

## ORIGINAL ARTICLE

### AN IN VITRO COMPARATIVE EVALUATION OF EFFECT OF MAGNIFERA INDICA (MANGO), AZADIRACHTA INDICA (NEEM) AND ACACIA NILOTICA (BABOOL) ON *STREPTOCOCCUS MUTANS*

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#### ABSTRACT:

**Introduction:** In India, mango, neem and babool chewing twigs are considered a common way of cleaning teeth. Dental caries is steadily increasing in the underdeveloped and developing countries. These twigs possess medicinal properties. The present study was conducted to evaluate the antimicrobial efficacy of these chewing sticks on the caries causing microorganism, *Streptococcus mutans*. **Aim:** An invitro Comparative evaluation of effect of *Magnifera indica* (mango), *Azadirachta indica* (neem) and *Acacia nilotica* (babool) on *Streptococcus mutans*. **Materials and Methods:** The sticks of mango, neem and babool were grounded into coarse powder and weighed into 50gm amount. These were mixed to 100 ml of distilled water. The sticks were soaked at 4°C for 48 hours and the mixture was filtered. Then each filtrate was inoculated onto blood agar plates and incubated at 37°C for 48 hrs. **Results:** *Azadirachta indica* (neem) extract produced maximum zone of inhibition on *Streptococcus mutans* at 50% concentration after 48 hours. **Conclusion:** From this study, it could be concluded that *Azadirachta indica* (neem) was more effective against *Streptococcus mutans* as compared to *Magnifera indica* (mango) and *Acacia nilotica* (babool) at the end of 1 week.

**Keywords:** Inhibition, *Streptococcus mutans*, Dental caries.

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#### INTRODUCTION

The word caries is derived from the Latin word “rot or rotten”. Dental caries, also known as tooth decay or cavity, is a disease where bacterial processes damage hard tooth structure (enamel, dentin, and cementum). Tissues progressively break down, producing dental caries (cavities, holes in the teeth). Two groups of bacteria are responsible for initiating caries: *Streptococcus mutans* and *Lactobacillus casie*.<sup>[1]</sup> Tooth decay is caused by specific types of acid-producing bacteria that cause damage in the presence of fermentable carbohydrates such as sucrose, fructose, and glucose. The mineral content of teeth is sensitive to increase in acidity from the production of lactic acid. Specifically, a tooth (which is primarily mineral in content) is in a

constant state of back-and-forth demineralization and remineralization between the tooth and surrounding saliva. When the pH at the surface of the tooth drops below 5.5, demineralization proceeds faster than remineralization (meaning that there is a net loss of mineral structure on the tooth's surface).<sup>[1]</sup> Oral hygiene measures have been practiced by different populations and cultures in a different way around the world. In various parts of the world where tooth brushing by modern method is uncommon or not possible, the practice of tooth cleaning by chewing sticks has been commonly observed.<sup>[2]</sup>

*Azadirachta Indica* commonly known as Neem is an evergreen tree, cultivated in several parts of the Indian subcontinent. Every part of the tree is used as traditional medicine for household remedy against

various human ailments, from ancient period<sup>51-56</sup>. Neem has been extensively used in ayurveda, unani and homoeopathic medicine and has become a cynosure of modern medicine.<sup>[3]</sup>

Acacia Nilotica are the species of Indian and Africans subcontinent. Antimicrobial function is believed to be due to tannins, phenolics compounds, essential oil and flavinoids and is effective against E-faecalis.<sup>[4]</sup>

Magnifera indica (mango) belongs to the family anacardiacea. It grows in the tropical & subtropical region & its parts are commonly used in folk medicine for a wide variety of remedies. Various parts of plant are used as a dentrifice, antiseptic, astringent, diaphoretic, stomachic, vermifuge, tonic, laxative and diuretic and to treat diarrhea, dysentery, anaemia, asthma, bronchitis, cough, hypertension, insomnia, rheumatism, toothache, leucorrhoea, haemorrhage and piles.<sup>[5]</sup>

Streptococcus mutans is the most common cariogenic bacteria associated with dental caries. This bacterium has the ability to metabolize dietary sucrose and synthesize glucan by cell surface and extracellular glucosyltransferase. This glucan is an insoluble sticky or slimy gel relatively inert and resistant to bacterial hydrolytic enzymes which causes plaque to adhere tenaciously to tooth surfaces.<sup>[6]</sup>

Thus the aim of the study was An invitro Comparative evaluation of effect of Magnifera indica (mango), Azadirachta indica (neem) and Acacia nilotica (babool) on Streptococcus mutans.

## MATERIALS AND METHODS

Small twigs of Magnifera indica (mango), Azadirachta indica (neem) and Acacia nilotica (babool) were cut into pieces and were tagged for identification and sun dried for two days.

Dried chewing twigs of Magnifera indica (mango), Azadirachta indica (neem) and Acacia nilotica (babool) were grounded into coarse powder,

weighed and transferred into labeled bottle and 10ml of sterile, distilled water was added to each bottle. The mixture was soaked at 4°C for 48 hours. It was then filtered to get extracts of Magnifera indica (mango), Azadirachta indica (neem) and Acacia nilotica (babool).

Freeze dried form of micro organism “Streptococcus mutans” MTCC 890 was obtained. The ampoule containing the microorganism was opened and content was added to nutrient broth which was then incubated at 37°C for 24hrs. A sterile cotton swab was dipped into the nutrient broth, inoculated onto agar plate which was then incubated at 37°C overnight.

The growth obtained on agar plate was transferred onto blood agar plate to test the antimicrobial activity of herbal extract.

Ditches were prepared onto agar plates at 3 individual quadrants and the extracts were filled in these wells. The plates were then incubated at 37 °C for 48hrs and the inhibition zones were measured using Vernier calipers.



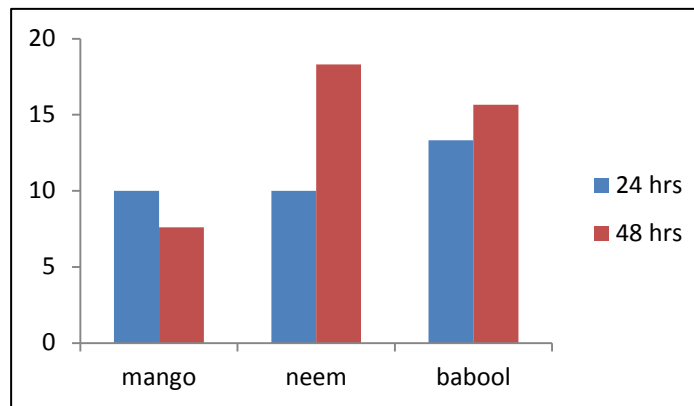
**Figure 1:** Blood agar plate

The collected data was statistically analyzed using Mean Value, One way analysis of variance (ANOVA) and Post Hoc Test using LSD.

## RESULTS

**Table 1:** Effect of concentration of mango, neem and babool extract on Streptococcus mutans at the end of 24 hrs and 48 hrs.

Concentration (50%)	Duration (in hours)	Mean diameter of zone of inhibition (in millimeters)	Duration(in hours)	Mean diameter of zone of inhibition (in millimeters)
Mango	24	10	48	7.6
Neem	24	13.33	48	18.3
Babool	24	10	48	15.66



## STATISTICAL ANALYSIS

**Table 2:** Paired sample statistics: Mean and Standard deviation of different groups at 24 hours and 48 hours were calculated

GROUPS	N	Mean± S.D	STANDARD ERROR MEAN	P
Mango (24hrs)	3	1± 0.000	0.000	0.667
Mango (48hrs)	3	9.2± 1.38	0.800	
Neem (24hrs)	3	13.3±1.52	0.88	
Neem (48hrs)	3	16.3±1.52	0.88	
Babool (24hrs)	3	9.6±0.577	0.33	0.557
Babool (48hrs)	3	14.6±0.577	0.33	

The correlation and t cannot be computed because the standard error of the difference is 0.

**Table 3:** Comparison of 3 groups at 24 hours using One Way Anova

Groups	Sum of Squares	df	Mean Square	F	P
Between Groups	304.222	2	152.111	97.786	.000
Within Groups	9.333	6	1.556		

**Table 4:** Comparison of 3 groups at 48 hours using One Way Anova

GROUPS	Sum of Squares	df	Mean Square	F	P
Between Groups	101.769	2	50.884	23.176	.002
Within Groups	13.173	6	2.196		

Since the “p” value was significant, Post Hoc test using LSD was applied.

**Table 5:** Comparison of 3 groups at 24 hours using Post Hoc Test

Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
1 (mango)	-12.33333*	1.01835	.000	-14.8251	-9.8415
	-12.33333*	1.01835	.000	-14.8251	-9.8415
2 (neem)	12.33333*	1.01835	.000	9.8415	14.8251
	.00000	1.01835	1.000	-2.4918	2.4918
3 (babool)	12.33333*	1.01835	.000	9.8415	14.8251
	.00000	1.01835	1.000	-2.4918	2.4918

\* The mean difference is significant at the 0.05 level.

**Table 6:** Comparison of 3 groups at 48 hours using Post Hoc Test

Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
				(Lower Bound)	Upper Bound
1 (mango)	-7.13333*	1.20984	.001	-10.0937	-4.1730
	-7.13333*	1.20984	.001	-10.0937	-4.1730
2 (neem)	7.13333*	1.20984	.001	4.1730	10.0937
	.00000	1.20984	1.000	-2.9604	2.9604
3 (babool)	7.13333*	1.20984	.001	4.1730	10.0937
	.00000	1.20984	1.000	-2.9604	2.9604

\*. The mean difference is significant at the 0.05 level

Statistically there was no significant difference between Group 1 and Group 2 whereas significant difference was seen between Group 2 and Group 3 and between Group 1 and Group 3.

## DISCUSSION

The increasing trends in dental caries and gingival periodontal diseases among the population in developing countries, the increasing costs of treating these diseases, and the potential side effects associated with conventional anti-plaque agents call for alternate strategies. The extracts derived from medicinal plants are staging a comeback, and herbal “renaissance” is occurring all over the globe. The plant medicines, now a day, symbolize safety, in contrast to the synthetics that are shown to be unsafe to humans and the environment to some extent.<sup>[7]</sup>

The extracts of *Magnifera indica* (mango), *Azadirachta indica* (neem) and *Acacia nilotica* (babool) chewing sticks were tested for antimicrobial activity. The microorganism used for the study was *Streptococcus mutans*.

*Magnifera indica* (mango) contains tannins, bitter gum and resins<sup>[8]</sup> At 24 hours, this herbal extract showed no antimicrobial activity but at 48 hours, higher antimicrobial activity was seen. Tannins and resins have an astringent effect on the mucous

membrane and they form a layer over enamel, thus providing protection against dental caries.

*Azadirachta indica* (neem) contains the alkaloid margosine, resins, gum, chloride, fluoride, silica, sulfur, tannins, oils and flavenoids and calcium.<sup>[9]</sup> Maximum antimicrobial activity was seen with a zone of inhibition of 18.3mm. This is due to the presence of fluoride which exerts anticariogenic action and silica which acts as abrasive.

Wolinsky *et al* reported that Neem plant belongs to the family of compounds known as gallotannins. The presence of gallotannins during the early stages of plaque formation could effectively reduce the number of bacteria available for binding to the tooth surface by increasing their physical removal from the oral cavity through aggregate formation. Additionally, the effective inhibition of glucosyl transferase activity and the reduced bacterial adhesion to SHA, as seen with the presence of gallotannin extracts, suggests anti-plaque activity. This result suggests that neem extract reduces the

ability of some streptococci to colonize tooth surface.<sup>[9]</sup>

Another study conducted by Khalid (1999)<sup>[10]</sup> at Saudi Arabia examined the effectiveness of antimicrobial activity of aqueous extracts of neem at various concentrations and reported that it showed maximum effectiveness at 50% concentration as it includes fluoride, which exerts an anticariogenic action, and silica acts as an abrasive and prevents plaque accumulation.

In a study conducted by Prateek et al (2012)<sup>[11]</sup>, *Mangifera indica* (mango) did not show any antimicrobial activity after 24 hrs whereas another study done by Prashant et al<sup>[8]</sup> concluded that *Mangifera indica* (Mango) showed minimum zone of inhibition against *Streptococcus mutans*.

From this study, it could be inferred that to maximize the antimicrobial effect of chewing stick extracts, they should be used in combination with better oral cleanliness and protection against oral bacteria.

## CONCLUSION

Within the limitations of this study, it could be concluded that *Azadirachta indica* (neem) was more effective against *Streptococcus mutans* at the end of 48 hours as compared to *Mangifera indica* (mango) and *Acacia nilotica* (babool).

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