



## RESEARCH ARTICLE

### COMPARITIVE EVALUATION OF THE ANTIMICROBIAL EFFICACY OF SIX DIFFERENT PLANT EXTRACTS AND THEIR SYNERGISTIC EFFECT AGAINST STREPTOCOCCUS MUTANS AND LACTOBACILLUS: AN INVITRO STUDY

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#### ABSTRACT

**Background:** Chewing of sticks from plants of neem, miswak, liquorice and babul have been used as a method of cleaning teeth in rural areas and as part of tradition in many parts of India. Pine was believed to be used in ancient times for cleaning teeth. The seeds and oil of Black cumin also finds wide use in oral hygiene and treating oral diseases. These plant parts are considered to have medicinal values.

**Objectives:** To assess the efficacy of miswak, neem, liquorice, babul, black cumin and pine extracts for their antibacterial effect also to assess for their probable synergistic effect.

**Materials and Methods:** Twigs of neem, miswak, liquorice, babul chewing sticks ; black cumin seeds and pine leaves were sun dried, ground into coarse powder. 5%, 10% and 50% sterile aqueous solutions were prepared. Two types of methodology was done, aqueous extraction and cold infusion method. The filtrate was inoculated onto blood agar plates containing Streptococcus mutans and Lactobacillus and incubated at 37°C for 48 h.

**Results:** The six plant extracts were found to inhibit Streptococcus mutans and Lactobacillus. Miswak and babul surfaced as strong antibacterials. The combination of these plant extracts showed significant zone of inhibition

**Conclusion:** Pine doesn't have earlier adequate research to validate its medicinal effects. Six plant extracts and their combination were effective against bacteria and can be used for preparation of antiplaque agents.

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## INTRODUCTION

Oral cavity is the window into the health of your body. Maintaining good oral hygiene has a major influence of health and wellbeing. It is well known that the high incidence of oral diseases and microorganisms are directly proportional. Streptococcus mutans and Lactobacillus acidophilus probably play a role in acid production in the plaque and involved in caries and periodontal diseases (Prashant, 2007). Because of the increased microbial resistance to antibiotics, toxic and harmful effects of few common antimicrobial agents, there is a continuous need for alternative therapies which are affordable, non-toxic and effective, such as medicinal plants (Deshpande and Kadam, 2013; Badria et al., 2011) which may overcome the high incidence of oral diseases. A few recent studies have focused on the antimicrobial activity against selected oral pathogens from natural sources.

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There is a need to screen medicinal plants for their promising biological activity.

### Aim and Objectives

This in vitro study was designed to evaluate and compare the antimicrobial effects of aqueous extracts of six naturally available plant extracts in India [miskwak (Salvadorapersica), neem (Azadirachta indica), liquorice (Glycyrrhizaglabra), babul (Vachellianilotica), black cumin (Nigella sativa) and pine (Pinus)] against Streptococcus mutans and Lactobacillus acidophilus.

## MATERIALS AND METHODS

### Material collection

Twigs of neem (Azadirachta indica), miswak (Salvadorapersica), liquorice (Glycyrrhizaglabra), babul

(Vachellianilotica) chewing sticks; black cumin seeds (*Nigella sativa*) and pine leaves (*Pinus*) from Chennai were bought from the open market. The experiments were carried out at SRM Dental College, Chennai.

### Preparation of extracts

**Water extraction method:** The sticks of miswak, neem, babul and liquorice; seeds of black cumin and leaves of pine were sun dried, ground into coarse powder and weighed into 5gm, 10gm, 50gm amounts of each powder. Combined equal amounts of each powder were taken and weighed into 5gm, 10gm, 50gm. These were added to 100 ml of deionised distilled water. After soaking for 48 hours at 4°C, the water was filtered using whatman filter. The filtrate was inoculated on wells containing specific organisms.

**Cold infusion method:** The sticks of miswak, neem, babul and liquorice; seeds of black cumin and leaves of pine were sun dried, ground into coarse powder and weighed 2gm each. Combined equal amounts of each powder were weighed 2gm. These were added to 10ml of ethanol. These were hand shaken every 2 hours for 15 minutes for 48 hours. The filtrate was filtered using whatman filter paper and allowed to dry completely. The dried extracts was then mixed with required quantity of DMSO/DMF to obtain the working concentration of the extract (100mg/ml).

equal efficacy with chlorhexidine. 10% miswak extract and the combined plant material extract again showed effective inhibition, with miswak being considerably higher. The efficacy of 50% aqueous babul extract increased compared to the effectiveness of chlorhexidine. Among the various extracts miswak and the combined plant materials as the most efficient and babul becomes equally efficient in increased concentration. Combined plant aqueous extracts were not obtained at 5, 10 and 50% concentrations, it was only obtained by cold infusion method. Antimicrobial efficacy of ethanol extracts obtained by cold infusion against *Streptococcus mutans* showed poor Zone of inhibition compared to aqueous extracts and none of the plant extract was as effective as that of the control (18mm). Among the extracts miswak showed the maximum zone of inhibition (12mm) and liquorice showed the least (9mm). (Table 1) The antimicrobial efficacy of extracts obtained by Cold infusion and aqueous extraction process against *Lactobacillus acidophilus*. 5% aqueous extract of black cumin and neem had a zone of inhibition (18mm) which is considerable with that of control chlorhexidine (22mm). Zone of inhibition of Miswak, neem, pine and liquorice extracts at 5% showed comparatively lower efficacy. Neem and pine 10% aqueous extracts showed zone of inhibition (>15mm). 50% aqueous extract showed a whopping 20 mm zone of inhibition. Pine, babul and black cumin showed an almost equal efficacy 23-24mm zone of inhibition being the highest among extracts followed by the combination on plant material producing a

**Table 1. Antimicrobial efficacy against *Streptococcus mutans***

Plant extract	Zone of inhibition (5% Aqueous extraction)	Zone of inhibition (10% Aqueous extraction)	Zone of inhibition (50% Aqueous extraction)	Cold infusion and using DMSO as solvent Zone of inhibition
Chlorhexidine	18mm	18mm	18mm	18mm
Miswak	16mm	16mm	Extract not obtained	12mm
Babul	14mm	12mm	17mm	11mm
Black cumin	13mm	12mm	12mm	10mm
Neem	13mm	12mm	13mm	9mm
Pine	13mm	12mm	Extract not obtained	11mm
Liquorice	11mm	11mm	Extract not obtained	9mm
Combined (6gm each) 36gm in 180ml		Extract not obtained		10mm

**Table 2. Antimicrobial efficacy against *Lactobacillus acidophilus***

Plant extract	Zone of inhibition (5% Aqueous extraction)	Zone of inhibition (10% Aqueous extraction)	Zone of inhibition (50% Aqueous extraction)	Cold infusion and using DMSO as solvent Zone of inhibition
Chlorhexidine	22mm	22mm	22mm	32mm
Miswak	14mm	>12mm	Extract not obtained	17mm
Babul	15mm	<15mm	20mm	23mm
Black cumin	18mm	<15mm	10mm	23mm
Neem	18mm	>15mm	10mm	19mm
Pine	12mm	>15mm	Extract not obtained	24mm
Liquorice	15mm	absent	Extract not obtained	12mm
Combined (6gm each) 36gm in 180ml		Extract not obtained		20mm

**Table 3. Combination of Powder**

Concentration in 100ml	<i>Streptococcus mutans</i>	<i>Lactobacillus acidophilus</i>
1 gm of each (6 gm)	15mm	11mm
2 gm of each (12gm)	13mm	15mm
8gm of each (48gm)	Couldn't extract	Couldn't extract
Chlorhexidine	18mm	22mm

## RESULTS

The antibacterial efficacy of varying concentration (5%, 10% and 50%) of aqueous extracts of different plant extracts and taking chlorhexidine as a control against *Streptococcus mutans* and findings were that 5% miswak had an almost

zone of 20mm but these are far lesser than the control 32mm. Liquorice showed the least inhibition (12mm) (Table 2) The antibacterial of combination powder at different concentrations. One gram of each plant extract in 100ml distilled water showed 15mm zone of inhibition for *streptococcus mutans* which is close to the control (18mm)

whereas two grams of each plant extract in 100ml distilled water showed similar zone of inhibition as chlorhexidine. (Table 3)

## DISCUSSION

The aqueous and ethanol extracts of six different medicinal plants were tested against the pathogenic microbes viz., *Streptococcus mutans* a most common bacteria of which virulent strains can cause dental plaque and caries. *Lactobacillus acidophilus* is other microbial species that knowingly cause several oral diseases, such as dental caries, endodontic infections, periodontal diseases. All the plant extracts produced antimicrobial activity to various extents to *Streptococcus mutans* and *Lactobacillus*. Of these miswak and babul showed highest activity against *Streptococcus mutans* whereas extracts of neem, black cumin, babul and the combined plant materials were more efficient against *Lactobacillus*. Pine. The efficacy of aqueous extract was significantly higher in almost all of the extracts suggesting the diffusion of the extracts is better in an aqueous extract and the antimicrobial action is better retained in the aqueous extract. In an earlier study Khalid *et al.* (Bhambal, Ajay, 2011) 1999 at Saudi Arabia 50% concentration of neem showed a highly significant inhibition against *Streptococcus mutans*. Wolinsky *et al.* (Prashant, 2007) reported that the pretreatment of saliva – conditioned hydroxyapatite with neem stick extract prior to exposure of bacteria yielded in less bacterial adhesion. Deshpande and Kadam (Al-Bayaty and Fouad Hussein, 2013) *et al* found the ethanolic extracts of Babul to be effective in inhibiting the growth of *S. mutans* (inhibition zone 31mm) and attributed this to presence of alkaloids, saponins, cardiac glycosides, tannins, flavonoids and anthraquinones. Dabur *et al.* (2014) found the extracts of babul to possess antifungal and antibacterial properties. Badria *et al.* (2011) found that the antimicrobial activity of liquorice extract against *E. faecalis* may be related to Glycyrrhizin. However in the present study liquorice is a poor antimicrobial against *S. mutans* and *Lactobacillus*. This study proves that though none of the materials could overcome the inhibition capacity of chlorhexidine, miswak is proven closely effective against

*Streptococcus mutans* and Neem against *Lactobacillus*. This study being the first of its kind to test the antimicrobial efficacy of pine is found not so effective compared to other plant extracts. Liquorice is found to have the least inhibition capacity against all the three microorganisms tested. It is evident that miswak and babul are strong antibacterials. Hence it is concluded that six plant extracts exhibit specific antibacterial properties and their combination would account for a better effect if used as antiplaque agent.

## REFERENCES

- Al-Bayaty, Fouad Hussein. 2013. "Effect of mouth wash extracted from *Salvadora persica* (Miswaak) on dental plaque formation: A clinical trial." *Journal of Medicinal Plants Research*, 4.14; 1446-1458.
- Badria CH. 2014. "Antimicrobial efficacy of the combinations of *Acacia nilotica*, *Murrayakoenigii* L. sprengel, *Eucalyptus* hybrid and *Psidium guajava* on primary plaque colonizers." *Journal of Basic and Clinical Pharmacy*, 5.4 115-119.
- Badria, A. E, Omar, and Badria. 2011. "A laboratory evaluation of the antibacterial and cytotoxic effect of Liquorice when used as root canal medicament." *International Endodontic Journal*, 44.1; 51-58.
- Bhambal, Ajay. 2011. "Comparative effect of neemstick and toothbrush on plaque removal and gingival health-A clinical trial." *Journal of Advanced Oral Research*, 2.3: 51-56.
- Deshpande, S. N. and D. G. Kadam. 2013. "Phytochemical analysis and antibacterial activity of *Acacia nilotica* against *Streptococcus mutans*." *Phytochemical analysis*, 15: 0-69.
- Morsi, Nagwa M. 2000. "Antimicrobial effect of crude extracts of *Nigella sativa* on multiple antibiotics-resistant bacteria." *Acta Microbiologica Polonica.*, 49.1; 63.
- Prashant, G. M. 2007. "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 Journal of Dental Research*, 18.4: 148.

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