

Effect of Neem Extracts (*Azadirachta indica*) on Bacteria Isolated from Adult Mouth

¹M.B. Yerima, ²S.M. JODI, ¹K. Oyinbo, ³H.M. Maishanu, ¹A.A. Farouq, ⁴A.U. Junaidu,
⁵M.N. Al-Mustapha and ⁶A.L. Shinkafi

^{*1}Department of Microbiology, Usmanu Danfodiyo University, Sokoto, P.M.B. 2346, Sokoto State, Nigeria.

²Dept. of Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto.

³Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto. P.M.B. 2346, Sokoto State, Nigeria.

⁴Dept. of Parasitology and Animal Health, Faculty of Veterinary Sciences, Usmanu Danfodiyo University, Sokoto.

⁵Department of Pure and Applied Chemistry, Usmanu Danfodiyo University, Sokoto.

⁶Department of Science Laboratory Sciences, Abdu Gusau Polytechnic, Talata Mafara, Zamfara State, Nigeria.

ABSTRACT: The antibacterial activity of the bark, Leaf, Seed and fruit extracts of *Azadirachta indica* (neem) on bacteria isolated from adult mouth was carried out using agar- well diffusion method. The test bacteria were isolated from patients attending Usmanu Danfodiyo University Teaching Hospital, Sokoto. The bark and leaf extracts showed antibacterial activity against all the test bacteria used. The zone of inhibition increased with increase in concentrations of the extracts. The seed and fruit extracts showed antibacterial activity only at higher concentrations. The results, therefore, confirm the traditional use of neem in maintaining oral hygiene.

Keywords: Neem extracts, Oral hygiene, antibacterial activity

INTRODUCTION

Neem tree (*Azadirachta indica*) is a very common tree and belongs to the family Meliaceae. It is a tall evergreen tree with clear foliage originally native of India. *Azadirachta indica* is one of the most widespread introduced tree species in Nigeria and is extensively naturalized in drier parts of Nigeria and the most successful shade and fuel plantation tree. Neem tree is up to 20-24 meters tall with hardy and fast growing stem. The leaves are divided into numerous leaflets, each resembling a full grown leaf, small white flowers which are auxiliary bunches and 1.5 to 2cm long, green or yellow fruits with a seed in each. It is commonly used in arid and sub-arid zone in afforestation programme (Kaura 1998).

The importance of *Azadirachta indica* has been recognized by U.S. National Academy of Science, which published a report in 1992 entitled "Neem a tree for solving global problems". More than 135 compounds have been isolated from different parts of the tree. They have been divided into isoprenoid and non-isoprenoid compounds (Kumar and Parmar, 1996; Dastagir and Haq, 1997; Biswas *et al.*, 2002.). Culter *et al.*, (1995) acknowledge the fact that the mouth is a unique anatomical site in the body which is composed of multiple epithelial and mucosal surfaces as well as calcified hard tissues. These tissues are constantly bathed by the saliva in which variable moisture and presence of dissolved food as well as small food particles would seem to make the mouth an ideal environment for bacterial growth. However, the continuous flow of saliva through the mouth exerts a mechanical flushing action that removes many microorganisms causing them to be

swallowed and destroyed by the acid of the stomach in addition to the phagocytic cells and immunoglobulin content of saliva (Pelczar *et al.*, 1986). Consequently the microbes that constitute the normal flora of the mouth resist such mechanical removal by being able to adhere firmly to various surface of the oral cavity (Pelczar, 1986).

Millions of people in India and Africa use twigs as "tooth brush" everyday. Dentists have endorsed this ancient practice, finding it effective in preventing periodontal disease. It is unclear whether the benefit is due to regular gum massage or preventing plaque building or due to Neem's inherent antiseptics action or all the three. This is why this research is sought with a view to achieving the following objectives:

- i. To isolate and characterize bacteria from adult human oral cavity.
- ii. To test the effect of different neem extracts on the bacteria

MATERIALS AND METHODS

Sample Collection

Samples of four parts of *Azadirachta indica* i.e. Bark, Leaves seeds and fruits were collected from shelter belts around Usmanu Danfodiyo University, Sokoto. The samples were washed using tap water. They were air dried and pounded into a paste using pestle and mortar. The paste of each of the neem aqueous extracts was allowed to dry in the sun for five days. It was then ground into powder. The seeds were treated in the same manner after the seed coat was removed.

The powder (10g) was weighed using a weighing balance (Mettler P 200) and dissolved into 1000mL of distilled water for each of the extracts. The same procedure was repeated for extracts of 30g, 60g and 90g to obtain various concentrations of 1%, 3%, 6% and 9% respectively (Lauk *et al.*, 2003).

Isolation of Bacteria

Swab samples were collected from the mouth of patients attending Usmanu Danfodiyo University Teaching Hospital Sokoto. Sterile swab sticks were used and immediately inoculated on to blood Agar using streaking method and incubated at 37°C for 24 hours. The colonies that developed were identified using microscopy and biochemical tests (Barrow and Feltham, 1993).

Morphological Characterization of the Isolates

From the colonies that developed on blood agar and 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. This was then tipped off with Lugol's iodine for 60 seconds and washed. The smear was then decolorized with 70% ethanol and washed. This was counter stained with safranin solution for 1minute followed by rinsing with distilled water. This was then allowed to air dry before viewing under the microscope using oil immersion objective (x100).

Biochemical Characterization of the Isolates

Relevant biochemical tests were carried out to aid in the identification of the bacteria down to species level. These were: catalase, indole, urease, growth on Triple Sugar Iron (TSI), Voges –Proskauer, Methyl red, Citrate utilization test and, motility test using standard procedures, described by Barrow and Feltham, (1993) as well as Cheesbrough, (2000).

RESULTS

Table 1 shows the results of Biochemical tests used in establishing identity of bacteria isolated from adult mouth. Figures 1 and 2 show mean zone of inhibitions (mm) of neem bark, leaves, seeds and fruits obtained against the test bacteria.

The leaf extracts showed antibacterial activity at all the concentrations except at 1% (the lowest concentration), against *P. aeruginosa* and *C. diphtheriae*. This is in line with the work of Morrisville, (1996) who found out that at optimal concentrations neem leaves can be used in the treatment of wounds

and neutralization of diphtheria toxins. *Bacillus* spp. is susceptible to the neem extracts only at the higher concentration. This could probably be due to their ability to produce spores which may serve as a protective material by limiting the contact between the fragile vegetative part of the cell and the neem extracts.

The seed and fruit extracts showed antibacterial activity only at higher concentrations. The resistance of bacteria to the fruit and seed extracts at lower concentrations may be due to many reasons including the strains of the isolates and probably the extracts may be bacteriostatic. On the whole antibacterial activity of neem extracts on the test bacteria isolated from the mouth is in line with the work of Popoola, (2005).

Table 1: Morphological and Biochemical Characteristics of Bacteria Isolated from Adult Mouth

Parameters	G-ve Rods	G+ve Rods	G+ve Rods	G+ve Rods
Hemolysis	α	α	-	-
Catalase	-	+	+	+
Citrate	+	-	S+	-
Urease	-	-	+	-
Methyl red	-	+	-	-
Voges proskauer	+	-	+	-
Indole	-	-	-	+
Glucose	+	+	+	+
Lactose	-	-	-	-
Sucrose	-	+	-	-
Motility	+	-	+	+
Gas	-	-	-	-
H ₂ S	-	+	+	-
Identity	<i>P.</i>	<i>C.</i>	<i>B.</i>	<i>B.</i>
	<i>aeruginosa</i>	<i>diphtheriae</i>	<i>spp</i>	<i>spp.</i>

α = Alpha haemolysis + = present - = absent

DISCUSSION

From the results obtained in this study it can be seen that the neem extracts had varying inhibition potential on the test bacteria. The bark extracts showed antibacterial activity at all the concentrations used against *Pseudomonas aeruginosa*, *Corynebacterium diphtheriae* and *Bacillus* spp. This agrees with the findings of Biswas *et al.*, (2002) who demonstrated that neem bark is useful in the treatment of cough, fever, lost appetite and wounds.

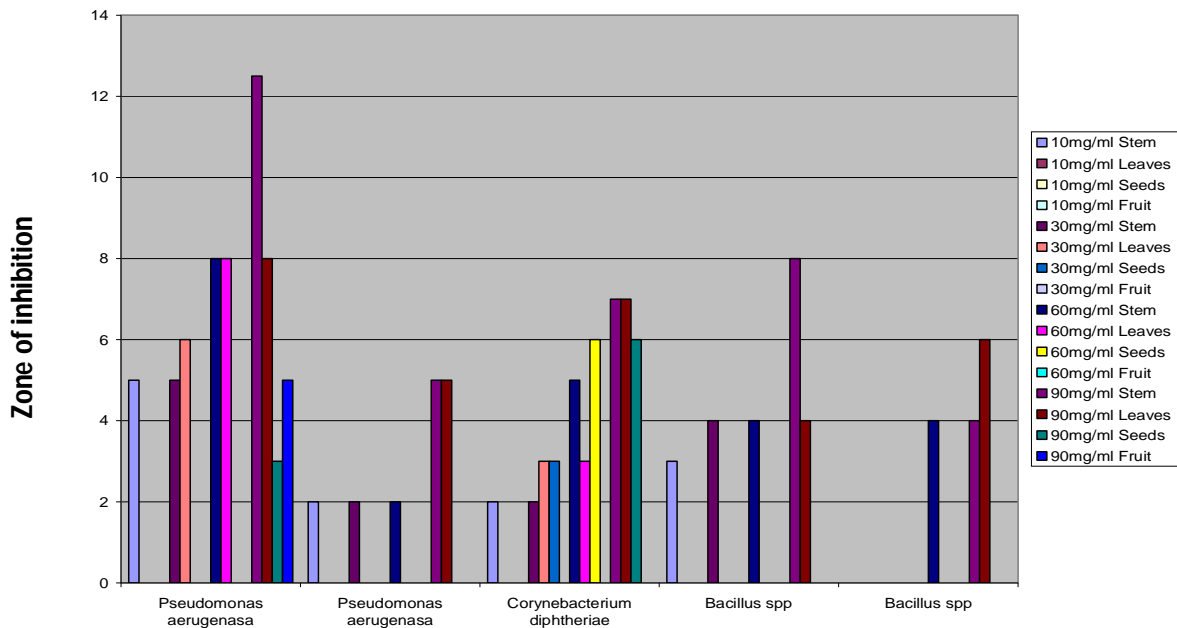


Figure 1: Mean zone of Inhibition (mm) of neem bark, leaves seeds and fruits extract from *Azadirachta indica* on bacteria isolated from adult mouth.

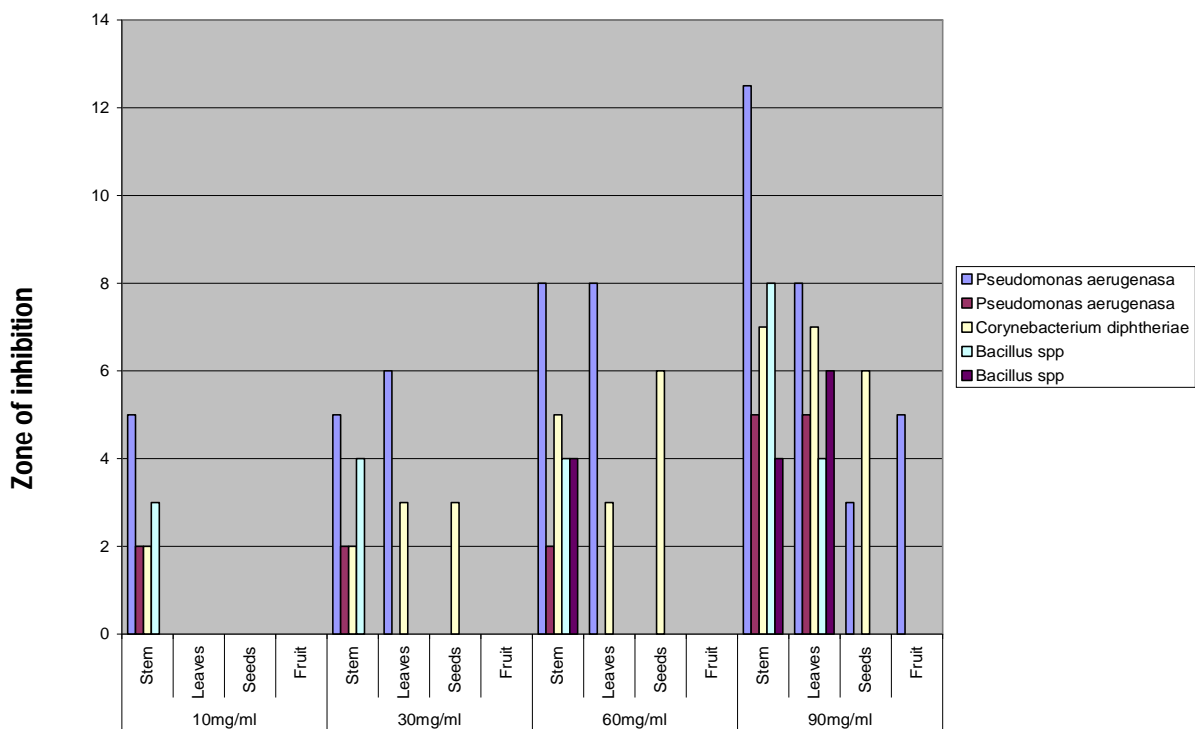


Figure 2: Sensitivity of bacterial isolates to Varying Concentrations of Neem Extracts

CONCLUSION

This study showed that extracts from *Azadirachta indica* possess antibacterial activity against bacteria present in the mouth, although the neem seed and fruit extracts failed to show antibacterial activity on the test bacteria. Thus, the results of this research

have established the use of chewing sticks made from neem in maintaining oral hygiene. Also the emerging healthcare products companies in Nigeria can explore the option of using raw materials from neem in manufacturing newer products for oral application.

REFERENCES

- Barrow, G.I. and Feltham, R.K.A. (1993). *Cowan and Steel's Manual for Identification of Medical Bacteria*, 3rd Edition,. Cambridge University Press, Great Britain. Pp. 50 –60 126
- Biswas, K., Ishiha, C.Y. and Rankjit, K. (2002). Biological Activities and Medicinal Properties of neem (*A. indica*) *Current Science* **5**: 1336-1345
- Cheesbrough, M. (2000). *District Laboratory Manual for Tropical Countries*, part 2 Cambridge Low Priced Edition. Pp. 62 -72
- Culter, C.W., John, R.K. and Corolone, A.G. (1995). Pathogenic Strategies of Oral Anaerobe. *Porphyromona gingivalis Trends in Microbiology* **2**: 46.
- Dastagir, G. and Haq, I.U. (1997). Testing Wood powder from Different plants on Human Teeth. *Journal of Science and Technology* **21(&)**: 57.
- Kaura, S.K. (1998). Plant Food for Human Nutrition. *Botanica* **52(4)**: 293
- Kumar, J. and Parmar, B.S. (1996). Compounds of medicinal Importance in Neem Tree. *Journal of Agricultural and Food Chemistry* **44(8)**: 2137-2143.
- Lauk, L., Lo Bue, A.M., Millazo, I., Rapisarda, A. and Blandino, G. (2003). Antibacterial Activity of Medicinal Plant Extracts Against Periodontopathic Bacteria. *Phytotherapy Research* **17(6)**: 599-604.
- Morrisville, P.A. (1996). *Neem leaf (Azadirachta indica)* Encapsulated Botanical Herbs html online vhsales@viableherbal.com
- Pelczar, M.J., Chan, E.C.S. and Krieg, N.R. (1986). *Microbiology*. Fifth edition, McGraw Hill, Inc. New York, St. Louis, San-Fransisco, Aukland, Bogota, New Delhi, Madrid, Mexico, Milan, Montreal, Sydney, Singapore, Tokyo, Toronto. Pp. 235-238
- Popoola, L. (2005). Poverty and Economic Viability of Neem Tree Growing in Nigeria. *A Seminar Presented at a Workshop Organized by Central Bank of Nigeria, Katsina State.*