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Evaluation of inhibitory effect of selected mouth rinses (chemical and herbal) on bacteria

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ABSTRACT

Dental caries and periodontal diseases are the two most common chronic diseases of the oral cavity. Bacterial accumulation on oral surfaces is a major factor in the development of most of the common dental diseases such as dental caries and periodontal disease. In the present investigation, four chemical mouth rinses and one herbal mouth wash were employed to assess antibacterial activity in liquid media against pure cultures of bacteria and oral flora in saliva. Different mouthwashes were found to exhibit varying effects on bacteria tested. The efficacy of different mouthwashes was found to be little less on *B.subtilis* and *E.aerogenes* when compared to other test bacteria. The mouthwashes were found to have appreciable activity against growth of oral flora. Among mouthwashes tested, Mougel was found to inhibit the growth of oral bacteria to more than 50% followed by Chlorhexidine and others. Among chemicals, Chlorhexidine was found to inhibit oral flora to large extent. The ayurvedic mouthwash Mougel which was found to affect oral flora to a greater extent when compared to some chemicals. The antibacterial activity of Mougel could be possibly due to the presence of phytochemicals. The study was done in *vitro* and similar results could be expected *in vivo*. The right use of mouthwashes at right time could be very effective in the prevention of dental caries and other periodontal diseases.

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KEYWORDS

Mouth rinses;
Antibacterial activity;
Chlorhexidine;
Dental caries;
Oral flora.

INTRODUCTION

Dental caries is an infectious, microbiological disease that results in localized dissolution and destruction of the calcified tissues of the teeth. Dental caries and periodontal diseases are the two most common chronic diseases of the oral cavity. Their prevalence is recorded along with the history of man after his appearance on earth. Experimental and epidemiologic studies have demonstrated that these diseases are dependent on the microbes present in plaque. Dental decay is the most common disease of mankind. It has reached epidemic proportion in modern times since a fine consistency diet rich in refined sugar has been consumed[1]. Bacteria form an important group of micro-organisms found in both healthy and diseased mouths. Bacterial accumulation on oral surfaces is a major factor in the development of most of the common dental diseases such as dental caries and periodontal disease. *Streptococcus mutans*, a bacterium in mouth, is the chief bacterium that causes

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plaque and may also cause dental caries[2]. Mouthwashes are very useful in reduction of microbial plaques. Mouthwashes (mouth rinses) are solutions or liquids used to rinse the mouth for a number of purposes: (a) to remove or destroy bacteria (b) to act as an astringent (c) to deodorize and (d) to have a therapeutic effect by relieving infection or preventing dental carries. Mouthwashes are manufactured into two forms: the wash and the "spray". Constituents of mouthwashes include water (chief constituent); ethanol, dyes, surface active agents, zinc chloride/acetate, aluminum potassium sulphate (astringent): and phenolic compounds, quaternary ammonium compounds and essential oils such as oil of peppermint (as antibacterial agents) among others. Mouthwashes also provide a safe, effective chemical means of reducing or eliminating plaque accumulation. A number chemical agents are currently available in the market and are designed to assist individuals in their efforts to achieve and maintain oral health. While many agents are commercially available, the relative therapeutic benefits of most are not clearly defined[3]. The aims and objectives of the present investigation were to determine antibacterial activity of mouthwashes (chemical and herbal) on pure culture of bacteria and oral flora in saliva.

MATERIALS AND METHODS

Collection of mouth rinses

The different mouth rinses were purchased from local medical shops. The description of mouth rinses selected for the study was given in TABLE 1.

Screening mouth rinses for antibacterial activity

The test bacteria were obtained from National Chemical Laboratory, Pune. Gram positive bacteria namely *Bacillus subtilis* NCIM 2063, *Staphylococcus aureus* NCIM 2079 and Gram negative bacteria namely *Escherichia coli* NCIM 2065, *Enterobacter aerogenes* NCIM 2340 were used. *Streptococcus lactis* was obtained from the department culture deposit. Test tubes containing sterile Nutrient broth were aseptically inoculated with the pure cultures of test bacteria maintained on nutrient agar slants and incubated at 37^0C for 18 hours. The broth cultures of test bacteria obtained after incubation were used for inoculation. The antibacterial activity of different mouth rinses was tested in liquid nutrient media with minor modifications[4]. The nutrient broth tubes (5ml) containing known volume of mouth rinses (5%) was sterilized by autoclaving. The sterile media containing tubes were inoculated with standardized volumes of 24 hours old broth cultures of test bacteria followed by incubation at 37^0C for 24 hours. A set of tubes having mouth rinses were inoculated with saliva sample in order to check the efficacy of mouth rinses in affecting the bacterial flora present in saliva. A set of nutrient broth tubes inoculated with bacterial cultures was kept as control without adding mouth rinses. After incubation, the contents in the tubes were mixed thoroughly using vortex mixer and the optical density was measured by spectrophotometer at a wavelength of 490 nm as a guide to microbial growth.

RESULTS AND DISCUSSION

Antibacterial activity of different mouthwashes is given in TABLES 2-5. Different mouthwashes were

TABLE 1: Mouth rinses selected for study

Name	Active constituent
Rexidine	Chlorhexidine gluconate 0.2% w/v
Piclin	Sodium picosulfate
CPREV	Sodium fluoride (0.2% w/v), Triclosan (0.3% w/v)
Tantum	Benzydamine hydrochloride BP (0.15% w/v), Alcohol IP (10% v/v)
Mougel	*Emblica officinalis* (0.5%), *Terminalia chebula* (0.5%), *Terminalia belerica* (0.5%), *Acacia catechu* (0.5%), Borax (0.1%)

TABLE 2: Antibacterial activity of selected mouthwashes on target bacteria

Test bacteria	Optical density at 490 nm					
	Control	Benzydamine	Chlorhexidine	Sodium picosulfate	Sodium fluoride	Mougel
Streptococcus lactis	0.707	0.274	0.220	0.470	0.248	0.233
Escherichia coli	0.605	0.314	0.309	0.210	0.341	0.332
Enterobacter aerogenes	0.606	0.280	0.262	0.438	0.382	0.375
Staphyloccus aureus	0.682	0.260	0.244	0.397	0.234	0.231
Bacillus subtilis	0.453	0.253	0.284	0.393	0.253	0.176

The results are average of three trails

found to possess varying effects on bacteria tested. More than 50% reduction in growth was observed in case of *S.lactis* by Benzydamine, Chlorhexidine, Sodium fluoride and Mougel. Over 40% reduction in the growth of *E. coli* was observed in case of all the mouthwashes. The mouth rinses were found to be very effective against *S.aureus* and over 60% reduction was observed in all mouth rinses except Sodium picosulfate. The efficacy of different mouthwashes was found to be little less on *B.subtilis* and *E.aerogenes* when compared to other test bacteria. The different mouthwashes were found to have appreciable activity against growth of oral flora. Among mouthwashes tested, Mougel was found to inhibit the growth of oral bacteria to more than 50% followed by Chlorhexidine (49.11%), Sodium fluoride (46.97%), Benzydamine (33.27%) and Sodium picosulfate (30.78%).

Oral flora is a complex ecosystem with a wide variety of bacterial species. The number of bacteria in the dental plaque can reach 10^8/mg (Wet weight). *Streptococcus* species occupy approximately 1/3rd of total viable organisms of plaque. In addition of *Streptococcus mutans,* several streptococcal species are frequently found in the human oral cavity. Among these are *S.sanguis, S.gordonii, S.oralis, S.mitis, S.salivarious* and others. It has been shown that these oral streptococcal species are not involved in the development of dental caries in experimental animals. However, it should be noted that all these species are highly acidogenic when sucrose, glucose, fructose is given[5]. Mouthwashes are very useful in reduction of microbial plaques. Among available mouthwashes, chlorhexidine was shown to be highly effective in reduction of dental plaques and pathogenic micro-organisms including *Streptococcus mutans*[6]. Seven different brands of mouthwashes were evaluated for inhibition of growth of oral microbes and found wide variations in the effectiveness of mouthwashes. Those containing cationic surfactants and complex organic nitrogenous compounds were more active than older formulations based on phenols[4]. The inhibition of growth of oral bacteria by three modern commercial mouth rinses containing cetylpyridinium chloride, CPC (Macleans), phenolic compound (Colgate plax) or glycerin/triclosan (Listerine) was assessed. The results showed wide variations in their effectiveness, those containing CPC reduced oral microbial count significantly than formulations based on phenols or glycerin/triclosan. The results suggested that inhibitory power of CPC was greater on oral microbes than others[3]. The antibacterial effect of 0.2% Chlorhexidine to 0.5% sodium hypochlorite as canal irrigating solutions was compared. Elimination percent mean of *Streptococcus mutans* and anaerobics with 0.2% Chlorhexidine were 99.9 and 99.02 respectively and for 0.5% hypochlorite were 99.7 and 92.7 respectively[7]. Recently, the use of herbal mouthwashes such as persica is increasing. Persica is prepared from *Salvadora persica* extract. It has been shown that using this herbal medicine or its extract would support periodontal health, and reduces the accumulation of microbial plaques, and bleeding during brushing and control gingivitis and periodontal diseases[6].

TABLE 3: Percentage reduction in growth of test bacteria by selected mouthwashes

Test bacteria	Percentage reduction in growth of test bacteria				
	Benzydamine	Chlorhexidine	Sodium picosulfate	Sodium fluoride	Mougel
Streptococcus lactis	61.20	68.80	33.50	64.90	67.04
Escherichia coli	48.09	48.92	65.20	43.60	45.12
Enterobacter aerogenes	53.79	56.76	27.72	36.96	38.11
Staphyloccus aureus	61.80	64.22	41.78	65.68	66.12
Bacillus subtilis	44.15	37.30	13.24	44.15	61.14

TABLE 4: Antibacterial activity of selected mouthwashes on bacterial load in saliva

Sample	Optical density at 490 nm					
	Control	Benzydamine	Chlorhexidine	Sodium picosulfate	Sodium fluoride	Mougel
Saliva	0.562	0.375	0.286	0.389	0.298	0.233

TABLE 5: Percentage reduction in growth of test bacteria by selected mouthwashes

Sample	Percentage reduction in growth of test bacteria				
	Benzydamine	Chlorhexidine	Sodium picosulfate	Sodium fluoride	Mougel
Saliva	33.27	49.11	30.78	46.97	58.54

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Chlorhexidine mouthwashes are the most commonly used antiseptic solutions (concentrations range from 0.10% to 0.12%). Bactericidal activity of antiseptics is achieved through highly concentrated application for prolonged periods of time, e.g., 2% Chlorhexidine for ten minutes to eradicate *P.gingivalis*[8]. Triclosan, a compound commonly used for disinfection, is another broad spectrum antibacterial agent manufactured specifically for use in oral care. Triclosan does not irritate the oral soft tissue or cause staining like chlorhexidine does. Triclosan works by disrupting the bacterial cytoplasmic membrane resulting in the leakage of cytoplasmic contents and the death of the bacteria. Fluoride inhibits plaque fluid, pH change and reduces lactate production following consumption of sugar. *In vitro*, fluoride also inhibits bacterial growth at concentrations less than dental plaque. The exact mechanism underlying this inhibition is not known, but fluoride has been shown to inhibit a variety of bacterial processes that are mediated by enzyme binding[2].

CONCLUSION

Bacteria in the mouth are an issue everyone has to deal with. Some of the bacteria can be helpful. However, most bacteria are harmful and cause plaque and bad breath. There are toothpastes and other remedies that help kill and prevent bacteria in mouths. The results of the study clearly revealed the potential of different mouthwashes on pure culture of bacteria was well as oral flora present in saliva. Among chemicals, Chlorhexidine was found to inhibit oral flora to large extent. The study made use of an ayurvedic mouthwash Mougel which was found to affect oral flora to a greater extent when compared to chemicals and its activity could be due to phytochemical groups present. Thus, plants can be exploited in place of chemicals as chemicals could cause irritation to some extent, discolor teeth etc. The study was done in *vitro* and similar results could be expected *in vivo*. The right use of mouthwashes at right time could be very effective in the prevention of dental caries and other periodontal diseases.

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