

Antibacterial efficacy of herbal mouthwash against oral microbes - *in vitro* assay

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ABSTRACT

The study was to evaluate *in vitro* antibacterial effect of herbal mouthwash containing Licorice root, Tulsi leaf against *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus sanguis*, and *Lactobacillus acidophilus*. ATCC strains were obtained from Hi media (Mumbai). Using Agar well diffusion method, susceptibility tests were performed overnight incubation at 37°C. All tests were performed in duplicate. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were also performed by broth dilution method. The herbal mouthwash showed significant antibacterial efficacy against the oral microbes tested. MIC/MBC shows good efficacy against *S. mutans* and related strains and moderate efficacy against *L. acidophilus*. The search for herbal compounds with antibacterial efficacy is a progressing challenge, and medicinal plants have been considered an interesting alternative to synthetic drug. In conclusion, the study reveals that the herbal mouthwash possesses significant anticariogenic/antiplaque efficacy.

Keywords: Agar well diffusion, antibacterial, broth dilution, herbal mouthwash.

Introduction

In India, as in other developing countries, a very significant proportion of dental problems is due to microbial infections.^[1-3]

Bacteria existing in the dental plaque or biofilm play an important role in the development of both dental caries and periodontal disease.^[2, 9-17] Dental caries, also known as tooth decay or a cavity, is an infection, generally bacterial in origin, localized and transmissible, that results in the destruction of hard dental tissue. It results from the accumulation of plaque on the surface of the teeth and biochemical activities of complex micro communities. *Streptococcus mutans* is one of the main opportunistic pathogens of dental caries.^[3, 18-23] The occurrence of dental caries is approximately 60-65% among the Indian population.^[4, 5, 24-30]

Mouth rinses have been used for centuries for medicinal and cosmetic purposes, but it is only in recent years that the rationale behind the use of chemical ingredients has been subject to scientific research and clinical trials.^[6, 31-34] There is also an increased resistance of pathogenic bacteria to currently used antibiotics and chemotherapeutics. Despite several agents being commercially available, these chemicals can alter oral microbiota and have undesirable side effects such as vomiting,

diarrhea, and tooth staining.^[7, 35-39] Hence, the search for alternative products exists. There have been numerous reports of the use of traditional plants and natural products for the treatment of oral diseases.^[8, 9]

Natural herbs such as tulsi patra, neem, clove oil, pudina, ajwain and many more used either as whole single herb or in combination have been scientifically proven to be safe and effective medicine against various oral health problems such as bleeding gums, halitosis, mouth ulcers, and preventing tooth decay.

Ocimum sanctum (OS), commonly known as tulsi is a small shrub belonging to the mint family Lamiaceae. It has small leaves with a strong smell and purple flower.^[10] Tulsi is a time-tested premier medicinal herb. It is a plant of Indian origin, worshipped by the Hindus and used in Ayurvedic medicine since ancient times. It is one of the holiest and most sacred herbs grown widely in India. It possesses significant antibacterial, antioxidant, and anti-inflammatory properties. It is a herb that is bestowed with enormous antimicrobial substances and is used to treat a variety of illnesses ranging from diabetes mellitus, arthritis, bronchitis, and skin diseases.^[10-12]

Licorice also known as Glycyrrhiza, an herb, is known to have few medicinal values. It was used in ancient times as a remedy for a great diversity of ailments and sweetener.^[13] Roots of Glycyrrhiza contain a high concentration of saponin and glycyrrhizin, which are supposed to be sweetening agents.^[14]

This herb was also used in this study as one of the components. Several studies have proved that tulsi has antibacterial effectiveness against various Gram-positive and Gram-negative bacteria.^[15-23] Thus, this study

was planned to evaluate the antimicrobial efficacy of OS extract and to assess its mode of action on key virulence factors of *S. mutans* in vitro.

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Materials and Methods

For this study, we prepared a herbal mouthwash with dilutions of tulsi extract and licorice root extract. Fresh leaves of OS were collected, which was identified by a botanist. The OS leaves were washed with distilled water, air dried and ground to powder form. The leaf powder was extracted with different solvents (water, 100% methanol, and 100% ethanol) for 30 hrs. Then, it was centrifuged at 12000 rpm for 10 min, supernatant was collected, and the solvent was evaporated in hot air oven at 50°C. The dried extract was suspended in 75% of the above-mentioned solvents and used as a stock and stored at 4°C.

Along with tulsi extract (100 mg), licorice extract (200 mg) was added. 50 ml of distilled water was added, sonicated and filtered. To the filtrate 0.5 ml clove oil which is used as an obtundent, 0.3 ml peppermint oil as flavoring agent, and 5 ml ethanol as a preservative were added and finally it was made up to 100 ml with water.

Standard strains and culture conditions

Pure strains of *Streptococcus salivarius* ATCC 13419, *S. mutans* ATCC 25175, *Streptococcus sanguis* ATCC 10556, and *Lactobacillus acidophilus* ATCC 4358 were purchased from Hi media (Mumbai). The medium which was used for the study is Tryptic Soy broth (TSB). The test organism was grown in TSB medium [MHA-Hi media, Mumbai] for 24 h at 37°C and concentration was adjusted to 0.5 McFarland standard.^[24]

Minimum inhibitory concentration (MIC)

Initially to 100 µL of sterile broth, 100 µg/ml of the sample was taken as an initial concentration in the first well of 96 well titer plates. Then, it was serially diluted to obtain the dilutions of 100 mg/mL, 50 mg/mL, 25 mg/ml, 15 mg/mL, 10 mg/mL, 5 mg/mL, 2.5 mg/mL, and finally 1 mg/mL. The test organisms were added to the wells. The plates were kept in sealed covers and incubated at 37°C overnight, and growth/no growth was detected. All the tests were done in triplicate to minimize the test error. The concentration which inhibits the bacterial growth is considered as the MIC. 0.2% chlorhexidine was used as a positive control.

Results

In this study, the herbal mouthwash tested showed significant antibacterial efficacy represented in Figures 1 and 2. It showed no growth at 25 mg/ml tested against *S. mutans* and *S. sanguis*, and at 100 mg/ml against *L. acidophilus*. 10 mg/ml against *S. salivarius*. Agar well diffusion method showed a maximum zone of inhibition against *S. mutans* and *S. salivarius* (19 mm and 22 mm), respectively, when compared to other microbes depicted in Table 1.

Discussion

India is a land filled with nature's medicinal plants.^[15] Herbal extracts are known to possess antimicrobial compounds, especially against bacterial pathogens.

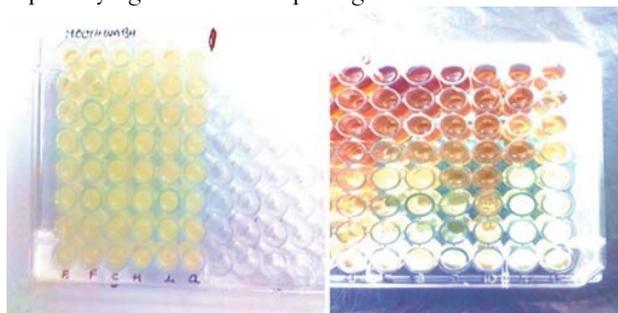


Figure 1: (a and b) Antibacterial assay of herbal mouthwash



Figure 2: Zone of inhibition for (a) *Streptococcus mutans*, (b) *Streptococcus salivarius*, (c) *Streptococcus sanguis*, and (d) *Lactobacillus acidophilus*

Table 1: MIC of herbal mouthwash

Chlorhexidine (+ control)	<i>S. mutans</i>	<i>S. salivarius</i>	<i>S. sanguis</i>	<i>L. acidophilus</i>
15 mg/ml	25 mg/ml	10 mg/ml	25 mg/ml	100 mg/ml

MIC: Minimum inhibitory concentration, *S. mutans*: *Streptococcus mutans*, *S. salivarius*: *Streptococcus salivarius*, *S. sanguis*: *Streptococcus sanguis*, *L. acidophilus*: *Lactobacillus acidophilus*

S. mutans is considered the main culprit among cariogenic microorganisms. A brief outline of *S. mutans* role in the pathogenesis of dental caries determines that initially there is adhesion mediated attachment of *S. mutans*, which adhere by hydrophobic bonds to the enamel surface and ferment sucrose. This sucrose metabolism is known to further promote firm adherence and aggregation of the bacteria to the tooth surface resulting in acid production and cavity formation.^[25-39]

The use of an antimicrobial agent in the oral cavity might inhibit the growth of microorganisms, thus preventing the development of dental caries. Commonly used anticariogenic oral rinses are fluoride and chlorhexidine. The herbal preparations are considered to be moderate in efficacy and are less toxic than the most commonly used pharmaceutical mouthwash.

Conclusion

It is suggested that the significant antibacterial efficacy of herbal mouthwash was found in this study and could be attributed due to the presence of polyphenols. The inhibitory effect shown by the herbal mouthwash against *S. mutans* and *S. salivarius* was significant. Thus, these herbal products can be used to replace the pharmaceutical mouthwash.

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