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Antimicrobial Activity of Propolis against *Streptococcus mutans* Compare with Chlorhexidine

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Introduction: Propolis is a compound produced by bee's which exerts antibacterial action against oral microorganisms especially streptococcus mutans. Propolis has different concentrations which exhibit different properties like antibacterial, antifungal, antiviral, antiparasitic, anti-inflammatory, antiproliferative and antioxidant.
Materials and Methods: Antimicrobial activity of propolis was first demonstrated through disk diffusion method and broth dilution method. In the disk diffusion method, 5 grams of propolis was diluted with 50ml of ethanol in an eppendorf tube. Different concentrations of propolis (ie) 50mm, 40mm, 30mm were added to sterile discs using a micropipette and were exposed to the bacteria and the results were observed. In the broth dilution method, different concentrations of propolis 1g, 1.25g, 1.5g were taken and dissolved in ethanol. Then 5ml of sterile distilled water was added to the propolis solution. The above solution was autoclaved for 1 and half hours. It was then exposed to the bacteria for half an hour and results were observed.
Results: This study was conducted to assess the antimicrobial activity of propolis against *Streptococcus mutans* in comparison with 0.2% chlorhexidine. When microorganisms were exposed to propolis for 24 hours, it affected the bacterial viability. There was a complete elimination of bacteria with propolis. Antimicrobial activity of chlorhexidine was evaluated and a similar result was obtained. Thus when we compare the antimicrobial activity of propolis and chlorhexidine there was no significant difference with a p value of 0.01 which is statistically significant.
Conclusion: This study showed a positive inhibitory influence of propolis with respect to the *Streptococcus mutans*.

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Keywords: Bacteria; chlorhexidine; novel method; propolis; Streptococcus mutans.

1. INTRODUCTION

Streptococcus mutans is one of the leading causes of dental caries which is the most cariogenic organism amongst the oral streptococci. Prevention can be obtained by brushing with fluoride containing dentifrices and topical application of fluorides. Dental caries can also be prevented by consuming low-sucrose diet meals and by the use of natural agents against oral pathogens. Propolis is a compound produced by bee's which exerts antibacterial action against oral microorganisms especially streptococcus mutans [1]. Propolis has different concentrations which exhibit different properties like antibacterial, antifungal, antiviral, antiparasitic, anti-inflammatory, antiproliferative and antioxidant [2]. Propolis in its raw form cannot be used for the analysis or treatment.So, it must first be dissolved and extracted for releasing the active ingredients. Many solvents can be used as the extractants like ethanol, methanol, water, hexane, acetone, dichloromethane and chloroform [3]. Antibacterial activity is due to the substances such as flavonoids and phenolic compounds [4].

It is important to study the mechanism of propolis as it allows to infer its effect on the cellular membrane permeability of microorganism, membrane potential disruption and the production of adenosine triphosphate (ATP) as well as a decrease in the bacterial mobility [5]. Previous studies have shown that propolis inhibits cell adhesion and water insoluble-glucan formation formed by streptococcus mutans. Studies have also shown that propolis possesses several other activities like anti-inflammatory, local anesthetic, hepatic-protective, antitumor and immunostimulating activities [6]. It has also been observed that the antimicrobial activity of propolis is higher in relation to Gram-positive than Gram-negative bacteria. The efficacy of propolis for surgical wound healing, caries prevention, treatment of dentin hypersensitivity, treatment of aphthous ulcers and propolis as a storage medium for avulsed teeth, root canal irrigating solution and mouthwash has been studied by various researchers and reported that propolis can be used as multipurpose product in oral health and dentistry [7].

Antiseptics like povidone iodine and chlorhexidine have many uses in medicine and dentistry. These oral antiseptics help in reducing bacterial contamination and controlling oral infections caused by cariogenic bacteria [8]. A study showed that propolis containing flavonoids show activity against the bacterial strains produced by campylobacter and streptococcus mutans. Another study showed that propolis in chewing candy preparations has antimicrobial activity - against streptococcus [9]. Certain studies done on iodine and chlorhexidine demonstrated that chlorhexidine mouthwash produced the greatest mean growth inhibition of S. sanguinis and S. mutans compared with other mouthrinses. Chlorhexidine and povidone iodine have been proved to be effective in inhibiting the growth of *S. mutans, S. sanguinis and L. acidophilus* [10]. Chlorhexidine and iodine have resulted in statistically significant reduction in *S. mutans and Lactobacilli* count. Thus the aim of this study is to evaluate the antimicrobial activity of propolis against *Streptococcus mutans and Lactobacilli* compared to iodine.

2. MATERIALS AND METHODS

The study was conducted in the Department of Microbiology in Saveetha Dental college. Antimicrobial activity of propolis was demonstrated through disk diffusion method and broth dilution method.

In the Disk diffusion method, 5 grams of propolis was diluted with 50ml of ethanol in an eppendorf tube. Different concentrations of propolis (ie) 50mm, 40mm, 30mm were added to sterile discs using a micropipette and were exposed to the bacteria. Through the disc diffusion method, antimicrobial activity was demonstrated. Thus broth dilution method was performed to obtain the results.

In the broth dilution method, different concentrations of propolis 1g, 1.25g, 1.5g were measured and dissolved in ethanol. Then 5ml of sterile distilled water was added to the propolis solution using a syringe. The above solution was autoclaved for 1 and half hours. It was then exposed to the bacteria for half an hour and introduced to the agar plates. After 24 hours, the observation was noted.

The obtained results and data were validated by the respective guide. The data and results were analysed using SPSS software. A p -value less

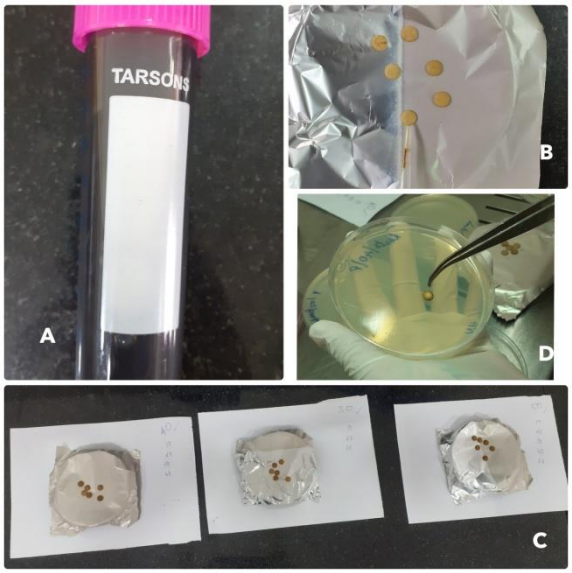

Fig. 1. Image depicts the disc diffusion method. (A) represents the propolis extract, (B) represents the addition of propolis to sterile discs, (C) represents the different concentration of propolis in sterile discs and (D) represents the exposure of propolis to the bacteria streptococcus mutans

than 0.05 (typically ≤ 0.05) was considered statistically significant. The results were tabulated and plotted in bar graphs. Dependent variables were *Streptococcus mutans,* propolis and chlorhexidine. Independent variables were bacteria other than *Streptococcus mutans* and other antimicrobial agents.

3. RESULTS

In the present study, antimicrobial activity of propolis and chlorhexidine were compared. Complete elimination of bacteria was observed in administration with propolis (Fig. 2). Therefore this confirms the antimicrobial activity of propolis. Then, the antimicrobial activity of chlorhexidine was evaluated. A similar result was obtained. Thus when we compare the antimicrobial activity of propolis and chlorhexidine there was no significant difference (Tables 1 and 2).

4. DISCUSSION

The present study examined the extract of propolis with ethanol which was demonstrated in vitro for evaluating the antimicrobial activity against the main cariogenic bacteria, that is, streptococcus mutans. When microorganisms were exposed to propolis for 24 hours, it affected the bacterial viability. There was a complete elimination of bacteria with propolis. Thus this confirms the antimicrobial activity of propolis. Then, the antimicrobial activity of chlorhexidine was evaluated. A similar result was obtained. Therefore, when we compare the antimicrobial activity of propolis and chlorhexidine there was no significant difference. A similar study demonstrated the antimicrobial activity of propolis where the ethanol extract of propolis had a strong antimicrobial activity and concluded that it is useful for the treatment of dental caries caused by streptococcus mutans [11]. Another study concluded that propolis was effective in inhibiting the growth of gram positive bacteria and it was as effective as chlorhexidine [12].

The mechanism of antimicrobial action demonstrated by propolis including cariogenic microorganisms is on the contrary and not completely understood. The biological activity of propolis has been reported to vary according to its composition. The significant variability of the chemical composition of propolis may be a limitation in terms of its quality control, comparability, and reproductive effect. There is several pieces of evidence to support the antimicrobial activity of propolis. Some studies have found propolis samples to be effective only against gram-positive bacteria and few fungi [13].

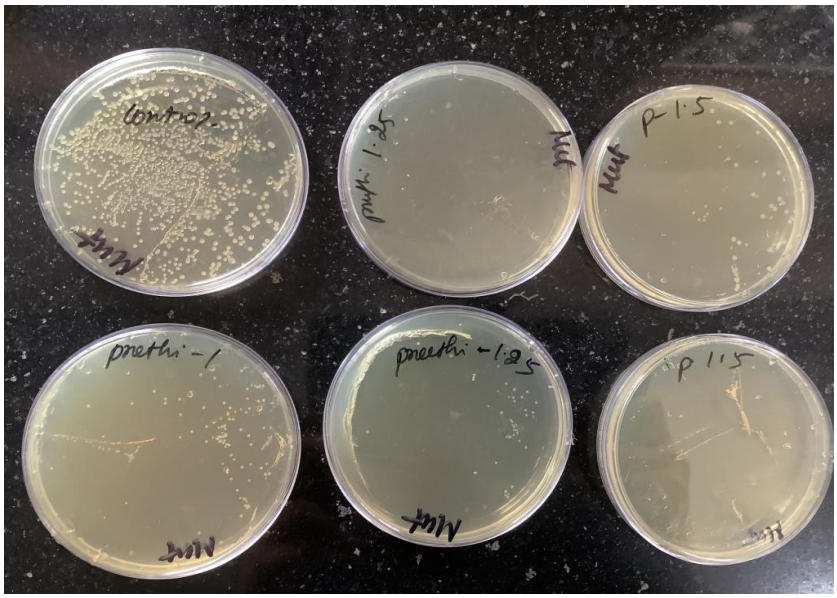

Fig. 2. Image represents the colony count of Streptococcus mutans

Table 1. Represents the mean colony forming unit of Streptococcus mutans with propolis

Concentration of propolis	1g	1.25g	1.5g
Streptococcus mutans	8	7	4

Table 2. Represents the mean colony forming unit of Streptococcus mutans with chlorhexidine

Concentration of chlorhexidine	2%	2%	2%
Streptococcus mutans	11	8	5

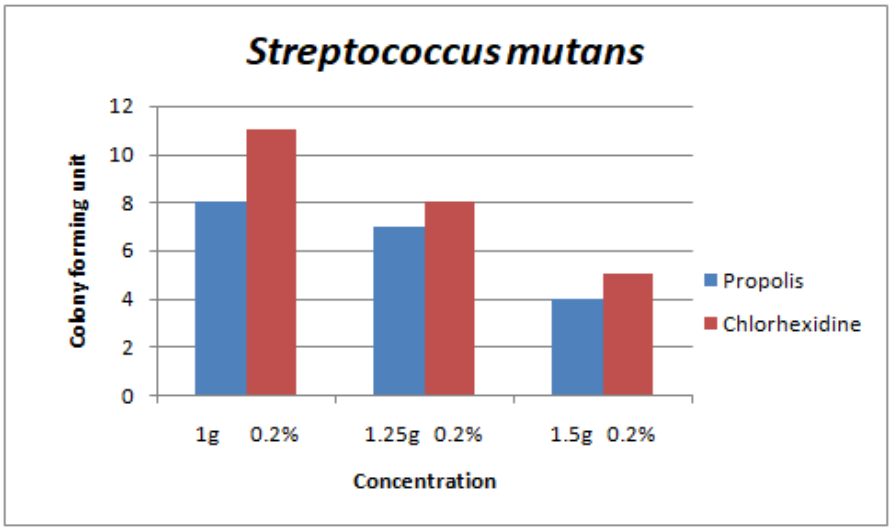

Fig. 3. The graph represents the comparison of antimicrobial activity of propolis and chlorhexidine against *Streptococcus mutans*. Blue colour denotes propolis and red colour denotes chlorhexidine. X axis represents the concentration of the extract and Y axis represents the antimicrobial activity in the colony forming unit. There was no significant difference in the antimicrobial activity of propolis and chlorhexidine with a p value of 0.01 (statistically significant)

Several studies conducted with antiseptics like chlorhexidine and iodine showed that 0.12% of chlorhexidine mouthwash had the best antimicrobial efficacy [14]. Another study demonstrated the highest activity shown by chlorhexidine (0.02) where a zone of inhibition of 13.9mm and 15.1mm was noted against *streptococcus mutans and lactobacillus acidophilus* [15]. A study showed the zone of inhibition to be ranging from 9 to 13mm in diameter for streptococcus mutans and candida albicans but there was no activity against lactobacillus acidophilus [16]. Another study demonstrated that the propolis supplemented chewing candy showed antimicrobial activity against streptococcus mutans and thereby indicating the formation of inhibition zones ranging from 1 to 8mm [17]. Thus propolis can be used in dentistry for several oral diseases as it possesses antimicrobial and anti inflammatory effects.

Our team has extensive knowledge and research experience that has translated into high quality publications [18-37].

5. CONCLUSION

Based on our results, we may conclude that the administration of propolis at appropriate concentrations can be effective on oral microorganisms. This study showed a positive inhibitory influence of propolis with respect to the oral microorganism growth. Thus it is evident that propolis exhibits antimicrobial activity against *Streptococcus mutans.*

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical clearance from the ethical committee of Saveetha dental college was obtained with registration.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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