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Comparing The Antibacterial Effectiveness Of Indigenously Derived Propolis In Various Vehicles Against Streptococcus Mutans Biofilm Formation: A Study With Contemporary Pulp Capping Agents

Ketaki Turbatmath[1] , Col.Sonali Sharma[2]

[1]Postgraduate Student
Department of Conservative Dentistry and Endodontics,
Saveetha Dental College and Hospitals,
Saveetha Institute of Medical and Technical Sciences (SIMATS)
Saveetha University,
Chennai-600077,Tamil Nadu,India
Email id- 152206006.sdc@saveetha.com
[2]Professor
Department of Conservative Dentistry and Endodontics,
Saveetha Dental College and Hospitals
Saveetha Institute of Medical and Technical Sciences (SIMATS),
Saveetha University,
Chennai-600077,Tamil Nadu,India
Email ID: colsonalisharma.sdc@saveetha.com
Corresponding Author:
Col.Sonali Sharma
Professor
Department of Conservative Dentistry and Endodontics,
Saveetha Dental College and Hospitals
Saveetha Institute of Medical and Technical Sciences (SIMATS),
Saveetha University,
Chennai-600077,Tamil Nadu,India
Email Id:colsonalisharma.sdc@saveetha.com

Introduction: Streptococcus mutans is one of the principle pathogens that significantly contributes to the etiology of dental caries. The virulence factor initiating the dental caries is the development of dental biofilm that is dominated by microorganisms.

Calcium hydroxide shows tunnel defects; hence, the need for the study was to develop a new product that can be used as a pulp capping agent with minimum drawbacks.
Material and methods: Propolis in concentrations of 0.2%, 0.5%, 1%, and 2%, calcium hydroxide 2%, and chlorhexidine 2% were prepared and mixed with dimethyl sulfoxide (DMSO). Agar diffusion by Mueller-Hington was done to assess biofilm inhibition at different concentrations. Antibiofilm assessment was done by Crystal Violet Stain.
Results: Aqueous propolis performed better than ethanolic propolis. However, though calcium hydroxide performed better than Propolis 2%, more optimization is needed to be performed.
Conclusion: When comparing biofilm inhibition, maximum biofilm inhibition was shown by calcium hydroxide 2%, followed by 2% aqueous propolis, 2% chlohexidine, and 2% ethanolic propolis. Aqueous extract of propolis showed better results when compared to ethanolic extract of propolis.
Keywords: anti-microbial efficacy, aparian-based product, apitherapy, indigenous, innovative, pulp capping agent, Streptococcus mutans

INTRODUCTION:

The virulence factor that starts dental caries is the development of dental biofilm that is dominated by microorganisms. [1] [2] Streptococcus mutans ferments a variety of carbohydrates, produces a large amount of acid, and secretes enzymes that synthesize extracellular polysaccharides and damage tooth enamel, ultimately leading to plaque or biofilm formation. [3] [4] [5]Traditionally, calcium hydroxide was used as a pulp capping agent. However, because of its alkaline pH, calcium hydroxide has natural antibacterial capabilities that can aid in the eradication of microbial penetration and consequent pulpal tissue unpleasant irritation. Calcium hydroxide has intrinsic limitations such as dissolving in oral fluids and the occurrence of tunnel defects in the development of dentin bridges. [6] [7]5] Chlorhexidine (CHX) is a widely used dental antibiotic that has been shown to be effective as an oral disinfectant and a non-specific matrix metalloproteinases (MMPs) inhibitor, with confirmed efficacy against MMP-2, -8, and -9 entrapped in dentin. As a pulp capping agent, calcium hydroxide and chlorhexidine were used to increase synergism and amplify antibacterial effectiveness. [8][9]

Propolis, where pro is ‘at the entrance’ and polis is ‘community’ made by honey bee Apis mellifera L. It is also called bee glue or hive dross. It is obtained from tree species like birch, poplar, pine, alder, willow, etc. It contains resin 50%, wax 30%, essential oil, pollen, and organic compounds. Propolis is well known as an immune-boosting flavonoid with numerous properties such as antiviral, antibiotic, antifungal, antioxidant, antibacterial, and anti-inflammatory. Propolis plays an important role in limiting plaque formation. Fatty acids in propolis provide a cariostatic effect; it decreases the tolerance of microorganisms to low pH and thus reduces acid production. Thus, the bacteriostatic, bactericidal, and antiadhesive activities of propolis on bacteria linked to caries imply that it may have an impact on the pathogenesis of caries. [10] [11]

The aim of the study was to compare and evaluate the indigenously developed apiarian-based formulation Propolis with calcium hydroxide and chlorhexidine against Streptoccocus mutans biofilm formation. The primary objective of the study was to assess biofilm inhibition by ethanolic propolis and aqueous propolis with calcium hydroxide and chlorhexidine by a crystal violet assay. The secondary objective was to evaluate the efficacy of aqueous propolis and

ethanolic propolis in biofilm inhibition at different concentrations by the agar diffusion method.

Description: Using the agar diffusion method and the crystal violet assay for antibiofilm assessment, 4 mg/mL of propolis was compared with 2% chlorhexidine and calcium hydroxide for biofilm inhibition.

Background: According to recent research, calcium hydroxide enhances biofilm growth rather than eliminating it. To increase the effectiveness of pulp capping, a propolis pulp capping agent is therefore to be developed. Propolis is the third most important component of bee products. It also contains vitamins and enzymes. [12] It has a variety of pharmacological attributes. As a result of its anti-oxidant, anti-inflammatory, immunomodulatory, antibacterial, and tissue-generating capabilities, it will aid in improving wound healing on an exposed tooth pulp. Therefore, propolis may increase tubular dentin synthesis and lessen pulp irritation in direct pulp capping. [13]

MATERIAL AND METHODS:

Ethanolic extract Propolis (ETOH) 0.2%, 0.5%, 1% & 2% concentration, Aqueous extracts of propolis (AQ) 0.2%,0.5%,1%& 2% concentration, Calcium hydroxide (CaOH2)2% and Chlorhexidine(CHX) 2% were mixed with Dimethyl Sulfoxide (DMSO).Evaluation of the antimicrobial activity through various tests such as Zone of inhibition and Biofilm viability assay was done. The study design contained three groups, with Group A- Propolis 0.2% , 0.5%, 1% and 2% as the experimental group, Group B-2% chlorhexidine as the control group, and Group C-Calcium hydroxide as the control group. Crude propolis was obtained from the Nature Honey, Chennai. 1 gram of grounded propolis was then dissolved in 50 ml of 70% ethanol. 1 gram of grounded propolis was dissolved in 50 ml of distilled water. A shaker by Orbitek was used to stir the solution at 155 rpm for 48 hours at room temperature. The ideal temperature required is 250 rpm. This process is known as maceration. Propolis with ethanol extract supernatant and propolis with distilled water extract supernatant are dried and stored. Chlorhexidine was obtained from Gluco-Chex (Cerkamed) 2% gel and Calcium Hydroxide from Dycal (Dentsply sirona), which is used for pulp capping.

The clinical strain of Streptococcus mutans was obtained from the Saveetha Microbiology Culture Unit and injected into Brain Heart Infusion Broth (BHIB). Following incubation, the S. mutans strains were kept in Brain Heart Infusion Agar (BHIA) at 4°C and 20% glycerol at 80°C. Streptococcus mutans ferments a range of carbohydrates, creates a high quantity of acid, lowering the pH, secretes extracellular polysaccharides (EPS), and forms plaques or biofilms. Agar diffusion was used to test the antibacterial effectiveness using Mueller-Hington agar media. The plates were incubated for 24 hours at 37 °C. For the results, the inhibitory zones on the plates were observed. Crystal violet staining was used to assess biofilm inhibition. After diluting the samples to the proper quantities (0.1 ml to 0.003 ml), they were added to the MHI broth in the wells of a 96-well microplate. The samples were then inoculated with 50 L of the broth culture and cultured at 37°C for 48 hours. Following the incubation period, the broth was aspirated from the wells with a sterile pipette and rinsed with PBS solution. Then, in each well, 150 L of crystal violet (0.2%) was added and allowed to stand for 15–20 minutes. The

dye is then removed and rinsed with PBS to eliminate unbound and excess dye. The dye was then dissolved in each well by adding 150 L of glacial acetic acid (30%). Readings were taken at 570 nm with an ELISA Plate Reader, and the absorbance value was recorded. Data was collected, and statistical analysis was performed under GPower.

RESULTS:

Graph 1: Antimicrobial efficacy against Streptococcus mutans

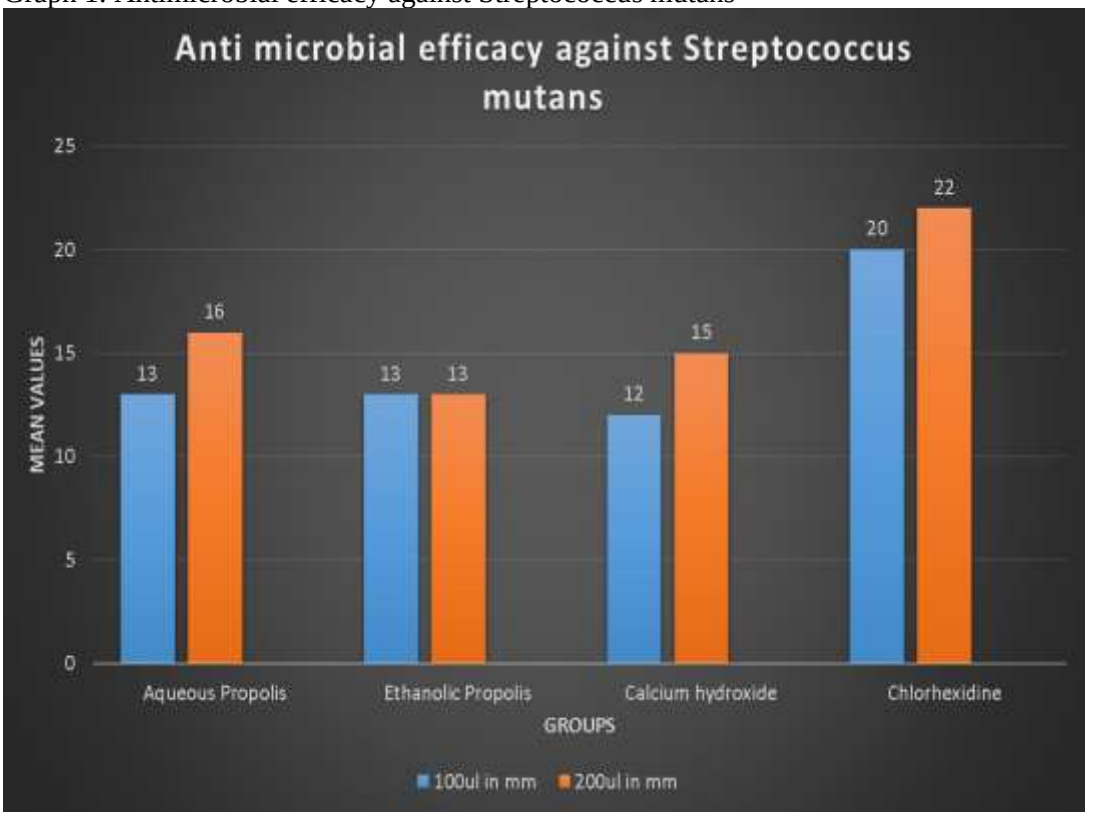
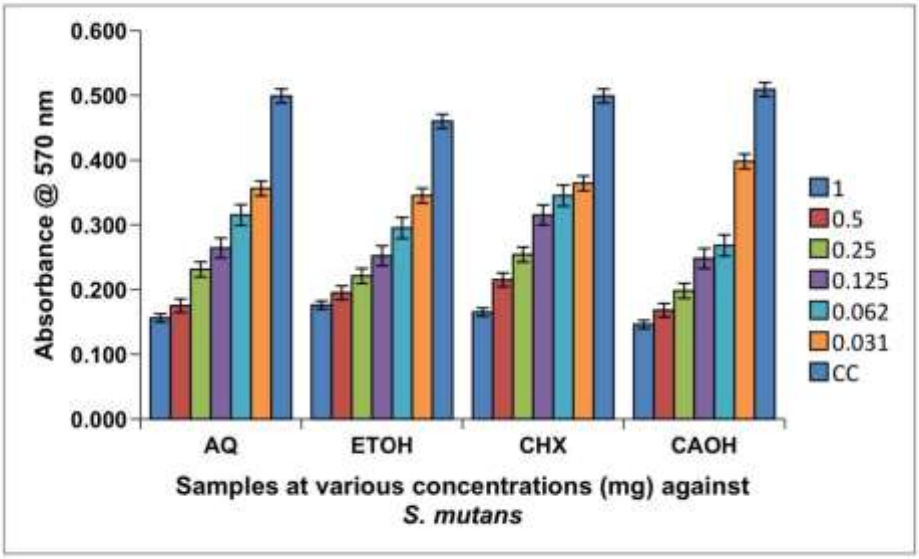


Table 1: Antimicrobial efficacy against Streptococcus mutans by the Agar diffusion method

Aqueous Propolis	Ethanolic propolis	Calcium hydroxide	Chlorhexidine
100ul (13mm) 200ul (16mm)	100ul (13mm) 200ul (13mm)	100ul (12mm) 200ul (15mm)	100ul (20mm) 200ul (22mm)

Graph 2: Antibiofilm assessment by Crystal Violet stain.



INDEX-

AQ	Aqueous Propolis
ETOH	Ethanolic Propolis
CHX	Chlorhexidine
CAOH	Calcium hydroxide

Table 2: Antibiofilm assessment by Crystal Violet assay

Concentration (mg)	% Biofilm formation

	AQ Propolis	Ethanolic propolis (ETOH)	CHX	CaOH
1	31.26	38.26	33.07	28.68
0.5	35.07	42.39	43.09	33.01
0.25	46.29	48.04	50.90	38.90
0.125	52.91	54.78	63.13	48.72
0.062	63.13	64.13	69.14	52.65
0.031	71.34	75.00	72.95	78.19

The highest antimicrobial efficacy was shown by 2% chlorhexidine, followed by 2% calcium hydroxide and 2% aqueous propolis, followed by 1% aqueous propolis and 0.5% aqueous propolis. (Graph 1, Table 1) It was seen that 0.2% aqueous propolis extract shows no significant action against Streptococcus mutans. The aqueous extract of propolis showed better inhibitory zones than the ethanolic extract of calcium hydroxide, which showed maximum biofilm inhibition against Streptococcus mutans. (Graph 2, Table 2) Though calcium hydroxide marginally performed better than propolis 2%, more optimization is needed to be performed as it is an indigenously developed product.

DISCUSSION:

Over time, pulp capping agents containing an antibiotic component prevented the establishment of resistant streptococci mutant strains. Pedrina et al. observed that calcium hydroxide does not destroy the biofilm but in fact contributes to it. [14] Chlorhexidine tolerance can also lead to genetic defects. Emislon et al. observed that the most persistant reduction in biofilm has been achieved by chlorhexidine. [15] But Kaspar et al. reported that Streptococcus mutans may develop resistance against the antimicrobial effects of flouride. [16] As a result, the purpose of this study was to compare and introduce novel, indigenous materials for pulp capping. Propolis, an apiarian product, was chosen as the indigenous new material. The purpose of this study was to evaluate the antibacterial activity of indigenous propolis in modern pulp. capping agents in suppressing Streptococcus mutans biofilm formation. Our findings provide crucial insights into the possible usage of these compounds in dental operations.

We discovered that indigenous propolis and modern pulp capping agents had antibacterial activity against Streptococcus mutans, and the inhibition zones observed in our experiments demonstrated these agents ability to impede biofilm formation, which is a critical factor in the development of dental caries. The current study's findings are consistent with prior research on the antibacterial capabilities of aparian products such as propolis and royal jelly and modern pulp capping agents. Yalcin et al. found similar antibacterial properties of Propolis

against oral infections, correlating with our findings. [17] Studies by Sanghavi et al. suggested that propolis can also be used as a storage medium for PDL cells. [18] Studies by Tyagi et al. reported that propolis can also be used as an effective antifungal agent similar to that of sodium hypochlorite on candida albicans biofilm. [19] Furthermore, our findings are compatible with the well-documented bactericidal capabilities of modern pulp capping agents, as described by Aljandan et al. [20] and Poggio et al. [21] However, studies by Djais et al. tested different samples of propolis concentration, and they showed no inhibitory effect against Streptococcus mutans. [22]

The antibacterial mechanisms of propolis, as well as comparisons with modern pulp capping agents, merit additional investigation. Future research should look into how these compounds interact with Streptococcus mutans and their ability to disrupt biofilm development.

In contrast, traditional pulp capping treatments frequently contain minerals such as calcium hydroxide or mineral trioxide aggregate, which are recognized for their alkaline pH and capacity to trigger tissue repair. These features may contribute to their antibacterial activities by making the environment unfavorable for bacterial development [23] [7] [24] [21] . These discoveries have major clinical implications. Both indigenous royal jelly and its antibacterial activity make it a promising natural alternative, notably in the prevention or inhibition of dental cavities and as an alternative pulp capping agent. [25] Depending on patient-specific characteristics and clinical settings, dentists may consider these medications as viable alternatives or complementing treatment adjuncts.

Long-term studies to assess the substantivity of the antibacterial properties should be among the future research topics. [26] [27] To assure patient safety, cytotoxicity tests should be performed on each element of propolis extract. Clinical trials will be conducted to assess the efficacy of these medicines in human patients. Further research could include combining Propolis extract with other restorative materials or intracanal medicaments to assess synergism.

It's critical to recognize our study's limitations, such as the use of in vitro experiments that may not fully reproduce the complexity of the oral environment. Furthermore, the study concentrated on a single bacterial strain of Streptococcus mutans, but the oral microbiome has a diverse range of species. [2]More study should be done to cover a broader range of cariogenic and other oral infections. The research shows that both indigenous propolis and modern pulp capping agents have antibacterial activity against Streptococcus mutans biofilm development. These findings pave the way for the creation of novel dental therapies as well as the improvement of existing pulp capping techniques. It`s potential as a natural alternative merits additional investigation in clinical settings, with the ultimate goal of enhancing oral health and treatment outcomes.

CONCLUSION:

Despite the study's limitations, it is possible to conclude that indigenously created apiarian compounds are effective antibacterial agents against S. mutans biofilm.

More research is needed to investigate the inhibitory concentrations and assess their long-term clinical efficacy and safety as pulp-capping agents in dental practice.

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