

Comparison of Antibacterial Efficacy of Different Herbal Mouthwashes – An in Vitro Study

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Abstract

Aim: To compare of antibacterial efficacy of different herbal mouthwashes

Background: Next to dentifrices, mouthwashes are one of the widely used modality to maintain oral hygiene. Chlorhexidine and Listerine are some of the commonly prescribed mouth rinses, but they come along with many disadvantages. To overcome this, in recent decade, the medical field has turned its attention towards indigenous techniques and medicines. Yet the efficacy needs to match the conventional products. Hence this trial was done to assess the anti bacterial efficacy of different commercially available mouth rinses.

Materials and Method: Three different herbal mouthwashes were taken. Saline was taken as control. 1000 μ L of Himalaya herbal mouthwash, K.P Namboodiri herbal mouthwash, Orafrsh mourthwash and saline was pipetted into three cuvettes each . Cuvette was then incubated for 1hour at 37 degree Celsius at microbiology department, saveetha dental college, Nutrient agar was prepared. 10 μ L of sample was pipetted out from one cuvette and poured into one nutrient agar plate. Metal loop was heated to red hot and sample was streaked using metal loop. Nutrient agar plates was then incubated at 37 degrees Celsius for 24 hours. Agar plates were taken out of the incubator and bacterial colony count was done.

Results: The mean CFU/10 μ L of streptococcus mutans in Himalaya herbals complete care mouthwash was 8 CFU/10 μ L. The mean CFU/10 μ L of streptococcus mutans in namboodiri's herbal mouthwash was 14.33 CFU/10 μ L. The mean CFU/10 μ L of streptococcus mutans in orafresh herbal mouthwash was 17.33CFU/10 μ L. Saline was compared as a control . The CFU/ μ L of streptococcus mutans in saline was 58.

Conclusion :The Antibacterial efficacy of different herbal mouthwash shows that Himalaya and namboodiri's herbal mouthwash has significantly antibacterial efficacy against S.mutans. Further research should be made against different organism and different herbal mouthwash.

Keywords: Herbal, Mouthwash, Micro Organisms, Oral Health, S.mutans

Introduction

Oral health can directly affect systemic health so prevention and maintenance of oral is very important. Products which are safe, effective and economical are necessary for improving oral health. Microorganisms are the main cause for disease of oral cavity. Use of dentifrice and mouthwash prevent the accumulation of plaque and calculus. Main method for prevention the progression of periodontal diseases is by use of mechanical methods like brushing and flossing. After brushing and flossing, use of mouthwash also help in prevention. Mouthwash can be used in mentally and physically challenged patients

but it should be used along with brushing and flossing^[1]. Mouthwash is an aqueous solution which is most often used for its deodorant, refreshing and antiseptic property or for control of plaque. Mouthwash contains alcohol, synthetic sweeteners, surface acting agents, coloring agent. Mouthwash contains anti microbial substances and hence proven to be effective in eliminating the microorganisms like streptococcus mutans (S. mutans), Lactobacillus, Enterococcus faecalis (E. faecalis) , Staphylococcus aureus (S. aureus)^[2,3,4].

Most of the commercially available mouthwash like chlorhexidine , Listerine contain alcohol leading

to side effects like brown staining of the teeth, changes in taste sensation, alter the oral mucosa leading to mucosal erosion, parotid swelling in rare cases and sometimes can also causes supra gingival calculus and burning sensation of mouth .The staining property of chlorhexidine can be due to degradation of chlorhexidine and release of parachloraniline, Maillard reaction ,metal sulphide formation with protein denaturation ,precipitation of dietary chormogens . Ethanol present in mouthwash can causes oral and pharyngeal cancer. Toxic metabolites of ethanol are formed from alcohol containing mouth and the concentration was similar to those after alcohol consumption. Mouthwashes which contain alcohol have also decreased the hardness of composite and resin restoration and also have altered the color of the composite restoration when it is used for a long time. Xerostomia can also be causes by alcohol present in mouthwash. Alcohol containing mouthwash is contraindicated in children as they can be toxic and even lethal if swallowed in large amounts. Sodium lauryl sulfate (SLS) is a chemical compound present in certain mouthwashes that has been proved to cause many health problem such as menopausal syndrome, premenstrual syndrome (PMS), decreased male fertility and also breast cancer .Benefit of alcohol in mouthwash is not yet scientifically proven.^[5,6] Fluoride containing mouthwash is not recommended for children below 6 years because of the risk of fluoride ingestion .

New age medicine is most popular nowadays; new age medicine includes uses of herbal products. Herbal products have lesser side effects than others. Current research indicate that the polyphenols, being secondary metabolites, are present in rich amount in various plants . Many of them possess antioxidant, anti-inflammatory, low antibacterial resistance and several others therapeutic properties than chlorhexidine mouthwash ^[4].

Mouthwash has been used for centuries for both medical and cosmetics purpose. Mouth rinsing was first described in Indian (Ayurveda) and Chinese medicine around 2700 BC. Ancient Indians and Chinese thought decay of the teeth was caused by worms and treatment included powder, tablet or ointments of various mixtures that contain different herbs, minerals, and esoteric agents like mouse bones. The Egyptians used honey, goose fat, cumin, and ochre for rinsing the mouth. Greeks recommended the uses of salt, alum, vinegar, leaves of olive tree, milk, pomegranate seed, and wine for rinsing the mouth. Tooth paste and mouth wash was first invented by Romans they used human urine in

mouthwash and paste. Till 18 century urine was added in paste and mouthwash due to its high ammonia content ^[6,7]. At present there are several herbal mouthwash made out of neem, miswak, pomegranates extracts, papaya extract, clove, basil, lemon grass oil, peppermint , turmeric ^[4,8].

Use of herbal mouthwash is both promotive and preventive when used. In this method various plant and there extract are used to treat disease and to maintain good hygiene. Natural plants like neem ,clove, tulsi , jyestiamadh, pudina, triphala, ajwain and more plants can be used either as whole single herb or in combination . Herbal products have been scientifically proven to be safe and effective medicine against various oral health problems like gingivitis, periodontitis , halitosis, mouth ulcers and preventing tooth decay. The major strength of these natural herbs is that their use has not been reported with any side affects till date and by use of a herbal mouth rinse, ingredients which causes halitosis are avoided which itself is one step forward towards better oral hygiene and better health ^[7].

Streptococcus mutans is a, gram-positive coccus, facultative anaerobic. Main stains of *S.mutans* were isolated from the oral cavity .This microorganism was first described by J Kilian Clarke in 1924. *S. mutans* is stongly associated with dental caries and the primary causative agent ^[8].

Hence the aim of the study is to compare the antibacterial efficiency of different commercially available herbal mouthwash - An in vitro study.

Material and Method

Study design: In vitro, microbiological analysis

Inclusion criteria: Herbal formulations available in the market under mouth rinse category were to be included in the study.

(An online search was conducted to find out the commercially available herbal mouth rinse preparations, a total of 10 products were identified, of which 3 were selected for this study)

Exclusion criteria:

Any mouthwash containing alcohol, Chemical constituents as in conventional mouth rinses and fluoride containing mouthwashes were excluded from the study.

Products tested and composition

1) Namboodiri herbal mouthwash

K.P.Namboodiri'S herbal fresh mouthwash ingredients are as follows:

- Sorbitol,
- Licorice extract,
- polysorbate 20,
- Thymol,
- Tea,
- tree oil,
- extracts of clove,
- ginger,
- nutmeg,
- Cardamom.

2) Himalayan herbal complete care miswak and pomegranate mouthwash(Manufactured by the Himalaya Drug Company Makali, Bangalore 562123 (India)

Himalaya herbal complete care ingredients are as follows:

- Salvadora Persica extract ,
- punica granataum fruit extract ,
- Melisa Azadirachta leaf extract ,
- Sorbitol, citric acid.

3) Orafresh mouthwash - manufactured by charak

Orafresh Mouthwash Ingredients are as follows:

- Camellia sinensis 100mg,
- Triphala 100mg,
- Piper cubeba 70 mg,
- Melisa azadirachta 60 mg,
- Acacia catechu 40 mg,
- Mentha spicata 75 mg,
- Purified alum 50 mg,
- Eucalyptus globulus 5mg.

4) saline



Fig1: different herbal mouthwash

Armamentarium:

- Cuvette
- Cuvette stand
- Micropipette
- Petri dish
- Nutrient agar
- Spirit lamp
- Metal loop
- incubator
- saline
- Gloves

Method:

Three herbal mouthwashes were taken. 1000 µL of each herbal mouthwash was pipetted into three cuvettes saline was taken as control .1000 µL of saline was pipettes and poured into a cuvette. S. Mutans bacterial suspension was prepared. 10 µL of the prepared bacteria suspension was pipetted out and add to each cuvette. Cuvette was then incubated for 1hour at 37 degree Celsius at microbiology department, Saveetha dental college, poonamalle. Nutrient agar was prepared. 10µLof sample was pipetted out from one cuvette sand poured into one nutrient agar plate. Metal loop was heated to red hot and sample was streaked using metal loop. Nutrient agar plates were then incubated at 37 degrees Celsius for 24 hours. Agar plates were taken out of the incubator and bacterial colony count was done.



Fig2: depicts methods for identification of antibacterial effect of different herbal mouthwash

Flow chart 1: Method for indentifying antibacterial efficacy of different herbal mouthwash

Statistical Analysis

- 1) Data was entered in Microsoft excel spread sheet and analysed
- 2) Numerical data were presented as mean and standard deviation

Result

The CFU/µL of streptococcus mutans in different commercially available herbal mouthwash was given in table 1.

Himalaya herbal mouthwash had less number of bacterial colonies and oral fresh had high number of bacterial colonies, but all the herbal mouthwash has better Antibacterial effect Thant saline.

Table 1: S.mutans colony forming unit per µl of different herbal mouthwashes

Herbal mouthwash	Colony forming units per µL			Mean
	Sample 1	Sample2	Sample3	
Saline	58			
Himalaya	13	7	4	8
Namboodiri's	13	12	23	14.33
Orafresh	21	13	18	17.33

Discussion

In current study *Streptococcus mutans* showed 8 CFU/10 μ L in Himalaya herbals complete care mouthwash (miswak and pomegranate) and this result was similar to the results obtained from the study made by Charuta Sadanand Dabholkar, Mona Shah which also showed Himalayan herbal mouthwash exhibited anti bacterial efficacy against *S. mutans*. Study made by Jonathan E Sam also suggested that Himalaya herbal mouthwash significantly reduced the *S. mutans* count. Rupali Mahajan et.al also suggested that Himalaya herbal mouthwash had the same effect of chlorhexidine [9, 10, 11].

Study done by Sanjukta also mentioned the anti bacterial effect of Himalayan herbal mouthwash HiOra R (manufactured by The Himalaya Drug Company, Bangalore, Karnataka, India). It is a herbal preparation, made from a combination of natural herbs with beneficial properties of anticarcinogenic and anti-plaque due to the presence of Pilu (*Salvadora persica*) 5 mg, antibacterial, anti-inflammatory, and immunity booster due to Bibhitaka (*Terminalia bellerica*) 10 mg, antioxidant, antimicrobial, and plaque inhibiting properties due to Nagavalli (*Piper betle*) 10 mg. Essential oils of Gandhapurataila (*Gaultheria fragrantissima*) 1.2 mg possess antimicrobial, anti-inflammatory, and analgesic properties. Oil extracted from Ela (*Elettaria cardamomum*) 0.2 mg is a potent antiseptic that is known to kill bacteria producing bad breath. Peppermint satva (*Mentha sp.*) 1.6 mg acts as a natural mouth freshener. Yavanisatva (*Trachyspermum ammi*) 0.4 mg also has antimicrobial properties. *S. persica* is one among the most commonly used antibacterial agent in traditional Ayurveda medicine. Its role as an anti-plaque agent has been reported extensively [12].

In the current study *Streptococcus mutans* showed 14.33 CFU/10 μ L in namboodiri's herbal mouthwash and this result was similar to the result obtained from the study by sham .s butt results suggest that against *Streptococcus mutans*, K. P. Namboodiri's toothpaste was found to be the most effective followed by Colgate herbal toothpaste. K [6]

In current study orafresh herbal mouthwash showed 17.33 CFU/10 μ L this herbal mouthwash contain acacia catechu leaf extract which has high levels of Rutin and Epicatechin and the results was similar to the results obtained by Ezhil.I et.al. The results showed that epicatechin has no growth at 25 mg/ml tested against

S. mutans and *E. faecalis*, chlorhexidine as positive control showed no growth in 10 mg/ml. Rutin showed no growth at 50 mg/ml against *S. Mutans* and at 25 mg/ml against *E. faecalis*. When compared to chlorhexidine the efficacy was less, but epicatechin and Rutin showed antibacterial activity against *S. mutans* and *E. faecalis* with a moderate action. Current research indicates that the polyphenols, being secondary metabolites, are present in rich amount in various plants. [13].

The results obtained by Rakshana et.al showed that the herbal mouthwash made up of *Ocimum sanctum* tested showed significant antibacterial efficacy represented in. It showed no growth at 25 mg/ml tested against *S. Mutans* and 25 mg/ml against *S. sanguis*, and at 100 mg/ml against *L. acidophilus*. 10 mg/ml against *S. salivations*. Agar well diffusion method showed a maximum zone of inhibition against *S. Mutans* and *S. Salivarius* (19 mm and 22 mm), respectively [14].

In the current study use of herbal mouthwash was recommended but this was contradictory to the result obtained by Amir Moeintaghavi. This review showed that, when compared to herbal mouthwash, chlorhexidine mouthwashes provided better results in its antimicrobial efficacy against *Streptococcus mutans*. Further research could study the antimicrobial efficacy of herbal mouth rinse in greater depth and in vivo clinical testing is essential to confirm the in vitro results. [15,16]

There were few limitations associated with this study, colony forming units was only test in this study further study has to be made to identify the zone of inhibition of *S. mutans* against several herbal mouthwash.

There have been numerous in vitro studies that have investigated the activity of natural plant substances against oral bacteria. These studies have focused on bacteria known to be involved in the aetiology of oral and dental diseases. Early studies have clearly established that a number of herbal substances had potential to be utilized as mouthwash, given their activity against carcinogenic bacteria and those bacteria associated with periodontal diseases. certain plants and herb extracts like clove bud oil, cinnamon bark oil, and papuaamace extracts constituents of these extracts, such as cinnamic aldehyde and eugenol [17,18,19].

Use of different commercial brands of mouthwash, use of different ingredients may differ from study to study hence they affect the results. Presiding the ingredients, concentration, commercial brands can

help the researchers to identifying the main causes of differences in the results. [20]

Conclusion

The Antibacterial efficacy of different herbal mouthwash shows that Himalaya and namboodiri's herbal mouthwash has significantly antibacterial efficacy against S.mutans. Further research should be made against different organism and different herbal mouthwashes.

Ethical Clearance : Nil

Source of Funding : Self

Conflict of Interest : Nil

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