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# Antimicrobial Effectiveness of Herbal and 0.2% Chlorhexidine Mouthrinse against *Streptococcus mutans*: An *In-vitro* Study

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***Abstract:***

**Background:** Microorganisms in the oral cavity are still considered as serious public health problems and inflict a costly burden to health care services in and around the world. Mouthrinses have been used for centuries with the objective of reducing the amount of microorganisms in the oral cavity. Mouthrinses are used as adjuncts to mechanical oral hygiene. Therapeutic mouthwashes are often recommended as an adjunct to mechanical plaque control for prevention of plaque accumulation and to maintain gingival and periodontal health. Mechanical control alone for reducing recalcitrant biofilms in the oral cavity has been challenged because it is considered to be rather time-consuming and most importantly insufficient for effective oral hygiene. The aim of this study was to evaluate the antimicrobial efficacy of herbal and 0.2% chlorhexidine gluconate mouthrinse against *Streptococcus mutans*.

**Materials and Methods:** The antimicrobial effectiveness (zone of inhibition) of an herbal mouthrinse and 0.2% chlorhexidine mouthrinse was determined by agar well diffusion method.

**Results:** The zone of inhibition of *S. mutans* was 19 mm for the 0.2% chlorhexidine mouthrinse. The arowash liquid mouthrinse shows that *S. mutans* do not produce a zone of inhibition.

**Conclusion:** Chlorhexidine mouthrinse (0.2%) has a better antimicrobial efficacy against the *S. mutans* when compared to herbal mouthrinse (arowash liquid).

***Key Words***: Antimicrobial effectiveness, chlorhexidine, herbal, *Streptococcus mutans*

## Introduction

Dental caries, gingival disease,[1] and periodontal disease are the most frequently occurring oral diseases in the world. The use of tooth brushing and fluoride toothpaste appeared to be almost universal.[2] There is difficulty in effectively removing plaque by only using toothbrushing and flossing, as these measures do not remove plaque completely.[3]

Many studies have shown that dental floss is superior in removing interproximal plaque compared to others, and it also reduces the incidence of proximal caries.[4] Therefore, an easy applicable alternative is needed.

The incorporation of broad spectrum antimicrobial mouthrinses as adjuncts to patient's daily oral hygiene regimens has assumed greater importance with the recognition that most individuals are unable to consistently maintain adequate levels of plaque control using mechanical methods alone.[1]

There is a necessity for adjuncts, in addition to mechanical aids for maximum plaque control. A variety of subsequent studies have demonstrated the antiplaque and anti-gingivitis effectiveness of chlorhexidine mouthrinses as adjuncts to usual oral hygiene methods. As a result, 0.2% chlorhexidine mouthrinses became widely used in many European countries.[1] Till present, chlorhexidine mouthwash is considered as gold standard for the best plaque control agent.[5]

Currently, there are commercialized oral rinses, which contain natural compounds with antimicrobial activity and are known to be safe for human use. However, there is inadequate clinical research on herb based mouthrinses and dentifrices in Asia.[6] Till present, there are many conditions affecting the oral health which can be prevented and/or using natural product-based drugs or formulations.[7] Natural extracts such as *Eucalyptys globules* (Eucalyptus), *Eugenia caryophyllus* (Clove), *Sanguinaria canadensis* (Sanguinarine), *Cinnamomum verum* (Cinnamon), and *Mentha spicata* (Spearmint) are among the extracts added to the formation

of many commercialized mouthrinses antimicrobial agents due to the broad range effects towards oral microbes and pathogens.[8]

Neem, garlic, and green tea extracts were also tested for its efficacy.[9] Pomegranate fruit also has antibacterial and anticaries properties comparable to 0.2% chlorhexidine mouthwash.[10] Lemongrass oil has also been considered as an alternative to 0.2% chlorhexidine mouthwash because of its antibacterial, antioxidant, antifungal, and anti-inflammatory properties.[11] Rosemary (*Rosmarinus officinalis* L.) extract has been proven to improve healing of mouth ulcers and bleeding gums.[3] Arowash liquid is a judicious combination of three herbs: *Acacia catechu* extract, *Glycyrrhiza glabra* extract, and *Syzigium aromaticum* extract, which are responsible for antiseptic, anti-inflammatory, astringent, local anesthetic, and mouth freshener effect.

To the best of our knowledge, no past researches have been conducted on these combinations of herbs against the *Streptococcus mutans*. Hence, the aim of this study was to evaluate the antimicrobial efficacy of herbal and 0.2% chlorhexidine gluconate mouthrinse against *S. mutans*.

## Materials and Methods

### *Study design*

An *in vitro* experimental study on microbiological analysis of herbal mouthrinse and 0.2% chlorhexidine gluconate mouthrinse against *S. mutans*.

### *Test materials*

- Control mouthrinse: Hexidine mouthrinse (ICPA Health Products Ltd.)
- Experimental mouthrinse: Arowash liquid mouthrinse (Cadila Pharmaceuticals Ltd.).

### *Test organism*

*S. mutans* (ATCC 25175).

### *Review board clearance*

Before the commencement, the study was submitted for approval and clearance was obtained from Scientific Review Board, Saveetha University, Chennai.

### *Approval from authorities*

The study was carried out in the Department of Microbiology, Sri Muthukumaran Medical College and Hospital, Chennai. Permission to conduct the study was obtained from the Dean and Head of the Department (Microbiology) of the college.

### *Scheduling*

The study was scheduled for a period of 1-month from 1st May 2013 to 31st May 2013.

### *Procedure for antimicrobial activity by zone of inhibition (agar well diffusion method)*[12]

For each test organism, 0.5 McFarland turbidity adjusted inoculum was prepared in brain heart infusion broth.

↓

Using a sterile cotton swab, a lawn inoculum was made in the blood agar for *S. mutans*, in the Mueller-Hinton agar for *Staphylococcus aureus* and *Enterococcus faecalis*, and in sabouraud dextrose agar for *Candida albicans* strains, respectively.

↓

With the help of the sterile Pasteur pipette (blunt end), 2 wells measuring 8 mm in diameter were made in the media (one in each half) and 80 µl of undiluted control and test solutions were dispensed in each well.

↓

Petri dishes were incubated upright at 37°C for 24 h to grow the microorganisms.

↓

Diameter of zones of inhibition of both the test solution as well as the control solution was measured in mm.

## Results

Table 1 describes the zone of inhibition for control and experimental mouthrinse against *S. mutans*. The zone of inhibition for control mouthrinse was 19 mm, and there was no zone of inhibition observed for the experimental mouthrinse.

## Discussion

The oral cavity represents a dynamic ecosystem; therefore, it would not be totally advantageous to eliminate all elements of the oral microflora in an effort to control dental plaque-associated infections. Rather, it may be more ideal to remove only most cariogenic and periodontopathic elements of the dental plaque microflora while permitting the most innocuous elements to remain.[13]

The classical experiments of Loe *et al.* (1965) demonstrated that the accumulation of microbial plaque for 3 weeks predictably resulted in the development of generalized gingivitis. Likewise, plaque removal reversed clinical inflammation to the healthy gingiva. A large number of studies have confirmed these findings both in humans and in experimental animals.[14]

**Table 1: Zone of inhibition for control and experimental mouthrinse against *S. mutans*.**

| Mouthrinse | *Streptococcus mutans* |
|---|---|
| Control mouthrinse | 19 mm |
| Experimental mouthrinse | 00 mm |

*S. mutans: Streptococcus mutans*

Studies among various populations in developing countries demonstrated that dental caries, gingivitis, and periodontitis are a common feature among adults. The data from the National Health Survey conducted by the United States (1969) showed that the prevalence of gingivitis for adolescents 12-17 years of age is 62%.[15]

In the third National Health and Nutrition Examination Survey (NHANES III, 1988-1994) of the United States Public Health Service, 50% of adults were identified as having gingivitis.[16] Gingival bleeding was more prevalent (63%) in the 13-17 years old group and declined (48%) gradually through the 35-44 years old age group. The prevalence increased (53%) again at 45-54 years old age group but remained fairly constant (53%) in older age groups. A study of the US school children aged 14-17 years reported that the prevalence of gingival bleeding was 61.5%, which is identical to the prevalence reported in NHANES III.[17]

Various devices have been used over the years to assist in the removal of plaque from the tooth surfaces and interproximal sites, as it has been demonstrated that tooth brushing alone does not adequately remove interproximal plaque and thereby control gingivitis.[18] The routine practice of twice daily brushing and once daily interdental cleaning has been the mainstay of oral hygiene recommendations by dental professionals to their patients for decades.[18] Of these devices, the daily use of mouth rinse and dental floss has received the most attention as an effective method of interproximal cleaning.

A relatively large number of chemical agents, which are mostly synthetic compounds, have been used for many purposes, control of dental plaque, elimination of oral pathogens, against malodor, etc.[19]

Arowash liquid is a judicious combination of three ayurvedic herbs such as *A. catechu*, *G. glabra,* and *S. aromaticum*. *A. catechu* extract contains Tannins, which have astringent and antioxidant effects, thereby protecting the mucosal layers. *G. glabra* extract, containing Glycyrrhizin, is a reputed drug for gastric and duodenal ulcers and possess soothing properties. Glycyrrhizin has also been reported to have anti-inflammatory action. Clove oil is a reputed antiseptic, antibacterial, antifungal, antiviral and a local anesthetic. It is an invaluable remedy in inflammation of the mouth and pharynx and as a local analgesic and dental antiseptic.

The present *in vitro* study was conducted to estimate the minimum inhibitory concentration of an herbal mouth rinse and to compare its antimicrobial efficacy with a commercially available mouthrinse (0.2% chlorhexidine gluconate) against 4 oral bacterial strains. The mouthrinses used in the present study were experimental mouthrinse (arowash liquid [combination of *A. catechu* extract, *G. glabra* extract, and clove oil]) and control mouthrinse (hexidine [0.2% chlorhexidine mouthrinse]).

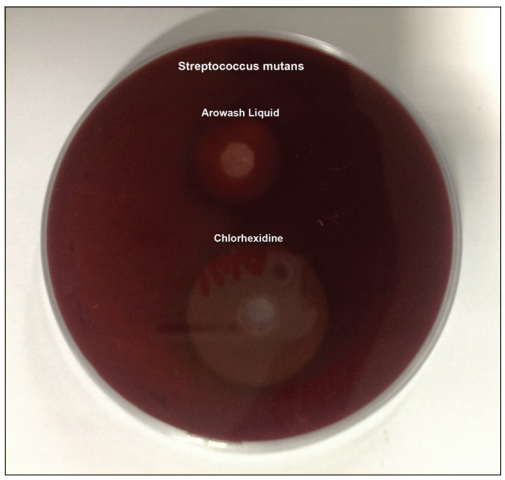


**Figure 1:** Antimicrobial efficacy of an experimental and control mouthrinse against *Streptococcus mutans.*

The strain used in the present study was internationally accepted *S. mutans* (ATCC 25175). The study was conducted in the Department of Microbiology, Sri Muthukumaran Medical College, for a period of 1-month. To determine the antimicrobial activity (zone of inhibition) for mouthrinses agar well diffusion method was used.

In the current study, the zone of inhibition for control mouthrinse (hexidine) for *S. mutans* was found to be 19 mm, which was lower than the study conducted by Sedighinia *et al*.[20] The reason for this difference in values is because of variation in product formulation.

In the current study, the experimental mouthrinse does not inhibit the zones in *S. mutans,* whereas in the study conducted by Geetha *et al*.[21] reported that the zone of inhibition of *A. catechu* extract for *S. mutans* was found to be 10 mm and Dhanyakumar *et al*.[22] reported that the zone of inhibition of *G. glabra* for *S. mutans* was found to be 16.8 mm. Again the difference could be attributed to the difference in product formulation, thus concluding that a combination is better when compared to a single ingredient (Figure 1).

## Conclusion

The result from this study claims that chlorhexidine gluonate mouthrinse (0.2%) has a better antimicrobial efficacy against the *S. mutans* when compared to herbal mouthrinse (arowash liquid). Further, studies are required to better evaluate of this extract as a root canal irrigants. *In vivo* clinical testing essential to confirm *in vitro* study results to be done in larger sample size.

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