

Antibacterial Efficacy of Azadirachta Indica (Neem) against oral microorganisms- An in-vitro study

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Abstract

Introduction: Neem Tree (*Azadirachta 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 comparison 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 Acidophilus*, chlorhexidine, antibacterial

Introduction:

Indian subcontinent, the Middle East & Africa marks the use of chewing sticks to clean oral cavity since ancient times and about 3500 BC by Babylonians. Neem contains the alkaloid margosine, resins, gum, chloride, fluoride, silica, sulfur, 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- *Salvadora Persica*, *Azadirachta Indica* (Neem), babool. Neem Tree (*Azadirachta 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 & 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:

1. Neem twig bark
2. Bacteria- a. *L.Acidophilus*, b. *S.Mutans*, c. *A.Viscosus*
3. BHI Broth
4. Ethanol
5. Chlorhexidine
6. Conical Flask
7. 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 comparison with Chlorhexidine as standard.

MIC procedure:[4]

1. 9 dilutions of each drug have to be done with BHI for MIC.
2. In the initial tube 20microliter of drug was added into the 380microliter of BHI broth.
3. For dilutions 200microliter of BHI broth was added into the next 9 tubes separately.
4. Then from the initial tube 200microliter was transferred to the first tube containing 200microliter of BHI broth. This was considered as 10^{-1} dilution.

5. From 10^{-1} diluted tube 200microliter was transferred to second tube to make 10^{-2} dilution.

6. The serial dilution was repeated up to 10^{-9} dilution for each drug.

7. From the maintained stock cultures of required organisms, 5 microliter was taken and added into 2ml of BHI (brain heart infusion) broth.

8. In each serially diluted tube 200microliter of above culture suspension was added.

9. The tubes were incubated for 24 hours and observed for turbidity.

Note: For facultative anaerobes, tubes were incubated at 37°C for 48-72 hrs in CO_2 jar.

For strict anaerobes, tubes were incubated in anaerobic jars for 48-72 hrs.

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

Result

Neem powder	100mg/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

Table1: Minimum Inhibitory Concentration of Neem as compared to Chlorhexidine against A. viscosus acidophilus

Neem powder	100mg/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 2: Minimum inhibitory concentration of Neem bark as compared to Chlorhexidine against L.acidophilus

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

Table3: Minimum inhibitory concentration of Neem as compared to Chlorhexidine against S.mutans

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 100mg/ml while that of Chlorhexidine was 25mg/ml.

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

Antibiotic resistance is a major concern and development of new agents from plants could be useful in meeting the demand for new antimicrobial agents with improved safety and efficacy.^[7]

The extracts of Neem bark were tested for minimum inhibitory concentration against *A.viscosus*, *L.acidophilus* and *S.mutans*. Neem bark extracts were most effective against *A.viscosus* as compared to *S.mutans* and *L.acidophilus* (P-0.001). (Table.1,2,3) .

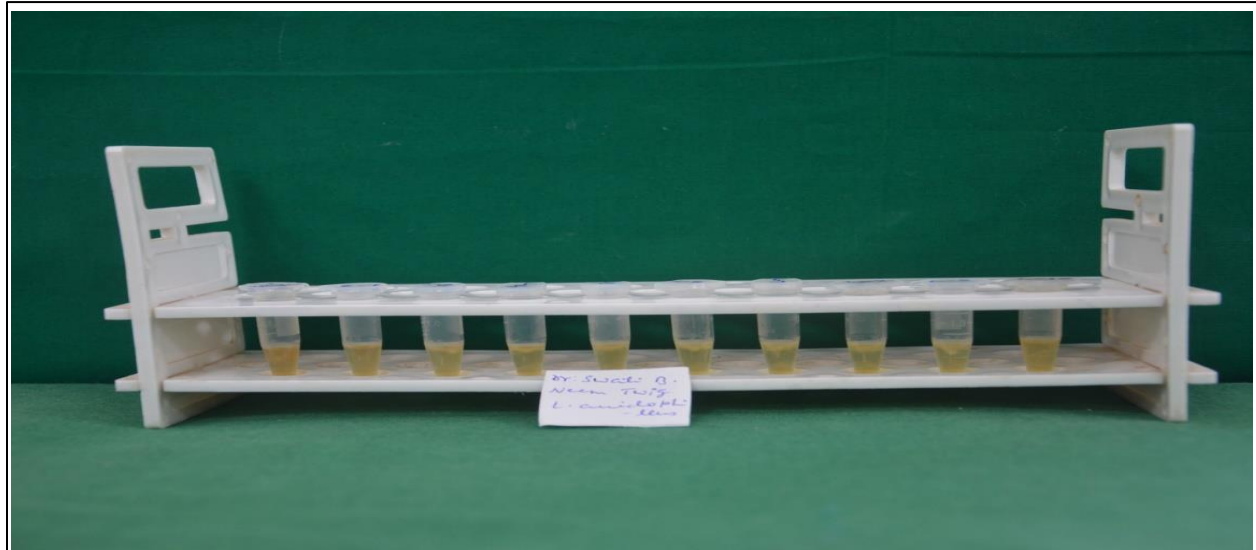
The neem bark extract showed sensitivity for *A. Viscosus* and *S.mutans* at lower concentration whereas it showed sensitivity at higher concentration for *L.acidophilus*.

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.

References:

1. Prashant GM, Chandu GN, Murulikrishna KS, Shafi ulla MD The effect of mango and neem extract on four organisms causing dental caries: *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus mitis*, and *Streptococcus sanguis*: An in vitro study .*Indian J Dent Res*, 18(4), 2007)
2. Ranjit R. Raut , Ajit RS and Bhagyashree BJ. Antimicrobial activity of *Azadirachta indica* (Neem) against Pathogenic Microorganisms. *Journal of Academia and Industrial Research (JAIR)* Volume 3, Issue 7 December 2014.
3. Santosh KS, Babita A, Akhilesh K and Archana P. Phytochemicals of *AzadirachtaIndica* Source of Active Medicinal Constituent Used for Cure of Various Diseases: A Review. *Journal of Scientific Research*, Volume 64, Issue 1, 2020
4. Antimicrobial susceptibility testing protocols. Schwalbe, Moore and Goodwin, Crc Press 2007.
5. Bansal V, Gupta M, Bhaduri T, Shaikh SA, Sayed FR, Bansal V, et al. Assessment of antimicrobial effectiveness of neem and clove extract against *Streptococcus mutans* and *Candida albicans*: An in vitro study. *Niger Med J* 2019;60:285-9.
6. Isha Jain, Pankaj Jain, Dakshina Bisht, Alosha Sharma, Binita Srivastava, and Nidhi Gupta- Comparative Evaluation of Antibacterial Efficacy of Six Indian Plant Extracts against *Streptococcus Mutans*. *J Clin Diagn Res*. 2015 Feb; 9(2): ZC50–ZC53.
7. Raja RRY, Krishna KC, Lokanatha O, Mamatha S, Damodar . Antimicrobial activity of *Azadirachta Indica* (neem) leaf, bark and seed extracts (2013) *Int. J. Res. Phytochem. Pharmacol.*, 3(1), 1-4



1. MIC -LACTOBACILLUS ACIDOPHILLUS AND NEEM TWIG



2.MIC S.MUTANS AND CHLORHEXIDINE.

