Evaluation And Comparison Of Antimicrobial Activity Of Tulsi (Ocimum Sanctum), Neem (Azadirachta Indica) And Triphala Extract Against Streptococcus Mutans&Lactobacillus Acidophilus: An In Vitro Study

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Abstracts: **Background and Objectives:** This in vitro study was designed to evaluate and compare the antimicrobial effects of extracts of Neem sticks, Tulsi leaves and Triphala against *Streptococcus Mutans* and *Lactobacillus Acidophilus*.**Materials and Methods:** Sterile solutions of 10% & 25% concentrations were prepared from Neem, Tulsi and Triphala extracts. After growing on culture plates micro-organisms were transferred to nutrient agar; antimicrobial activity of the extracts was tested after 48 h. **Results:** Mean zones of inhibition against *Streptococcus Mutans*&*Lactobacillus Acidophilus* after 24hrs for 10% neem extract is 0.7 & 0.2mm and for 25% neem extract is 1.1 & 0.9 mm respectively. For 10% tulsi extract is 0.3 & 0.2mm and for 25% tulsi extract is 0.5 & 0.6 mm respectively. And for 10% triphala extract is 0.0 & 0.0mm and for 25% triphala extract is 0.1 & 0.0 mm respectively. **Interpretation and conclusion:** Extracts of neem and tulsi demonstrated an antimicrobial activity but triphla has been failed to show antimicrobial activity against *S Mutans* and *L Acidophilus*. This in vitro evaluation is an attempt to encourage further studies comparing the antimicrobial effects of different ayurvedic extracts on prevalence of caries. [Shah SNJIRM 2014; 5(4) :17-21]

Key Words: Lactobacillus Acidophilus, Neem, Streptococcus Mutans, Triphala, Tulsi.

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Introduction: Dental caries is an irreversible microbial disease of the calcified tissues of the teeth, characterized by demineralization of the inorganic portion and destruction of the organic substance of the tooth, which often leads to cavitation. Dental caries is probably a disease of modern civilization. By about 17th century, there was a significant increase in the total caries experience[1] , The dental caries burden in India has been on the rise and it is important to explore all avenues possible to reduce this disease burden[2].Treatment is expensive and not a realistic option for the poor. Hence, there is an urgent need to promote traditional preventive measures that are acceptable, easily available, and cost effective[3].

The etiology of dental caries is generally agreed to be a complex which may be complicated by many indirect factors that obscure the direct cause. It involves interplay among the teeth, the oral host factors of saliva, microflora and the external factor of diet[1]. Variations in its prevalence are also ascribed to differences in dietary habits (especially the consumption of sugar), variations in the patterns of oral hygiene, changes in the virulence of oral and dental plaque microflora, and alterations in the oral protective mechanisms (including the immune status). Role of microorganisms is very vital in its etiology[4]. It was discovered that *Streptococcus Mutans*, were the main causative agent of dental caries[5]. *Mutans Streptococci* are the most cariogenic pathogens as they are highly acidogenic, producing short-chain carboxylic acids which dissolve hard tissues such as enamel and dentine[6]. *S Mutans*, being a facultative anaerobe, can survive anywhere in the oral cavity[7]. It can ferment most of the sugars and sugar alcohols present in food such as glucose, sucrose, lactose, trehalose, mannitol, sorbitol, and melibiose[8,9].*Lactobacillus Acidophilus* is also known as a specific etiologic factor responsible for the initial stages.Hence effective strategies are required to reduce the levels of *S Mutans*&*Lactobacillus* as a preventive measure for dental caries[1].

Material and Methods: The following materials were used in this study:

1) Dried chewing sticks of:
Neem (Azadirachta indica)
2) Dried leaves of:Tulsi (Ocimum sanctum)
3) Dried powder of:Triphala

4) Species of microorganisms:
   i) *Streptococcus Mutans* (MTCC 890)
   ii) *Lactobacillus Acidophilus* (MTCC 447)
5) Peptone water
6) Nutrient agar medium
7) Vernier callipers
8) Sterile plastic containers
9) Sterile deionised water
10) Incubator
11) Inoculating wire loop
12) Sterilized 6mm paper discs

Preparation of Extracts: Twigs of locally available neem were collected and were identified by their colour and scent. They were cut into pieces of approximately 15 cm length. All the sticks were authenticated by the Department of Botany, Akhandanand Ayurvedic College and Hospital, Ahmedabad. The twigs were sun dried for 2 days and were grounded separately into coarse powders. Aqueous extracts of chewing sticks were prepared by adding 10 gm and 25 gm of chewing stick powder to 100 ml of deionised distilled water. The mixture was then shaken well by hand and allowed to soak for 48 h at 4°C. It was then filtered to get extracts of 10%, and 25% concentration of chewing stick. Tulsi leaves were obtained from courtyards and were dried in sunlight. The dried leaves were then powdered finely. Hundred grams of finely powdered Tulsi was then macerated with 100% ethanol. It was then subjected to filtration with Whatman filter paper to obtain a clear filtrate. The filtrate so obtained was reduced at a low temperature of less than 60°C to obtain a solid residue of Tulsi extract. Then from the solid residue two different concentrations (10% & 25%) of solutions were made.Readily available powder of Triphala powder was obtained from market. Aqueous extracts of powder were prepared by adding 10 gm and 25 gm. of powder to 100 ml of deionised distilled water. The mixture was then shaken well and allowed to soak for 48h at 4°C. It was then filtered to get extracts of 10%, and 25% concentration.

Group Distribution Was As Follows:

1. Group 1 (neem)
   - 1A- 10% neem
   - 1B- 25% neem
2. Group 2 (tulsi)
   - 2A- 10% tulsi
   - 2B- 25% tulsi
3. Group 3 (triphala)
   - 3A- 10% triphala
   - 3B- 25% triphala
4. Group 4 (positive control; 0.2% chlorhexidine)
5. Group 5 (negative control; dimethyl formamide)

Procurement Of The Microorganisms: Ampules containing pure forms of *S Mutans* (MTCC 890) and *L Acidophilus* (MTCC 447) were obtained from Microbial Type Culture Collection (MTCC) and gene Bank, Institute of Microbial Technology, Chandigarh, India.

*Preparation Of The Culture Media For The Study:*Culture plates for *S Mutans* and *L Acidophilus* were prepared separately in the Department of Microbiology, College of Dental Sciences & Research Centre, by inoculating the contents of the ampules in chocolate agar and blood agar plates for *S Mutans* and *L Acidophilus*, respectively, at 37°C for 12 h. Growth obtained from agar plates was transferred to nutrient agar for testing the antimicrobial activity of the extracts.

Evaluation Of Antimicrobial Sensitivity By Disc Diffusion Method: Standard 6 mm sterile discs were dipped in the prepared extracts (i.e., concentrations of 10%, and 25%) and placed over the agar plates, which were then incubated at 37° for 48 h. dimethyl formamide was used as a negative control & 0.2% clorhexidine was used as positive control. Zones of inhibition were measured using the vernier callipers. The same experiment was repeated thrice for each extract and the mean values of the zones of inhibition for different concentrations against *S Mutans* and *L Acidophilus* were calculated.

Results: The effect of various concentrations of Tulsi, Neem and Triphala extract on *Streptococcus Mutans* and *Lactobacillus Acidophilus* is tabulated in Tables 1-8 respectively.

Table 1: Mean Zones of Inhibition against S Mutans&L Acidophilus After 24hrs for Neem Extract

CONCENTRATION USED	MEAN ZONE OF INHIBITION (IN MM)	
	S MUTANS	*L ACIDOPHILUS*
10%	0.7	0.2
25%	1.1	0.9

Table 2: Mean Zones of Inhibition against S Mutans & L Acidophilus After 48hrs for Neem Extract

CONCENTRATION USED	MEAN ZONE OF INHIBITION (IN MM)	
	S MUTANS	*L ACIDOPHILUS*
10%	2.5	0.5
25%	4.6	1.8

Table 3: Mean Zones of Inhibition against S Mutans & L Acidophilus After 24hrs For Tulsi Extract

CONCENTRATION USED	MEAN ZONE OF INHIBITION (IN MM)	
	S MUTANS	*L ACIDOPHILUS*
10%	0.3	0.2
25%	0.5	0.6

Table 4: Mean Zones of Inhibition against S Mutans & L Acidophilus After 48hrs For Tulsi Extract

CONCENTRATION USED	MEAN ZONE OF INHIBITION (IN MM)	
	S MUTANS	*L ACIDOPHILUS*
10%	0.6	0.8
25%	0.9	1.0

Table 5: Mean Zones of Inhibition against S Mutans & L Acidophilus After 24hrs for Triphala Extract

CONCENTRATION USED	MEAN ZONE OF INHIBITION (IN MM)	
	S MUTANS	*L ACIDOPHILUS*
10%	0.0	0.0
25%	0.1	0.0

Table 6: Mean Zones of Inhibition against S Mutans &L Acidophilus After 48hrs for Triphala Extract

CONCENTRATION USED	MEAN ZONE OF INHIBITION (IN MM)	
	S MUTANS	*L ACIDOPHILUS*
10%	0.2	0.1
25%	0.4	0.1

Table 7: Mean Zones of Inhibition against S Mutans & L Acidophilus After 24hrs For 0.2% Chlorhexidine

CONCENTRATION USED	MEAN ZONE OF INHIBITION (IN MM)	
	S MUTANS	*L ACIDOPHILUS*
0.2%	9	11

Table 8: Mean Zones of Inhibition against S Mutans & L Acidophilus After 48hrs For 0.2% Chlorhexidine

CONCENTRATION USED	MEAN ZONE OF INHIBITION (IN MM)	
	S MUTANS	*L ACIDOPHILUS*
0.2%	15	19

Discussion: Dental caries is one of the most common dental diseases. It can be controlled by various preventive and therapeutic measures[10]. There is substantial evidence that suggests that *Streptococcus Mutans*&*Lactobacillus Acidophilus*is one of the main culprit microorganisms responsible for dental caries. Reducing their levels in the oral cavity would provide an additional rationale for the prevention of dental caries[11]. Ayurvedic medicines can be used as an alternative for the preventive measures in dental caries. De et al evaluated antimicrobial activities of 35 different species. The study indicated that clove, cinnamon, bishop's weed, chilli, horseradish, cumin, tamarind, black cumin, pomegranate seed, nutmeg, garlic, onion, tejpat, celery, cambodge, have potent antimicrobial activities against the organisms[12]. Neem has been used to treat infections, skin conditions and reduce swelling. Neem has shown anti-plaque, anti-carious and anti-bacterial effects[13].Neem as a chewing stick has been widely used for cleaning teeth in

rural areas for a long time. Neem contains the alkaloid margosine, resins, gum, chloride, fluoride, silica, sulphur, tannins, oils, saponins, flavonoids, sterols, and calcium. Fluoride exerts an anti-cariogenic effect. Silica acts as an abrasive and prevents accumulation of plaque. Alkaloids are known to exert analgesic action. The oils have anti-septic and analgesic effects. Tannins exert an astringent effect and form a coat over the enamel, thus protecting against tooth decay. Neem-stick extract can reduce the ability of some streptococci to colonize tooth surfaces[3]. Tulsi is one of the holiest and most sacred herbs grown widely in India. It is an herb that is bestowed with enormous antimicrobial substances and is used to treat a variety of illnesses. Eugenol (1-hydroxy-2-methoxy-4-allylbenzene) is the active constituent present in Ocimum sanctum. The other important constituents include ursolic acid and carvacrol. The antimicrobial activity of Tulsi can be attributed to these constituents[11]. Triphala is a key ingredient used in Ayurveda since time immemorial. It is a botanical preparation consisting of equal parts of three herbal fruits: Harada (Terminalia chebula), Amla (Emblica officinalis), and Bihara (Terminalia bellerica). Triphala has been proven to have antibacterial, antiviral, and antifungal actions. It is also said to possess antihistamine, anti-inflammatory, antioxidant, antitumor, blood pressure lowering, cholesterol lowering, digestive, diuretic, and laxative properties[2]. The present study was designed to evaluate and compare the antimicrobial action against *S Mutans* and *L Acidophilus* of aqueous extracts of Neem, Tulsi and Triphala.

Conclusion: Extracts of neem and tulsi demonstrated an antimicrobial activity against *S Mutans* and *L Acidophilus*up to certain extent.
Extract of triphala has been failed to show an antimicrobial activity.

Among the agents used in the present study, neem aqueous extracts showed the most antimicrobial activity against *S Mutans.*

This study was conducted in vitro. The duration of the contact of the extract with the microorganisms in the oral cavity in vivo is not clear. This in vitro evaluation is an attempt to encourage further studies comparing the antimicrobial effects of different ayurvedic extracts on prevalence of dental caries.

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