Evaluation of antimicrobial activity of commercially available herbal toothpaste - An *in vitro* study

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**ABSTRACT**

**Introduction:** The presence of *Streptococcus mutans* has been consistently linked with the etiology of many oral problems such as the dental caries due to their mechanisms of plaque biofilm development and accumulation of acids. As synthetic dentifrices commonly used contain chemical agents, which are known to produce harmful side effects on prolonged use. Several antimicrobial chemicals, incorporated into toothpaste, can be beneficial in the prevention of dental caries. Inhibition of each step in this process of caries formation contributes to the prevention of dental caries. Several medicinal plants have been recently found to have antimicrobial properties. Hence, the extracts of these plants have been used to improve dental health and oral hygiene. The aim of the study is to evaluate the antibacterial activity of herbal toothpaste on *S. mutans* level - an *in vitro* study. **Materials and Methods:** Eight commercially available herbal toothpastes were included in the study. Antimicrobial efficacies of dentifrices were tested by measuring the maximum zone of inhibition at 24 h on the Muller-Hilton agar media inoculated with microbial stain using disk diffusion method. **Results:** The study showed that herbal toothpaste containing clove was effective against *S. mutans* than the fluoridated toothpaste with a zone of inhibition of 22 mm. **Conclusion:** The antibacterial properties of eight dentifrices were studied *in vitro* and concluded that the use of herbal toothpaste may reduce the streptococcus count due to their antimicrobial activity and could help lower the prevalence of dental problems and maintain oral hygiene in the population.

**KEY WORDS:** Antimicrobial, Muller-Hilton agar, Plaque, *Streptococcus mutans*, Zone of inhibition

**INTRODUCTION**

Dental caries is a localized and transmissible pathological infectious process that results in the destruction of hard enamel tissue.[¹] The oral biofilms associated with caries and periodontal diseases are generated by multispecies interactions.[²] The mouth contains a wide variety of oral bacteria, but only a few species of bacteria are believed to play an important role in causing dental caries; *Mutans streptococcus* and *Lactobacilli*. The primary strategies in prevention of dental caries are elimination of cariogenic bacteria from the oral cavity using antibacterial agents.[³] Biofilm formation is a natural process in the oral environment but needs to be controlled through regular brushing to prevent the development of caries and periodontal diseases. Despite the efficacy of many synthetic toothpaste available with antibacterial and anticariogenic properties, there is an increased desire to rely on naturally occurring compounds for health care, which has found its way into dentistry.[⁴] As synthetic dentifrices commonly used contain chemical agents, which are known to produce harmful side effects on prolonged use, herbal extracts are becoming popular and used in various formulations.[⁵] Ingredients used in modern toothpaste formulations include abrasive agents, tensoactives, humectants, thickening agents, flavoring, coloring agents, and antimicrobial agents. These antimicrobial agents include metal salts, phenols, herbal extracts, enzymes, essential oils, and biguanides. Toothpastes have been formulated to contain chemotherapeutic agents, to improve oral health, and to produce inhibitory action plaque formation. Nature has provided a complete storehouse of remedies to cure all ailments of humankind[⁶] because of their great potential against infectious agents and can be used for therapeutic purposes.[⁷] Several antimicrobial chemicals incorporated into these herbal extracts help to improve dental health and
oral hygiene without side effects. They have natural cleansing and healing property to teeth and gums.[3] Furthermore, few research efforts are directed toward addressing the potency or quality of herbal ingredients used in these dental products. While many herbal toothpastes claim to have antimicrobial properties, very little research was conducted to investigate these claims.[8] Hence, this study is thus aimed at comparing the antimicrobial activity of eight commercially available herbal toothpastes which has the following ingredients.

**Myrrh and Sage (Commiphora myrrha and Salvia officinalis)**

Sage is believed to have antibacterial, antifungal, antiviral effects, and decrease tissue bleeding. Sage has been used effectively for throat infections, dental abscesses, infected gums, and mouth ulcers. Myrrh has well known biblical connections and widespread use in the ancient world. Myrrh is used occasionally as part of topical dressings, but it is an herb that probably deserves more frequent use. It is particularly useful for the mucous membranes of the mouth and gums, and for horses with sores from bit trauma, poor teeth, or gum disease (which is being recognized more frequently now). It improves the appetite, stimulates digestive processes, and acts as an astringent (Grieve, 1931). Myrrh is reputed to have antiseptic properties and a strong cleaning and healing action and is used to treat gingivitis, oral ulcers, and stomatitis.[9]

**Aloe vera**

*A. vera* is a cactus plant that belongs to the Liliaceae family. It is extremely helpful in the treatment of gum diseases such as gingivitis and periodontitis. It reduces bleeding, inflammation, and swelling of the gums. It is a powerful antiseptic in pockets where normal cleaning is difficult, and its antifungal properties help greatly in the problem of denture stomatitis, aphthous ulcers, and cracked and split corners of the mouth.[10]

**Miswak**

Miswak is a teeth cleaning twig made from the *Salvadora persica* tree. Apart from their antibacterial activity which may help control the formation and activity of dental plaque, they can be used effectively as a natural toothbrush for teeth cleaning. Such sticks are effective, commonly available, and contain many medicinal properties. It prevents dental plaque formations and associated with multiple medicinal benefits and uses such as it prevents plaque and gingivitis, gums strengthening, bad breath, strengthens teeth enamel, tooth whitening, anticariogenic, and prevents tooth decay.[3]

**Neem (Azadirachta indica)**

Conventionally, slender neem twigs were called as datun which are first chewed as a toothbrush and then split as a tongue cleaner. It has been found to be as effective as a toothbrush in reducing plaque and gingival inflammation. Neem has antiviral, antimicrobial, antibacterial, antipyretic, anti-inflammatory, antitumor, antihelminthic, anticariogenic, and antioxidant activity. Studies have shown that neem is used in the treatment of dental caries, gingivitis, and periodontitis.

**Clove (Syzygium aromaticum)**

Cloves are the aromatic flower buds of a tree in the family Myrtaceae, *S. aromaticum*. Clove oil is commonly used in the preparation of some toothpastes and Clovacaine solution, which acts as a local anesthetic used in oral ulceration and inflammation. Eugenol (or clove oil generally) is mixed with zinc oxide to form a temporary tooth cavity filling. It has analgesic, antibacterial, antiviral, anti-inflammatory, and antioxidant property. It has been used to relieve toothache, in periodontitis, as an anesthetic and also to treat bleeding gums.[11]

**Pudina Satva (Peppermint) and Tomar Beej (Zanthoxylum armatum)**

Peppermint (mentha × piperita, also known as Mentha balsamea Willd.) is a hybrid mint, a cross between water mint and spearmint. Peppermint oil has a cooling effect which when applied topically can be used to relieve muscle pain, nerve pain, relief from itching, or as a fragrance. Peppermint oil is used for toothache and to relieve gum inflammation.[12] *Z. armatum*, also called winged prickly ash, enriched with medicinal properties. *Z. armatum* helps in removal of bad odor has antiseptic and antibacterial property.

**Babool (Acacia arabica)**

The tender twig of this plant is used as a toothbrush in Southeast Africa, Pakistan, and India. This ayurvedic herb is great astringent and is used as antihemorrhagic agent and anti diarrheal. It helps in the reduction of plaque and gingival inflammation and remineralization of enamel lesions.[13]

**Peelu (S. persica)**

It is claimed that the Vitamin C and sterol content of this plant have great roles in strengthening the gum capillaries and preventing gum inflammation. Calcium salts and fluoride are quite effective in preventing dental caries.[11]

**MATERIALS AND METHODS**

The organism *Streptococcus mutans* was isolated from sample of saliva using special media (*Mutans*-sanguis agar which is recommended for differentiation of *S. mutans* and *Streptococcus sanguis* associated with oral microflora) and maintained in tryptone soya agar at 4°C in the Department of Microbiology, Saveetha Dental College and Hospitals.[14]
Eight commercially available herbal toothpastes and its main composition: (i) Herbal toothpaste 1 - Myrrh and sage, (ii) herbal toothpaste 2 - A. Vera, (iii) herbal toothpaste 3 - Miswak, (iv) herbal toothpaste 4 - Neem, (v) herbal toothpaste 5 - clove, (vi) herbal toothpaste 6 - Pudina satva, (vii) herbal toothpaste 7 - babool, and (viii) herbal toothpaste 8 - peelu control - fluoridated toothpaste. The agar well diffusion technique was used for screening the antibacterial activity.

**Screening of Antibacterial Activity (Agar Well Diffusion Technique)**

Broth culture of the test organism compared to 0.5 McFarland’s standard was prepared. Lawn culture of the test organism was made on the Muller-Hinton agar (HiMedia M1084) plates using sterile cotton swab and the plates were dried for 15 min. A well measuring 4 mm depth was made on the agar with sterile cork borer. Different herbal toothpastes were added to the wells. The plates were incubated at 37°C overnight and the zone of inhibition of growth was measured in millimeters. The tests were repeated triplicate to avoid test error.

**RESULTS**

The antibacterial activity of commercially available herbal toothpaste at different concentrations was screened by agar well diffusion technique and their zone of inhibition was measured in mm diameter. The results are given in Table 1. The results of this study have shown that the herbal toothpaste 5 (clove) is more significant with a zone of inhibition of 22 mm at concentration of 100 μl when compared to other herbal toothpastes 6, 3, 4, 1, 7, 2, and 8 which had a zone of inhibition of 21, 21, 21, 20, 20, and 19 mm, respectively, at 100 μl concentration when compared with standard fluoridated toothpaste which had a zone of inhibition of 19 mm at 100 μl concentration [Figure 1].

**DISCUSSION**

Periodontal diseases encompass multifactorial diseases involving bacterial biofilms and the generation of an inflammatory response including the production of cytokines, eicosanoids, and matrix metalloproteinase. Bacterial biofilms have been proven to be the primary etiological factor in the initiation of gingivitis and subsequent destruction of periodontal tissues. It is well established that supragingival plaque is the cause of gingivitis and plays a primary role in the initiation of periodontitis. The removal of microbial plaque leads to resolution of gingival inflammation, and cessation of plaque control leads to a recurrence of inflammation. The control of plaque in the maintenance of gingival health has been well established in the literature. It has been shown that rigorous self-performed plaque control over long periods of time reduced the levels and altered the composition of subgingival bacteria and reduced the frequency of deep periodontal pockets.

Since the 1980s, fluoride has been the most commonly used remineralizing agents. It is known to control caries predominantly through its topical effect. Fluoride inhibits demineralization, enhances remineralization, and inhibits bacterial activity. When the acid attacks the enamel surface, the pH begins to rise and fluoride present in the microenvironment causes enamel dissolution to stop. It acts by creating phases of calcium and phosphate, thereby increasing the surface fluoride content in the enamel. The other contributing factors for fluoride are its antimicrobial property, reduction in bacterial adherence, and increases the plaque pH. Thus, the fluoride toothpaste reduces the number of streptococcal colony despite the fact that fluoride was added to the toothpastes first with the aim to preserve the product and then to protect the teeth from caries. The effectiveness of fluoride toothpastes as an antimicrobial agent is concentration dependent.

According to the results of the present study, herbal toothpaste can cause inhibition of bacterial growth. The organisms employed in the present study include both the normal flora and the pathogens of the oral cavity. S. mutans has been strongly associated with the initiation of caries, while there is a correlation between Lactobacilli and the further development of carious lesions. It was observed that the dentifrice with multiple herbal components is more effective in inhibiting both the organisms as herbal extracts have received special attention because of being

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Figure 1: Antibacterial activity of herbal tooth pastes against Streptococcus mutans
non-chemical and non-synthetic in nature, and have been used in traditional medicine.[5] The antimicrobial activity of the herbs is due to the presence of secondary metabolites such as alkaloids, flavonoids, polyphenols, and lectins. Synergistic interactions between the principal components of these herbal products are considered as a vital part of their efficacy.[13] Our study mainly concentrates in comparing efficacy of herbal dentifrices with conventional ones in inhibiting cariogenic bacteria, suggesting that efficacy of herbal dentifrices is similar and has superior antibacterial efficacy.[20] Hence, the addition of natural plant extracts to toothpastes can increase the antimicrobial spectrum, thus reducing, controlling, or preventing oral diseases.[21,22]

CONCLUSION

The herbal toothpaste formulations studied in our experiments, appear to be more effective than the fluoride dental formulations. Hence, herbal toothpaste has been proved to have superior antimicrobial activity against S. mutans than the fluoridated toothpaste due to the excess use of fluoride that can cause the dental fluorosis, stomach ailments, acute toxicity, and skin rashes (perioral dermatitis), these herbal products are symbol of safety in contrast to synthetic toothpaste that is regarded as unsafe to human beings and environment. Inhibit plaque may be expected to be of value in both the prevention and management of periodontal disease, thus exhibiting major effect on improving the oral health of the individual.[23] However, further studies are needed to know the efficacy of these toothpastes.

REFERENCES