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Research Article

Antibacterial Efficacy of *Azardirachta indica* (Neem) Against Oral Microorganisms- An *In-vitro* Study

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Abstract

Introduction: Neem Tree (*Azardirachta indica*) is a common evergreen tree with medicinal values. Neem contains the alkaloid margosine, resins, gum, chloride, fluoride, silica, sulfur, tannins, oils, saponins, flavenoids, sterols, and calcium.

Methodology: Antimicrobial susceptibility testing protocols. Schwalve, Moore and Goodwin, Crc Press 2007 were followed after the extract was prepared. The extract were stored under 5 deg.C. and used for minimum inhibitory concentration (MIC) determination in comparision with Chlorhexidine as standard.

Results: The extracts of Neem bark were tested for minimum inhibitory concentration against *A. viscosus, L. acidophilus* and *S. mutans*. Neem bark extract. s were most effective against *A. viscosus* as compared to *S. mutans* and *L. acidophilus.*

Conclusion: Neem has many medicinal benefits apart from antibacterial activity. The current study paves way to study in deep about the antibacterial activity of neem i.e. how does it works on biomolecular level.

Keywords: Neem; BHI; Lactobacillus Acidophillus; Chlorhexidin

Introduction

Indian subcontinent, the Middle East and Africa marks the use of chewing sticks to clean oral cavity since ancient times and about 3500 BC by Babylonians. Neem contains the alkaloid tannins, oils, saponins, flavenoids, sterols, and calcium [1].

Miswak is an Arabic word it means tooth cleaning stick. Numerous plant species are used as Miswak to name a few- *Salvodara persica*, *Azardirachta indica* (Neem), babool. Neem Tree (*Azardirachta indica*) is a common evergreen tree with medicinal values. It belongs to family Meliaceae. It possess antibacterial, anti cariogenic, astringent and anti-inflammatory properties [2].

The phytochemical analysis shows presence of constituents such as nimbidin, nimbin, nimbolide, azadirachtin (most effective), gallic acid, epicatechin, catechin and margolone. All are potent antibacterials [3].

Neem twig bark has shown potent antibacterial activity against oral microorganisms such as *S. mutans*, *S. mitis*, *S. sanguis*, *C- Albicans*. The present study was aimed to assess the antibacterial efficacy of neem twig bark against oral microorganisms.

Material and Methods

Material

- Neem twig bark
- Bacteria- a. *L. Acidophilus*, b. *S. Mutans*, c. *A. Viscosus*
- BHI Broth
- Ethanol
- Chlorhexidine
- Conical Flask
- Test tubes

Methodology

Collection of Neem tree bark: The Neem tree was identified by a botanist in a Garden in Campus of college premises. The fresh bark from the stem was collected and washed and dried under sun for 2 days.

It was then crushed to powder in a blender. 20 g of the ground stem bark soaked into 250 ml of ethanol (95 %) in conical flasks.

The mixture was shaken periodically and allowed to stand for about 48 hours for extraction. Then the resulting solutions were filtered using a filter paper. The filtrates were then evaporated in order to get the final extracts. The extract were stored under 5 deg .C. and used for minimum inhibitory concentration (MIC) determination in comparision with Chlorhexidine as standard.

MIC procedure

The tube with least concentration of extract without growth after incubation was taken and recorded as the minimum inhibitory concentration.

Result

(MIC)

Neem powder	100	50	25	12.5	6.25	3.125	1.6	0.8	0.4	0.2
S. mutans	S	S	S	S	S	R	R	R	R	R
CHX	S	S	S	S	S	S	S	S	S	S

Table 1: Minimum Inhibitory Concentration of Neem as compared to Chlorhexidine against *A. viscosus*.

Neem powder	100 mg/ml	50	25	12.5	6.25	3.125	1.6	0.8	0.4	0.2
A. viscosus	S	S	S	S	S	S	R	R	R	R
CHX	S	S	S	S	S	S	S	S	S	R

Table 2: Minimum inhibitory concentration of Neem bark as compared to Chlorhexidine against *L. acidophilus.*

Neem powder	100 mg/ml	50	25	12.5	6.25	3.125	1.6	0.8	0.4	0.2
L. acidophilus	S	R	R	R	R	R	R	R	R	R
CHX	S	S	S	R	R	R	R	R	R	R

Table 3: Minimum inhibitory concentration of Neem as compared to Chlorhexidine against *S. mutans*.

Figure 1: MIC – *Lactobacillus acidophilus* and neem twig.

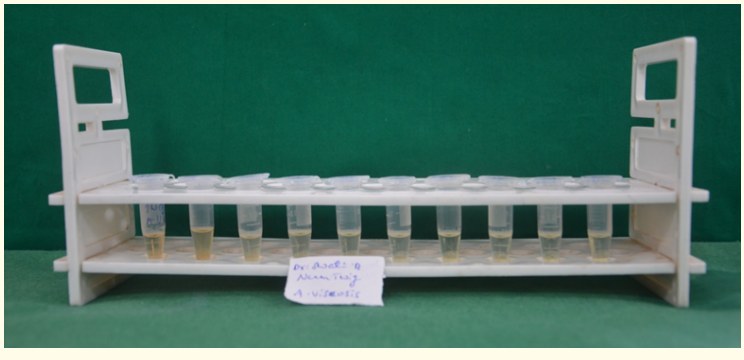

Figure 2: MIC *S. mutans* and chlorhexidine.

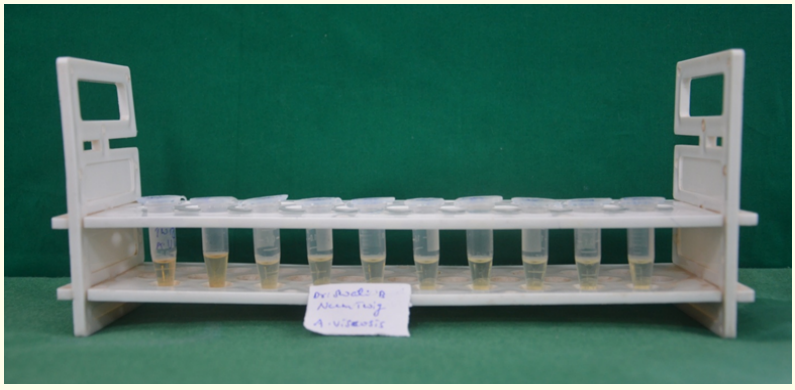

Figure 3: MIC. *A. viscosus* and neem twig.

Statistical analysis

Statistical analysis was done using SPSS software. The tests used were ANNOVA and t- test. P value was 0.05.

Discussion

The minimum inhibitory concentration of neem for *A. viscosus* was 3.125 mg/ml whereas that of chlorhexidine for *A. viscosus* was 0.4mg/ml.

The MIC of Neem for *L. acidophilus* was found to be 100 mg/ml while that of Chlorhexidine was 25 mg/ml.

The MIC of Neem for *S. mutans* was 6.25 mg/ml whereas that of Chlorhexidine was at 0.2 mg/ml.

In a study done by Bansal V., *et al*. (2019), MIC of neem for *S. mutans* was (4.2 mg/ml) [5]. In another study done Jain I., *et al*. (2015) by the zone of inhibition against *S. mutans* was 17.09 mm(result statistically significant) [6].

The antibiotics used routinely in dental practice have developed resistance which is a major concern [7].

In one study MIC of neem was more for *Candida* (5 mg/ml) as compared to *S. mutans* (4.2 mg/ml). MIC of the clove was equal against both the test organisms (5.5 mg/ml) [5].

In a study the MIC was 11mm to 21mm against *E. coli* at 25mg/ml and 125 mg/ml respectively. Thus, *E. coli* was most sensitive to the extract which is in line with a study done by Ranjit., *et al*. in the year 2014. The *S aureus* showed inhibition at 0 mm to 14 mm at the concentration of 25 and 125 mg/ml, which was same as Nwakaeze., *et al*. (2013) report. The Minimum Inhibitory Concentration (MIC) of leaf extract of *A. indica* was found to be effective against *E. coli* at the lowest concentration (50 mg/ml) [8].

In another study done by Nayak Aarti., *et al*. in 2011, the aqueous neem leaf extract at 7.5% was able to inhibit the growth of *E. faecalis*, *S. mutans* and *C. albicans* and MIC of an ethanolic neem leaf extract was 1.88%, 7.5% and 3.75% against three important dental organisms [9].

The extracts of Neem bark were tested for minimum inhibitory concentration against *A. viscosus, L. acidophillus* and *S. mutans*. Neem bark extracts were most effective against *A. viscosus* as compared to *S. mutans* and *L. acidophilus* (P-0.001) (Table 1-3).

The neem bark extract showed sensitivity for *A. viscosus* and *S. mutans* at lower concentration whereas it showed sensitivity at higher concentration for *L. acidophilus.*

Conclusion

Neem has many medicinal benefits apart from antibacterial activity. In Ayurveda, Neem tree has been considered a panacea for many ailments and is very useful in rural parts of India. The water boiled with neem leaves is excellent to control many infectious diseases. Fewer studies were available to compare with the current study which studied the minimum inhibitory concentration (MIC) of neem bark against oral microorganisms. The current study paves way to study in deep about the antibacterial activity of neem i.e. how does it works on biomolecular level.

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