agar

Mueller Hington Agar was prepared according to the manufacturer's instruction. 7.6g of Mueller Hington Agar was weighed in a weighing balance and dissolved in 200ml of distilled water. It was sterilized in an autoclave at 121^{0}C for 15 minutes at 15 Pascal.

Test Organisms

The micro-organisms used for this study are clinical samples, which are *Staphylococcus aureus, Streptococcus mutan and Streptococcus oralis.* Swab sticks were used to swab the infection area of the oral cavity of patients. The swab stick was then put into test tubes containing nutrient broth and was incubated at 37^{0}C for 24hours. This was then sub-cultured into nutrient agar to obtain pure colonies.

Gram Staining

From the colonies that developed on Nutrient Agar, a smear was made on a clean glass slide using sterile wire loop. It was dried and heat fixed. The smear was flooded with crystal violet solution for 60 seconds and washed, tipped off and covered with Lugol's iodine for 2 minutes. The stain was then decolorized with acetone and washed off immediately with distilled water. It was then counter stained with safranin for 2 minutes and rinsed with distilled water. The back of the slide was wiped clean; the smear was placed on a draining rack and allowed to air dry. The smear was then viewed under the microscope using oil immersion objective x 100. Biochemical tests were carried out as describe in Oyeleke and Manga (2008).

Antibacterial Activity

The antibacterial testing was carried out according to the method proposed by Mohan *et al.* (2011) as well as Oyeleke and Menga (2008). Mueller-Hing agar was prepared and the plates were allowed to solidify, the sterilized filter paper discs were soaked into the various concentrations of the Stem bark of *Azadirachta indicia (*ethanol and Aqueous) extracts. The soaked discs were dried and then placed on the plates containing the test bacteria *(Staphylococcus aureus, Streptococcus mutan and Streptococcus oralis).* This was done in duplicate. Ampicillin antibiotic impregnated disc was used as positive control. The plates were then incubated at 37^{0}c for 24 hours and the zone of inhibition was measured, recorded and expressed in millimeters (Mohan *et al.*, 2011).

Determination of (MIC) and (MBC) of the Crude Extracts

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the crude extracts on the test bacteria *(Staphylococcus aureus, Streptococcus mutant and Streptococcus oralis)* were determined according to the method proposed by Samie *et al.* (2005) and Omori *et al.* (2012).

The MIC was the concentration in the tube that failed to show evidence of growth (turbidity), just immediately after the last one that showed growth. MBC were the tubes that fail to show any growth including the MIC and were cultured on nutrient agar. The absence of growth after incubation indicated a positive result for MBC.

Phytochemical Screening

The physiochemical screening of the plants extract was conducted using standard procedure of Oyewole et al. (2010). The extract was tested for the presence of alkaloids, saponins, tannins, flavanoids, anthraquinones, glycosides and phenolic compounds.

Results and Discussion

The results of this research work are presented on tables as follows:-

Table 1: Shows Morphology and Biochemical characteristics of the test bacteria isolates obtained from the oral cavity.

	Bacteria Isolates confirmed		
Biochemical Test	**SA**	**SM**	**SO**
Grain reaction	+ve	+ve	+ve
Catalase	+ve	-ve	-ve
Coagulase	+ve	-ve	-ve
Mannitol	+ve	-ve	-ve
Citrate	+ve	+ve	+ve
Motility	-ve	+ve	+ve
Indole	-ve	-ve	-ve
Urease	+ve	+ve	-ve
Methyl Red	+ve	+ve	+ve
Voges Proskauer	-ve	-ve	+ve
Lactose	+ve	+ve	+ve
Sucrose	+ve	+ve	-ve
Glucose	+ve	-ve	-ve
H_2S	-ve	-ve	-ve
Gas	-ve	+ve	-ve

Key:
SA = *Staphylococcus aureus*
SM = *Streptococcus mutan*
SO = *Streptococcus oralis*
+ve = present
-ve = absent.

Table 2: Shows mean zone of inhibition of *Azadirachta Indica* ethanol stem bark crude extract obtained against the test bacteria.

Test Bacteria	**Mean zone of inhibition in (mm)/Concentration in (mg/ml)**				
	10	**50**	**100**	**150**	**APX control**
Staphylococcus Aureus	11.0	14.0	21.0	21.0	21.0
Streptococcus Multan	11.0	12.0	8.0	18.0	16.0
Streptococcus Oralis	11.0	12.0	14.0	18.0	18.0

Key:
APX = Ampicillin
Mm = millimeter and
mg/ml = milligram/ml

Table3: Shows mean zone of inhibition of *Azadirachta Indica* stem bark aqueous extract obtained against the test bacteria.

Test Bacteria	**Mean zone of inhibition in (mm)/Concentration in (mg/mi)**				
	10	**50**	**100**	**150**	**APX control**
Staphylococcus aureus	11.0	14.0	21.0	16.0	21.0
Streptococcus mutan	8.0	12.0	13.0	18.0	16.0
Streptococcus oralis	8.0	14.0	14.0	16.0	18.0

Key: APX =ampicillin, mm=millimeter and mg/ml=milligram/ml.

Table 4: Shows the Phytochemical Components of *Azadirachta Indica* stem bark Extracts.

Phytochemical Components	**Levels of their presence**	
	EE	AE
Alkaloids	++	++
Tannins	++	++
Flavonoids	++	++
Saponins	+	+
Steroids	++	+
Terperiods	+	+
Glycosides	+	+
Anthraquinones	-	-

Key:
EE= Ethanol extract
AE = Aqueous extract
++= highly present
+= Slightly present
-= Absent

Discussion

All over the world especially in developing countries, disease caused by microorganisms account for high rate of morbidity and mortality that is imposing burden on health care facilities. The use of antimicrobial drugs in reducing and controlling these infections and diseases caused by microorganisms has been practiced and many of which are synthetic drugs causing various side effects and also allergic reactions. However, drug development plant based compounds could be useful in meeting this demand for newer drugs with minimal side effects. It then became important to search for cheap, available and safer remedy to address the problem of these diseases.

The antibacterial activity of *Azadirachta indica* stem bark ethanol crude extract and aqueous extract against bacteria isolates *(Staphylococcus aureus, Streptococcus mutan, and Streptococcus oralis)* from the oral cavity of patients with dental carries as shown in Table 1, 2, and 3. From Table 4 the ethanol crude extract of *Azadirachta indica* stem bark indicates high activity against the test bacteria at all the concentrations of the ethanol extract used. Highest activity of 21.0mm recorded against *S. aureus* at the highest concentration of 150mg/ml of the ethanol extract used. From Table 3, the aqueous extract of *Azadirachta indica* stem bark indicated high antibacterial activity against the test bacteria; the most potent activity was recorded against Streptococcus

mutan with activity of 18.0mm at the highest concentration of 150mg/ml used. This is similar to the report of Okemo *et al.* (2001), that the crude extract of *Azadirachta indica* was very effective against both gram positive and gram negative bacteria and fungi isolates. Ampicillin impregnated antibiotic was used as a positive control which was also effective against the test bacteria.

The minimum inhibitory concentration (MIC) and the minimum bacteriocidal concentration (MBC) of *Azadirachta indica* both ethanol crude extract and aqueous extract study against the test bacteria revealed that both extracts have bacteriostatic and bacteriocidal properties and it shows increase in activity with increase in the concentrations of the extract. This is similar to the findings of Delbano (2004) which says that substance of the same extracts has potentials for antimicrobial activity.

From Table 4, the phytochemical screening carried out on both the ethanol crude extract and aqueous extract of *Azadirachta indica* stem bark revealed the presence of many potential biologically active compounds such as Alkaloids, Tannins, Flavonoids, Saqpoonins, Steroids, Terpenoids and Glycosides while Anthraquinones was absent. The presence of biologically active compounds could be associated with antimicrobial activity of plant extracts (Barreto *et al.*, 2008).

Conclusion

The test bacteria used in this research were obtained from patients attending dental unit of Federal Medical Centre, Birnin Kebbi. The antibacterial properties studied indicated high activity of the extracts against the test bacteria it isolates. The minimum inhibitory concentration and minimum bacteriocidal concentration studied revealed the stem bark of *Azadirachta indica* has great bacteriostatic and bacteriocidal effects and the responses of these test bacteria to the extracts could be as a result of the presence of potential phytochemical compounds. This suggests that the stem bark of *Azadirachta indica* could be used for medicinal oral preparations to take care of bacterial infection of the oral cavity or to take care of infections or disorders that may be caused by these test bacterial isolates.

Reference

Andargarchew, M., Belay. T. and Fetene, D. (2004): Invitro assessment of the antimicrobial potential of Azdiranchta indica on common human's pathogen ethiop. Journal Health Development 2004" 18(2): 107-111.

Barreto, J.C. Trevisan M.T.S., Hull W.E., Erben G., De Brito E. S., Pfundstein B., Wurtele G. Speigelhalder, B. and Owen r.W. (2008): "Characterization and quantitation of polyphenolic compounds in bark, kernel, leaves, and peel of mango (Mangifera indica L.)". Journal of Agricultural and Food Chemistry 56 (14): 5599-5610.

Biswas, K., Chattopadhyay, I., Banerjee, R.K. and Bandyopadhyaya, U. (2002): Biological Activities and Medicinal Properties of Neem (Azadiranchta Indica). Curr. Sci. 82 (11): 1336-1345.

Delbano, T. L. (2004): Unconvential medicine in the United States, Prevalents, coats and pattern of use. The New England Journal of Medicine (NEJM), London. 328:246-252.

El-Kamali, H.H. and Mahjoub, S.A.(2009): "Antibacterial activity of Francoeuria cripsa, Pulicaria undulate, Zizphus spina-Christ and Cucurbita pepo against seven standard pathogenic bacteria.". Ethnobotanical Leaflets, vol. 13: pp 722-723.

Elliott, T., Worthinton. T., Osman, H. and Gill, M. (2007): "Medical Microbiology and infection. Fourth Edition, Blackwell Publishing, Massachusetts, U.S.A." pp 153 – 166.

Kubmarawa, D., Ajoku, G.A., Enwerem, N.M. and Okorie, D.A. (2007): Preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Nigeria. African journal of biotechnology. 6 (14): 90-1696 (http://www.academicjournals.org/AJB)

Marjorie, M.C. (2006): Plant products as antimicrobial agents. Clinical Microbiology Reviews, American Society for Microbiology. Department of Microbiology, Miami University, Oxford, OH, USA, 12, 564-582.

Mohan, N., Jassal, P.S., Kumar, V. and Singh, R.P. (2011): Recent research in Science Technology; vol.3(9): pp 78-83.

Okemo, P. O., Mwatha, W.E., Chabra, S.C. and Fabry, We. (2001): The kill kinetic of Azadiranchta indica a joss extract on staphylococcus aureus, E. coli, Pseumdomonas aeruginosa and Candida albicans. African journal technology. 2;113-118.

Omori, R., Cowling, B.J. and Nishiura H. (2012): How vaccine effectiveness scaled by the transmission dynamics of interacting pathogen strains with cross-protective immunity? Journal pone, 7(11), 507-51.

Owuor, B, Chege J, Orwas C, Simon A.J., Salimm A.S. and Mutua A. (2002): World Agroforestree database. Nairobi Center, Kenya (http://www.worldagroforestrycenter.org).

Oyeleke, B.S. and Manga, S.B. (2008): Essential Laboratory Practical in Microbiology, 1st Ed, Tobest Publisher Minna, 23-62.

Oyewole, O.I., Owoseni, A.A and Faboro, E.O, (2010): "Studies on medicinal and toxicological properties of Cajanus cajan, Ricinus communis and Thymus vulgaris leaf extracts." Journal of Medicinal Plants Research, Vol. 4(19): Pp 2004 – 2008.

Samie, A., Obi, C.L., Bessong, P.O. and Namrita, L. (2005): "Activity profiles of fourteen selected medicinal plants from Rural Venda communities in South Africa against fifteen clinical bacterial species." African Journal of Microbiology Research, 1(3): pp 46-50.

Sharif, M.D.M. and Banik G.R. (2006): Status and Utilization of Medical plants in Rangamati of Bangladesh. Res. J. Agric. Biol. Sci. 2(6): 268-273.

World Health Organization, (2001): Quality Control Methods for Medicinal Plant Materials. World Health Organization, Geneva. Pp. 1 and 2. Pp 164-180.