

Comparative Evaluation of the Effect of Propolis and Chlorhexidine Mouthwashes on *Streptococcus mutans* Counts in Saliva: An *In Vivo* Study

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ABSTRACT

Background: The role of *Streptococcus mutans* as the causative agent of dental caries is well demonstrated and documented. Besides chlorhexidine being the widely used synthetic medicament, a natural antibiotic substance called propolis (bee glue) has attracted the attention of researchers due to its multiple pharmacological properties. This natural product is produced by honey bee and showed antibacterial activity against a range of commonly encountered Gram-positive microorganisms including *Streptococcus mutans*, preventing dental caries.

Aims and objectives: This study aimed to compare the efficacy of 5% propolis and 0.2% chlorhexidine mouthwashes in reducing *Streptococcus mutans* colony counts in saliva.

Materials and methods: Prerinse and 0.2% chlorhexidine mouthwash postrinse unstimulated salivary samples were collected from sixty subjects. The samples were then transferred to laboratory, using a transport medium, where they were cultured on trypticase soy yeast 20% sucrose bacitracin agar (TYS20B), a selective media for the growth of *Streptococcus mutans*. After one week, prerinse and 5% propolis mouthwash postrinse salivary samples were collected from the same subjects and were cultured in a similar manner. Each sample was serially diluted upto 1:10⁴ dilutions with the help of automated pipettes. *Streptococcus mutans* colonies were identified by various tests and counted by the use of a digital colony counter.

Results: The reduction of *Streptococcus mutans* by both the mouthwashes was found to be statistically significant and compared. 0.2% chlorhexidine showed better results in reducing *Streptococcus mutans* colonies than 5% propolis, which individually showed a good efficacy against the culprit microorganism.

Conclusion: Chlorhexidine has statistically better anticariogenic efficacy in comparison to propolis against *Streptococcus mutans* in saliva. On the other hand, propolis itself showed a significant anticariogenic activity against the growth of *Streptococcus mutans*. It may be considered as an alternative natural mouthwash to enhance oral health with minimum side effects.

Keywords: Chlorhexidine, Propolis, *Streptococcus mutans*.

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INTRODUCTION

Ever since the start of dental profession, dentists have been attempting to treat and prevent dental caries but it still continues to be a commonly found public health problem. Oral cavity harbors a wide variety of microorganisms and there is considerable evidence incriminating *Streptococcus mutans* as the most important organism in the initiation of dental caries. It causes demineralization due to its strong adhesion to tooth surface and acid production after fermentation.¹ Inhibition of *Streptococcus mutans* colonization on tooth surface can prevent the formation of dental plaque and the subsequent development of dental caries.

Chlorhexidine is a broad-spectrum antimicrobial bis-biguanide having potent anticariogenic activity and being more aggressive on Gram-positive microorganisms. The suppression of *Streptococcus mutans* by antimicrobial agents, especially locally administered chlorhexidine, is of clinical importance. The antibacterial action of chlorhexidine is based on its adsorption onto bacterial surfaces. At low concentrations, the bacteriostatic effect is based on the disturbance of bacterial cell functions, enzymes, and cell receptors, and at high concentrations, chlorhexidine causes cytoplasmic precipitation or coagulation.² Although chlorhexidine has proven to be an efficacious antibacterial agent, it may show side effects such as altered taste perception and discoloration on regular use.^{3,4} Till date, the prevention and control of dental caries is not restricted to a

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single procedure. In addition to the traditional methods, researchers are currently interested in natural substances that may offer alternatives in prophylaxis of dental caries with lesser side effects.

Propolis has been the material of interest for being a natural antibiotic substance. It is a complex resinous material collected by honey bee mixed with secreted beeswax. It consists of 50% resin,

vegetable balsam, 30% wax, 10% essential, aromatic oils, 5% pollen, and 5% other substances which include organic debris. Propolis is nontoxic and have multiple pharmacological effects due to its main chemical classes, i.e., flavonoids, phenolics, and aromatic compounds, where flavonoids provide antibacterial, antifungal, antiviral, antioxidant, and antiinflammatory properties. Propolis has been found to inhibit cell adhesion as well as water-insoluble glucan formation by *Streptococcus mutans*, thus may help in preventing dental caries formation. It may be used as a natural prophylactic agent for the control of dental caries with minimal side effects.^{5,6}

AIMS AND OBJECTIVES

The present study aimed to compare the antibacterial efficacy of 0.2% chlorhexidine and 5% propolis mouthwashes in reducing *Streptococcus mutans* colony counts in saliva.

MATERIALS AND METHODS

The current study was conducted in the Department of Pedodontics and Preventive Dentistry, Dasmesh Institute of Research and Dental Sciences, Faridkot, in collaboration with the Department of Microbiology, Guru Gobind Singh Medical College, Faridkot (Punjab). Sixty children in the age group of 8–12 years were selected for the study with no sex differentiation. An approval from the ethics committee was obtained prior to start of the study. Parents of the selected children were educated about the study and the written informed consent was obtained.

Inclusion Criteria

- Children in the age group of 8–12 years with DMFT <2 were identified and selected for the study.
- Willingness of the patients to participate in the study.

Exclusion Criteria

The following subjects were excluded from the study:

- Patients with abnormal oral or medical conditions.
- Those currently using any mouthwash.
- Subjects on antibiotic therapy or on any other medication 2 weeks prior to the start of study.
- Patients with xerostomia.
- Those having any systemic or allergic diseases.
- History of hypersensitivity to any of the products to be used in this study.
- Subjects or parents not willing to participate in the study.

Materials Used

- 0.2% chlorhexidine gluconate: alcohol-free 0.2% chlorhexidine gluconate mouthwash (hexidine, ICPA Health Products Ltd, Ankelshwar)
- 5% propolis: 5 mL of commercially available propolis (propolis Platinum™ K-Link Healthcare (India) Pvt. Ltd, Chennai) was diluted in 95 mL of distilled water to make 5% solution to be used as mouthwash.
- Culture medium: TYS20B-trypticase soy yeast 20% sucrose bacitracin agar (Hi Media Laboratories Pvt. Ltd, Mumbai).
- *Streptococcus mutans* MTCC 890 strains (Institute of Microbial Technology, Chandigarh) as quality control.

COLLECTION OF SALIVARY SAMPLES

Samples were collected on two occasions. On the first visit, prerinse (baseline) followed by 0.2% chlorhexidine postrinse salivary samples were collected. On the second visit, after an interval of one week, the same subjects were recalled and their prerinse (baseline) followed by 5% propolis postrinse salivary samples were collected. Prerinse and postrinse 1–2 mL unstimulated saliva samples were collected using sterile disposable syringe from the floor of the mouth (Fig. 1). A measured amount of saliva from the samples was immediately transferred into transport media containing test tubes.

SAMPLE CULTURING

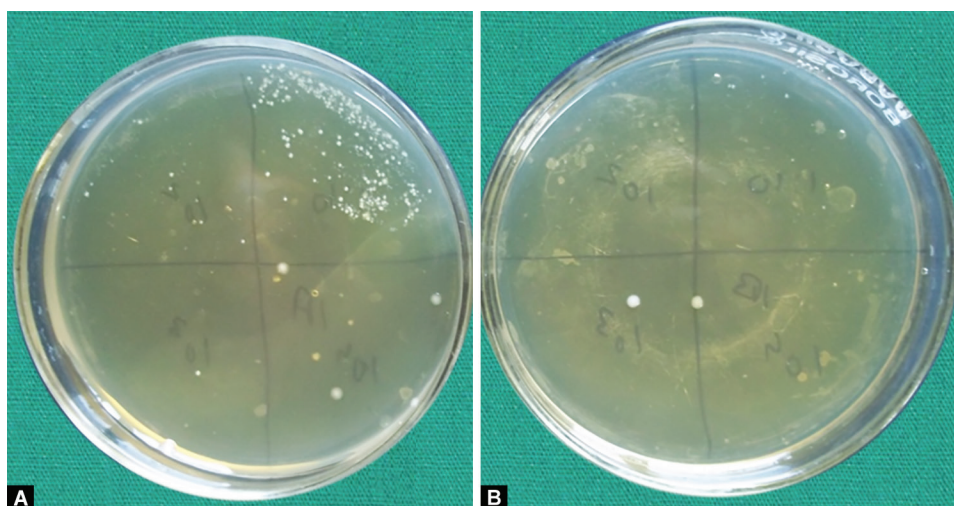
Salivary samples were transported to the microbiology department in test tubes containing thioglycollate broth (transport media). Each test tube contained a total of 1 mL solution (0.9 mL transport media and 0.1 mL salivary sample). Samples were serially diluted upto dilutions of $1:10^4$ using automated pipette and shaken manually to disperse bacteria. Culture media used for inoculation was trypticase soy yeast 20% sucrose bacitracin agar (TYS20B) which is a selective media for *Streptococcus mutans* growth.⁷ The petri dishes containing culture medium were labeled for each sample with dilution number. Incubation was done in candle jar/desiccator at 37°C for 48 hours. Colonies of *Streptococcus mutans* were identified by making smear on glass slide and observing under 100× magnification after Gram's staining. Colony characters such as color, size, shape, convexity, surface margins, consistency, opacity, hemolysis on blood agar, and pigmentation were studied. Pre- and postrinse colonies of both the mouthwashes were counted and compared (Figs 2 and 3).

RESULTS

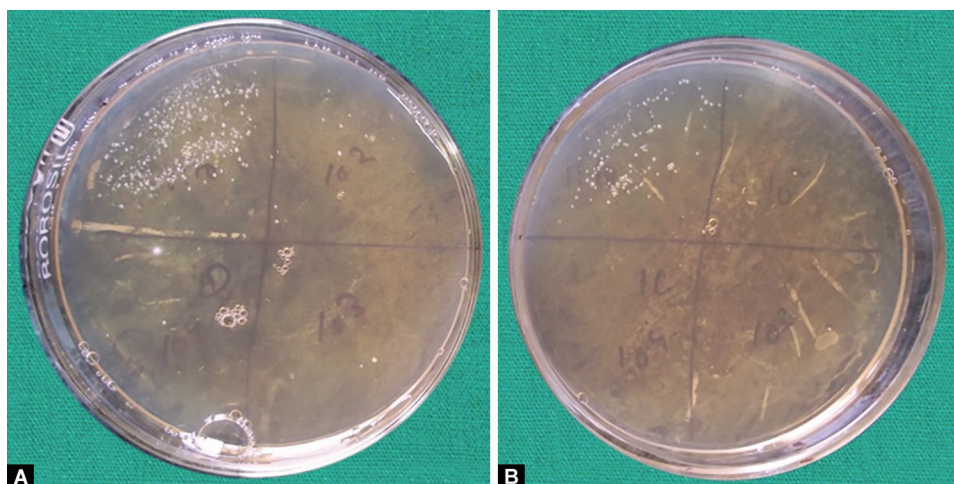
Data were collected, tabulated, and put to statistical analysis using SPSS (Statistical Package for Social Sciences) version 15.0 Statistical Analysis Software. The collected data did not have a normal distribution, so nonparametric Mann-Whitney *U* test was used for statistical analysis. Prerinse and postrinse *Streptococcus mutans* colonies in saliva were counted at $1:10$, $1:10^2$, $1:10^3$, and $1:10^4$ dilutions. There was no statistically significant difference in the mean prerinse colony counts on both occasions of saliva collection. Postrinse results with 0.2% chlorhexidine and 5% propolis



Fig. 1: Method of collection of saliva



Figs 2A and B: (A) Prerinse and (B) postrinse colonies on culture plates with 0.2% chlorhexidine mouthwash



Figs 3A and B: (A) Prerinse and (B) postrinse colonies on culture plates with 5% propolis mouthwash

mouthwashes showed significantly reduced *Streptococcus mutans* colonies, and the antibacterial efficacies of both the mouthwashes were compared.

- The mean salivary *Streptococcus mutans* colony counts after using 0.2% chlorhexidine mouthwash showed a statistically high significant reduction ($p < 0.001$), at all dilutions as shown in Table 1.
- 5% propolis mouth rinse showed a statistically high significant reduction ($p < 0.001$) in the mean salivary *Streptococcus mutans* colonies at 1:10 dilution and statistically significant reduction at dilutions 1:10², 1:10³, and 1:10⁴ ($p < 0.05$) as shown in Table 2.
- When both the mouthwashes were compared, 0.2% chlorhexidine showed a statistically high significant difference ($p < 0.001$) in the mean reduction of salivary *Streptococcus mutans* counts than 5% propolis, at dilutions 1:10, 1:10², 1:10³ but at dilution 1:10⁴; the difference in reduction was statistically significant ($p < 0.05$) as shown in Table 3.

DISCUSSION

The cariogenic microbiota includes *Streptococcus mutans*, which is the most frequently associated microorganism during the

Table 1: Comparison of mean prerinse and postrinse salivary *S. mutans* colony counts 0.2% chlorhexidine mouthwash ($n = 60$)

Dilution	0.2% chlorhexidine	Mean CFU/ mL	Std. deviation	p value
1:10	Prerinse counts	70.88	49.582	0.00
	Postrinse counts	22.98	27.466	
1:10 ²	Prerinse counts	36.82	33.625	0.00
	Postrinse counts	7.62	13.357	
1:10 ³	Prerinse counts	13.82	18.650	0.00
	Postrinse counts	2.28	5.773	
1:10 ⁴	Prerinse counts	5.43	10.162	0.00
	Postrinse counts	0.80	2.570	

initial phase of caries. Burt et al. stated that none of the teeth with nondetectable levels of *Streptococcus mutans* developed caries.⁸ Chlorhexidine inhibits plaque formation, reduces gingival inflammation, and prevents dental caries by limiting *Streptococcus mutans* colonization. Zanela et al. reported that 0.2% chlorhexidine gluconate reduced the highest percentage of plaque accumulation in comparison to 0.12% chlorhexidine gluconate associated to 0.05% sodium fluoride and 0.5% stevioside mixed with 0.05%

Table 2: Comparison of mean preinse and postrinse salivary *S. mutans* colony counts with 5% propolis mouthwash (n = 60)

Dilution	5% propolis	Mean CFU/ mL	Std. deviation	p value
1:10	Prerinse counts	59.38	39.882	0.00
	Postrinse counts	46.98	28.122	
1:10 ²	Prerinse counts	26.30	27.145	0.004
	Postrinse counts	21.67	21.019	
1:10 ³	Prerinse counts	11.02	15.357	0.002
	Postrinse counts	5.43	12.078	
1:10 ⁴	Prerinse counts	4.00	7.323	0.018
	Postrinse counts	2.33	3.639	

Table 3: Comparison of mean reduction in salivary *S. mutans* colony counts with 0.2% chlorhexidine and 5% propolis mouthwashes (n = 60)

Dilution	Mouthwash	Mean reduction	Std. deviation	p value
1:10	0.2% chlorhexidine	47.90	36.691	0.000
	5% propolis	12.40	21.630	
1:10 ²	0.2% chlorhexidine	29.20	28.234	0.000
	5% propolis	4.63	12.087	
1:10 ³	0.2% chlorhexidine	11.53	15.612	0.000
	5% propolis	5.58	11.843	
1:10 ⁴	0.2% chlorhexidine	4.63	8.356	0.021
	5% propolis	1.67	5.048	

Highly significant at $p < 0.001$; significant at $p < 0.05$

sodium fluoride, which suggested the use of 0.2% concentration of chlorhexidine in the present study.⁹ Although chlorhexidine has marked anticariogenic effect, it also possesses certain drawbacks. Helms et al. found that chlorhexidine mouthwash reduced the perceptual taste intensity of sodium chloride (salty) and quinine hydrochloride (bitter) when used twice a day,¹⁰ whereas Solis et al. reported tooth staining with 0.2% chlorhexidine mouthwash, fifteen days after its use.⁴ Also, the taste of chlorhexidine mouthwash is not well accepted by children. These drawbacks have necessitated the search for alternate agents.

Propolis serves as a natural antibiotic produced by bees. The medicinal properties of propolis have been widely described which includes antibacterial capabilities against *Streptococcus mutans*, suggesting the use of propolis as a cariostatic agent.¹¹ Malhotra et al. stated that antimicrobial efficacy of propolis against *Streptococcus mutans* was similar to that of chlorhexidine.¹² A study conducted by Hegde et al. on the effect of propolis on *Streptococcus mutans* counts in saliva concluded that 90% samples showed reduction in bacterial load after the mouthwash.⁶ These studies led to the present study. 5% propolis mouthwash used in the present study was alcohol-free. The reason for not including alcohol in the mouthwash was based on both social and health reasons. It has been recognized that in some individuals, oral mucosa is sensitive to alcohol and possible increased risk of developing oral premalignant lesion.¹³ Also, alcohol has an antiseptic/antibacterial effect which would have enhanced the antibacterial activity of propolis. To check the real efficacy of 5% propolis against *Streptococcus mutans*, alcohol-free mouthwash was used.

In the present study, 0.2% chlorhexidine mouthwash decreased *Streptococcus mutans* colony counts in 98.33%, 95%, 85%, and 65% of the salivary samples at 1:10, 1:10², 1:10³, and 1:10⁴ dilutions, respectively. These results suggested that chlorhexidine is very effective against *Streptococcus mutans* and are in similarity with the study conducted by Jarvinen et al. who worked on the *in vitro* susceptibility of *Streptococcus mutans* to chlorhexidine and six other antimicrobial agents and found chlorhexidine was highly effective against all the *Streptococcus mutans* isolates.¹⁴ Whereas 5% propolis mouthwash decreased *Streptococcus mutans* colonies in 76.67%, 70%, 53.33%, and 43.33% of the salivary samples at 1:10, 1:10², 1:10³, and 1:10⁴ dilutions, respectively. Serial dilutions 1:10, 1:10², 1:10³, and 1:10⁴ of the salivary samples were carried out for easier counting of the colonies of *Streptococcus mutans*. Serial dilution method is used to identify the number of viable microorganisms in a fixed amount of liquid. Most specimens have high enough numbers of microorganisms that the specimen has to be serially diluted to quantitate effectively.^{15,16} The present study used in this method was similar to the study conducted by Gronroos et al., in which clinical mutans streptococcal isolates were studied using 10-fold dilutions.²

Liberio et al. reviewed the potential use of propolis as a cariostatic agent and its actions on mutans group streptococci. They stated that propolis showed reductions in *Streptococcus mutans* counts and interfere with the adhesion capacity and glucosyltransferase activity. *In vivo* studies have demonstrated that propolis reduce *Streptococcus mutans* colonies in saliva, the plaque index, and insoluble polysaccharide formation. These findings indicated that propolis can be a promising cariostatic agent.¹⁷

A comparison of both mouthwashes in the present study indicated that 0.2% chlorhexidine is more efficacious than 5% propolis mouthwash in reducing *Streptococcus mutans* counts in saliva, at all dilutions. This result is supported by the Dodwad and Kukreja study, where the subjects received a propolis mouthrinse or Saline or 0.2% chlorhexidine and concluded that 0.2% chlorhexidine mouthwash was better than propolis and saline in inhibiting plaque formation.¹⁸ Despite its significant anticariogenic property, the long-term use of chlorhexidine is not advisable due to local side effects, such as soreness of oral mucosa, irritation of taste buds, discoloration of the teeth, tongue, restorations, and dentures. Ozan et al. compared the effects of mouthrinse containing propolis and 0.2% chlorhexidine on oral microorganisms and their cytotoxic effects on human gingival fibroblasts. The study reported higher cytotoxic effects with chlorhexidine. On the other hand, propolis proves to be nontoxic to host cells, does not cause a significant imbalance in the oral micro biota, and has lesser side effects.^{1,19,20}

Jahromi et al. cited that propolis prevents bacterial cell division and broke down bacterial walls and cytoplasm similar to the action of some antibiotics.²¹ In the present study, 5% propolis mouthwash showed a statistical significant reduction in *Streptococcus mutans* colonies which suggest that propolis has a good potential against *Streptococcus mutans* and to be used as an anticariogenic agent. However, our study had a short-term evaluation with limited sample size and involved a multistep procedure to attain the final values, which may have influenced the results. Therefore, further long-term evaluations are recommended with a larger sample size and concentration variation, to observe its effect on the reduction of *Streptococcus mutans*, and to assess its practical and economic feasibility.

CONCLUSION

Chlorhexidine has a statistically better anticariogenic effect in comparison to propolis. But it is seen that in its own capacity, 5% propolis is a promising natural anticariogenic agent as it statistically reduced the *Streptococcus mutans* colonies in saliva. We can chemically standardize it further and it can be used as an alternative to chlorhexidine as a mouthwash to reduce the growth of *Streptococcus mutans* in the wake of many drawbacks to its use. Also, for the long-term usage, propolis can be a valuable option.

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