



ANTIBACTERIAL EFFECT OF NEEM *Azadirachta indica* STEM BARK EXTRACT ON SOME DENTAL PATHOGENS

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Abstract

The present study was carried out to evaluate the activities of neem stem bark extract against some dental pathogens using disc diffusion method. The pathogenic bacteria used were *Streptococcus mutans* and *Escherichia coli*. The organic extracts of the stem bark extract were prepared using different solvents Methanol and distilled water and were screened for its antibacterial activity. Among the two extracts of neem stem bark, methanolic extract showed stronger antibacterial activities against *S. mutans* with inhibition zone of 14 mm at 1000 µg, 10mm at 500 µg, and 8mm at 300 µg concentrations while the aqueous extract showed activity at 1000 µg with inhibition zone of only 10mm and 8mm at 500 µg. The results demonstrate that the methanolic extracts of neem stem bark have a strong antibacterial activities and suggested that it has potentials to be use in the treatment of dental caries. The neem stem bark extract did not have antibacterial activity against *E. coli* except at 1000 µg.

Key Words: Neem extract, Dental pathogens, antibacterial, *Streptococcus mutans*, *Escherichia coli*

INTRODUCTION

Bacterial plaque plays the primary role in the pathogenesis of the disease. Dental plaque is a general term for the diverse microbial community (predominantly bacteria) found on the tooth surface, embedded in a matrix of polymers of bacterial and salivary origin. Plaque is an example of a biofilm; current researches have shown that the properties of bacteria associated with a surface in a biofilm can be different from those



of the same cells growing in liquid broth (planktonic cells). Plaque is found preferentially at protected and stagnant surfaces, and this is one the causes of Tooth decay .

Azadirachta indica commonly known as neem belongs to the *Maliaceae* family, it is native of India and naturalized in most of tropical and subtropical countries, it is of great medicinal value and distributed wide spread in the world. The Chemical constituents contain many biologically active compounds that can be extracted from neem, including alkaloids, flavonoids, triterpenoids, phenolic compounds, Carotenoids, steroids and ketones. *Azadirachtin* is actually a mixture of seven isomeric compounds labeled as *azadirachtin* A-G and *azadirachtin* E is more effective (Verkerk *et al.*, 1993). Other compounds that have a biological activity are salannin, volatile oils, meliantriol and nimbin (Jacobson, 1990).

Extracts from the Neem tree (*Azadirachta indica* A Juss) also called 'Dogonyaro' in Nigeria are most consistently recommended in ancient medical texts for gastrointestinal upsets, diarrhoea and intestinal infections, skin ulcers and malaria (Schmutterer, 1995). All parts of Neem plant such as leaves, bark, flower, fruit, seed and root have advantages in medical treatment and industrial products. Its leaves can be used as drug for diabetes, eczema and reduce fever. Barks of Neem can be used to make toothbrush and the roots has an ability to heal diseases and also as an insecticide. (Puri, 1999). The seed of Neem tree has a high concentration of oil which is widely used as insecticides, lubricant, drugs for variety of diseases such as diabetes and tuberculosis (Ragasa *et al.*, 1996).

S. sobrinus, *S. mutans* play a major role in tooth decay, metabolizing sucrose to lactic acid using the enzyme glucansucrase. *S. mutans* is one of a specialized organisms equipped with receptors that improve adhesion to the surface of the teeth. Sucrose is used by *S. mutans* to produce a sticky, extracellular, dextran-based polysaccharide that allows them to cohere, forming plaque. Sucrose is the only sugar that *S. mutans* can use to form the sticky polysaccharide. However, the combination of plaque and acid leads to dental decay. (Madigan *et al.*, 2005).

About 80 percent of the populations in the world who live in rural areas still start their day with a chewing stick. There are at least six types of sticks used which include Neem, Mango, Guava, and roots of Pilu. Among all, neem stick is most commonly used all over the world. There is therefore a need to study the antibacterial effect of neem stem bark extract on some dental pathogens.



MATERIALS AND METHODS

Collection of samples

The plant samples were randomly collected around the compound of Bayero University Kano.

The plant was first identified in the Botanic Garden of Bayero University, Kano it was further confirmed and authenticated at the herbarium section.

Preparation of extract and Disc

The bark was thoroughly washed under running tap water, rinsed with distilled water and finally air dried (indoors). The dried barks were manually powdered using mortar and pestle as described by (Adoum *et al.*, 1993). The powdered content was then stored in an air dried container.

In the methanolic extract, 25g of neem stem bark powder was soaked in 250ml of methanol and left for 72hours to form percolates. For the aqueous extract, 25g of same powder was soaked in 250ml of distilled water and left for 72hours to form percolates. The percolated mixtures were filtered and the filtrates were evaporated to dryness using evaporating dish on a water bath at 50°C. The extracts were separated and kept in a suitable container for further use. The discs were prepared from sterile Whatman no.1 filter paper.

Discs of 6mm diameter were punched with the aid of a paper puncher and placed into bijou bottles in batches of 10 discs per bottle. The bijou bottles containing the discs were sterilized by autoclaving at 121°C for 15mins and allowed to cool. The stock solution of the methanolic extract was obtained by dissolving 1g of the extract in 1ml of dimethyl sulphur oxide (DMSO) in separate bijou bottles to yield 1000000ug/ml. From the stock solution, 0.01ml was transferred to a bottle containing 0.09ml of DMSO. This gives the concentration of 100ug/ml to which 10 discs were added such that after even distribution with the help of shaking at equilibrium each one of the discs absorbs 0.01ml. Also from the stock, 0.03ml was pipetted into another clean bottle containing 0.07ml of DMSO with 10 discs to yield the concentration of 300ug/ml. 0.05ml from the stock was also added to 0.05ml of DMSO in a separate bottle to yield 500ug/ml. Lastly, 0.1ml was pipetted into another bottle containing 0.9ml of DMSO. They were all shaken and the discs were added to yield a concentration of 1000ug/ml. All the solutions in the bottles were 100ug/disc, 300ug/disc, 500ug/disc and 1000ug/disc respectively.

Standadization of Inoculum

The standard solution (Barium Sulphate standard turbidity solution) was prepared as described by (Cheesbrough, 2000).

A measure of 1ml of concentrated tetraoxosulphate VI acid (H_2SO_4) was added 99ml of distilled water after which 0.5g of Barium Chloride ($BaCl_2 \cdot 2H_2O$) (BDH) was dissolved in water until the final volume of the solution reached 50ml mark. Then 0.6ml of Barium Chloride was then added to 99.4ml of the 1% H_2SO_4 solution and shaken. The above processes resulted in turbid solution; a small volume of the turbid solution was taken in test



tube which was used for comparison during standardizing inoculums of the test organisms. (Cheesbrough, 2000)

For standardizing the inoculums, the procedure of (Kirby, 1996) was employed as follows: The test organisms were subcultured on nutrient agar plates and incubated overnight. A Colony of the test organism was carefully picked using loop and transferred into a tube containing 2.0ml of normal saline until the turbidity of the suspension matches that of the standard turbidity solution. This is easier by viewing against a printed white sheet of paper. Where the inoculums are more turbid, a little more normal saline was added until its turbidity matched with the McFarland standard.

Antibacterial Assays

In the sensitivity test carried out, the activity of the extracts was evaluated by disc diffusion method as described by Kirby, 1996. During the test, nutrient agar plates and chocolate were prepared. The surface of the agar was dried in a hot-air oven. Using sterile swab sticks, the agar plates were aseptically inoculated with the test organisms which were previously standardized by the Mcfarland standard to ensure even distribution of the test organism off the plates. With the aid of a sterile forceps and syringe needle, discs containing different concentration of the extract (100ug, 300ug, 500ug and 1000ug) were impregnated firmly on the surface of inoculated plates. Control discs were also incorporated onto the inoculated plates, two controls were used these are: the positive and negative controls. The negative control discs were impregnated with the diluent i.e. DMSO while the positive control is a standard disc of ofloxacin. Both discs were sufficiently spaced to prevent overlapping of the zones. The plates were then allowed for the pre-diffusion time of 15mins and they were incubated at 37°C for 24hours. The zones of inhibition formed were measured after incubation with the aid of a meter rule to determine the effectiveness of the extract on the organisms (Reagor *et al.*, 2002)

RESULTS

The result analyses carried out on Neem (*Azadirachta indica*) stem bark extract using two different solvents, which are the Methanolic and Aqueous (distilled water) it was observed that the physical characteristics are found to be soft red and dark red respectively as shown in Table 1.

Table 1: Some Physical characteristics of the extracts of *Azadirachta indica*

EXTRACT	SAMPLE (g)	EXTRACT YIELD (g)	COLOUR	TEXTURE
Methanolic	25	2	Red	Soft
Aqueous	25	1	Dark-red	Soft

Table 2. Shows the antibacterial activity pattern of the methanolic extract, which from the zones of inhibitions, the extract has more effect on the *Streptococcus mutans* and at the highest concentration (1000ug).



Also, the bioassay shows the antibacterial activity pattern of the Aqueous extract in table 3. The extract shows no effect on *Escherichia coli*.

Neem has many antimicrobial properties against bacterial strains causing dental caries. The pathogenic bacteria such as *Streptococcus mutans*, *Streptococcus salivarius* and *Fusobacterium nucleatum* were isolated from dental caries.

Table 2: Antibacterial activity of methanolic stem bark and Aqueous stem bark extract of *Azadirachta indica*

Bacterial Isolates	Diameter of zone of inhibition(mm)/ concentration of extract (µg/ml)											
	Methanolic Stem Bark						Aqueous Stem Bark					
	100	300	500	1000	(-) Control	(+) Control	100	300	500	1000	(-) Control	(+) Control
<i>Streptococcus mutans</i>	0.0	8.0	10.0	14.0	0.0	20.0	0.0	0.0	8.0	10.0	0.0	20.0
<i>Escherichia coli</i>	0.0	0.0	0.0	8.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

DISCUSSION

From the results obtained in the study it can be seen that the neem extracts had varying inhibition potential on the test bacteria. The methanolic bark extract showed antibacterial activity at most of the concentrations used against *Streptococcus mutans*. Similar results have been reported by (Biswas *et al.*, 2002) with the aqueous extract of neem, where it was demonstrated that the bark extract showed antibacterial activity at all the concentrations used against *Pseudomonas aeruginosa*, *Corynebacterium diphtheriae* and *Bacillus spp* this is in agreement with the findings of (Biswas *et al.*, 2002) who demonstrated that neem bark was useful in the treatment of cough, fever, lost appetite and wounds. Prashant, 2007 conducted a study to evaluate the antimicrobial effects of these chewing sticks on the microorganisms *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus mitis* and *Streptococcus sanguis*. Which are involved in the development of dental caries. (Vanka *et al.*, 2001) studied the antibacterial effect of Neem mouthwash against salivary levels of *Streptococcus mutans* and *Lactobacillus* has been experienced over a period of 2 months. There is therefore a need to study the antibacterial effect of neem (*Azadirachta indica*) stem bark extract on some dental pathogens.

The effect exhibited by methanol extract was higher than that produced by aqueous extract . The aqueous extract showed no effect on *Escherichia coli*. Similar results were obtained with the methanolic leaf extracts of *Azadirachta indica* on *Escherichia coli*; extract concentrations of 1 mg, 3 mg and 5 mg of *A. indica* had no inhibitory effect on *E. coli* . The zero zone of inhibition on *E. coli* at concentrations of 1, 3 and 5 mg also agreed with reports by Yagoub *et al.* (2007) who in their preliminary screening for anti-microbial activity of different plants against different organisms, methanolic extracts of *A. indica* produced zero zone of inhibition against *E. coli*. The difference in the effect of this plant extracts within the organisms suggested that there are different antibacterial compounds in the plant extracts and that the



compound that acted on one may not be the same as the one that acted on the others since antibacterial agents have different modes of action (Aliu, 2007). This phenomenon of varied susceptibility was also observed by Ergene *et al.*, (2006). The kill-time of both the methanol and aqueous extracts of *A. indica* on Gram-negative organisms was much longer than on Gram-positive organisms. This might be due to the more complex nature of the cell wall of Gram negative organisms as compared with Gram positive organisms. The cell wall of Gram-positive organisms is single-layered; while that of Gram-negative bacteria is multilayered and also bound by an outer cell membrane .

Some phenolic compounds isolated from *F.carica* exhibit anticaries activity either due to growth inhibition against *mutans Streptococci* or due to the inhibition of glucosyltransferases (Kwasi, 2011). Also the methanol extract of neem leaf was tested for its antibacterial, antisecretory and antihemorrhagic activity against *Vibrio cholerae*. *A. indica* extract had significant antibacterial activity against the multi-drug-resistant *Vibrio cholerae* of serotypes O1, O139 and non-O1, non-O139 [Mukherjee *et al.*,2007]. Also the acetonic extract of *T.chebula* was more potent against *S.mutans* compared to other tested extracts. Methanol and aqueous extracts of *Azadirachta indica*, the tested chewing sticks had inhibitory effect on clinical isolate of *Streptococcus mutans*.

A study has shown that neem extract produced the maximum zone of inhibition on *Streptococcus mutans* at 50% concentration. Even at 5% concentration neem extract showed some inhibition of growth for all the four species of organisms, i.e. *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus mitis* and *Streptococcus sanguis* (Prashant , 2007)

Study done by (Bhuiyan, 1997) has shown that acetonic extract from the bark of neem was bactericidal at concentration <1% (w/v) which indicates that neem bark constituents are considered to have the ability to suppress the growth of bacteria. .

CONCLUSION

The present work has shown that *Streptococcus mutans* and *Escherichia coli* were susceptible to extracts of *A. indica in vitro* which means the plant has antibacterial property. The results of this study suggest that the bark of *A. indica* can be used as an antibacterial agent against infections caused by *Streptococcus mutans* and *E. coli*.

RECOMMENDATIONS

It is recommended that the phytochemical screening of the neem (*Azadirachta indica*) should be carried out to determine the specific phytochemicals that are of effect to the dental pathogens.



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