ORIGINAL ARTICLE

Antimicrobial Efficacy of *Triphala* and Propolis-modified Glass Ionomer Cement: An *In Vitro* Study

Jessy Paulraj[1], Priya Nagar[2]

Abstract

Background: The antimicrobial activity of restorative materials has a major role in preventing recurrent caries.

Aim: To assess the antimicrobial activity of *triphala* and propolis-modified glass ionomer cement (GIC) against *Streptococcus mutans* and *Lactobacillus*.

Materials and methods: The samples were prepared using cylindrical molds (6 mm in diameter and 2 mm in thickness). A total of 30 samples were prepared containing 10 samples in each group. Group I, 10 samples of glass ionomer with aqueous extract of *triphala* were prepared; group II, 10 samples of glass ionomer with 50% of ethanolic extract of propolis (EEP); and group III as control consisting of 10 samples of glass ionomer. The samples were placed in to agar plates containing inoculum of *S. mutans* and *Lactobacillus* and incubated at 37°C for 48 hours and using a digital caliper, zones of inhibition formed around specimens were measured.

Results: Data obtained were analyzed using nonparametric Kruskal-Wallis test followed by pairwise comparison was done using Dunn-Bonferroni test. Group I and group II showed highest antimicrobial efficacy against *S. mutans* and *Lactobacillus* with no statistical significant difference, i.e., (p value > 0.05) but in both groups I and II, there was a statistical significant difference when comparing with group III i.e., ($p < 0.05$).

Conclusion: Thus, *triphala* and propolis-modified GIC provided higher antibacterial effect with increased level of inhibition against the *S. mutans* and *Lactobacillus*; hence, it can be used as a choice of restorative material to treat dental caries. Further studies are required to determine the physical and mechanical characteristics of the material.

Keywords: Antibacterial effect, Glass ionomer cement, *Lactobacillus*, Propolis, *S. mutans*, *Triphala*.

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Introduction

The cariogenic bacteria responsible for dental caries are fixed in the dental plaque which are predominantly *Streptococcus mutans* and *Lactobacillus acidophilus*.[1,2] Microbial infection is the main cause for the inflammation of the dental pulp and periodontium. Previous literature indicates the existence of residual traces of infection in the site affects the success of restoration resulting in secondary caries.[3] Secondary caries process is difficult to diagnose and cannot be permanently treated by operative management.

Glass ionomer cements (GICs) are widely used in permanent restorations as a cavity liner, fissure sealants, and adhesives. It releases fluoride ions that act as anticariogenic agent and helps in prevention of oral problems such as enamel demineralization, remineralization, and also interferes with the bacterial growth and metabolism,[4] but it can reduce the microbial count to a certain extent. It is effective against some pathogens but not all oral pathogens causing cariogenic and periodontal problems. With the spectrum of bacteria inhibited by fluoride being inadequate, therefore, a restorative material that can create persistent antimicrobial environment around the restoration would be considerable to provide clinical benefit in reducing dental caries, plaque accumulation, and periodontal problems. Due to increased occurrence of recurrent caries after restorative treatment, much attention is required in the use of direct filling materials. Different modifications of GICs have been suggested in previous literature to enhance its antimicrobial properties. *Triphala* is an ayurvedic herbal formulation contains three medicinal plants *T. chebula*, *T. belerica*, and *Phyllanthus embelica*, which has been proven to have numerous benefits. This magical preparation has action on all the three components considered in Ayurveda—*Vata*, *Pitta*, and *Kapha*. *Such* a large range of action of *triphala* is supposed to be because of the 47 tannins and 35 phytochemicals.[5,6] Surprisingly, its mechanism of action has been less studied, and it has been expected to have an antimicrobial effect as said by previous literature. The study done by Srinagesh et al. suggested the anti-oral streptococci efficacy of *triphala*.[7]

Propolis, known for its antioxidant properties, was widely consumed since ancient times as a folk medicine.[8] In addition to antioxidant activity, it also contains numerous other benefits[9,10] that have been used till date in current medicine with a trend of "back to nature". The study reported by Yang et al. and Uzel et al. stated that there was a antimicrobial activity of propolis against *S. mutans*.[11,12]

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Another study done by de Carvalho Duailibe et al. proved that extract of propolis has an excellent antimicrobial activity against *S. mutans* which can be an alternate measure to prevent dental caries.[13] Keeping this in mind, the present study was planned to evaluate the antimicrobial efficacy of GIC-modified *triphala* and propolis against *S. mutans* and *Lactobacillus*.

Materials and Methods

Preparation of Ethanolic Extract of Propolis (EEP)

Propolis was supplied by Hitech Natural Lab, Delhi. The samples were grinded and preserved in container in 10-g portions. Using the magnetic mixer, the grinded samples were dissolved in 20 mL of ethanol. The rough particles were filtered, and the final extract of propolis was obtained.

Preparation of Aqueous Extract of *Triphala* (AET)

Triphala (IMPCOPS Ltd., Chennai, India) powder was transferred to solution by dissolving with 10% dimethyl sulfoxide (DMSO) (S.D. Fine Chem Pvt. Ltd., India). The previous literature states that pure properties of the herb can be attained even after mixing it with DMSO, as it is a highly polar, aprotic solvent[14,15] and hence it was used.

Bacterial Strain and Inoculum Preparation

Streptococcus mutans (ATCC 25175) and *Lactobacillus acidophilus (*ATCC 4356) were obtained from Bioline laboratories, Delhi. The agar well diffusion method was done to test the antimicrobial efficacy of ethanolic extract of propolis (EEP) and aqueous extracts of *triphala* (AET) against these bacterial strains.

A sterile complete loop of the pure culture of *S. mutans* was taken, and the facultative strains of *S. mutans* were fully grown on brain heart infusion agar. The microorganisms were subcultured in appropriate culture media and under gaseous conditions to improve purity, and it was inoculated individually in tubes containing 5 mL of sterile saline. The suspension was then adjusted to 0.5 Mcfarland scale = 1.5×10^8 colony-forming unit (CFU).

Determination of Minimal Inhibitory Concentration (MIC)

The MIC of EEP and AET against the bacterial strains. i.e., *S. mutans* and *Lactobacillus* was determined using the agar dilution method. The solution was serially diluted till the least concentration, the level at which inhibition was achieved toward the growth of *S. mutans* and *L. acidophillus* and was recorded as the MIC of the extract.

Specimen Preparation

The type IX GIC (GC corporation, Tokyo) was used in the present study.

- Group I: *Triphala* extract (L^{AET}) mixed with powder (P^{GIC}) and liquid (L^{GIC}) of GIC(P^{GIC}:L^{GIC}:L^{AET} ratio = 1:0.5:0.5)
- Group II: 50% EEP (L^{EEP}) added with powder (P^{GIC}) and liquid (L^{GIC}) of GIC(P^{GIC}:L^{GIC}:L^{EEP} ratio = 1:0.5:0.5)
- Group III: Conventional GIC (P^{GIC}:L^{GIC} ratio = 1:1) (Fig. 1).

After mixing the powder and liquid of conventional GIC, the liquid extract of propolis and *triphala* was incorporated. The final obtained cement was placed into cylindrical molds measuring diameter of 6 mm and 2 mm in thickness (Fig. 2), and the prepared specimens were carried to the cylindrical wells in less than 1 minute using the sterile cement carrier, and the upper surface of the cement layer was pressed to the equal level using sterile glass slide. After setting of the cement, the disk-shaped specimens were removed from the mold. The precise specimen was measured using calipers and recorded. Overall total of 30 specimens were obtained (Fig. 3).

Antimicrobial Assay Using Agar Disk Diffusion Test

Standard strains *S. mutans* and *Lactobacillus* were used to test the antimicrobial efficacy of two different restorative materials. Brain heart infusion broth is used for culture. Ten agar plates were used. Using a sterile swab, the surface of each agar plate was swabbed 3 times to ensure even distribution of the inoculum. After drying the agar plates, three wells of 6 × 2 mm diameter were made in each agar pate using sterile agar punchers, and set disk-shaped specimens were inserted into the wells after which the plates were set for incubation aerobically at 37°C for 48 hours. The zones of inhibition were measured based on the concept of Takahashi et al.[16] Digital caliper was used for measuring the inhibition zones.

Statistical Analysis

The obtained values were entered in MS excel spreadsheet, and the data were imported to SPSS (Statistical Package for Social Sciences) Version 20.1 (Chicago, USA Inc.). The descriptive and analytical statistics were done, and the normality of the data was checked using Kolmogorov-Smirnov test. Since the normality for the distribution was not met, the nonparametric Kruskal-Wallis

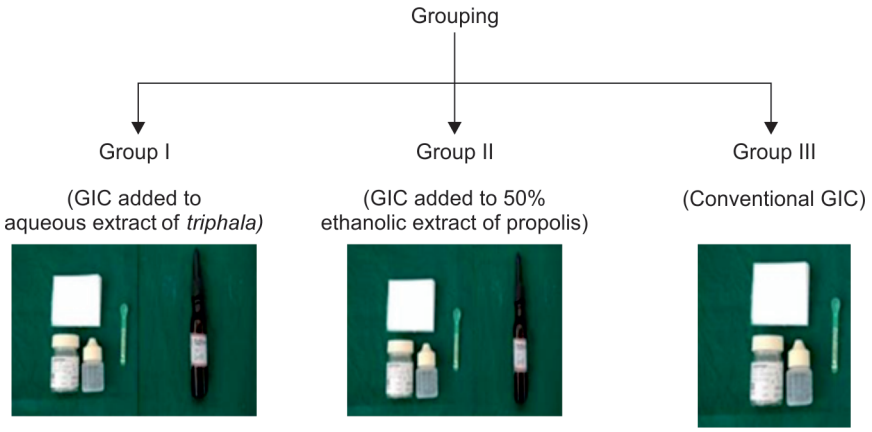

Fig. 1: Grouping

test was used to check differences between the groups, and the pairwise comparison was done using Dunn-Bonferroni test. The level of significance was set at <0.05.

Results

MIC Values

For aqueous extract of *triphala* solution and for ethanolic extract of propolis, inhibition of *S. mutans* and *Lactobacillus* was at 0.15 mg/mL and 0.1 mg/mL, and 0.025 mg/mL and 0.022 mg/mL, respectively. This present study proves superior antimicrobial activity of the *triphala* and propolis-modified GIC against *S. mutans* and *Lactobacillus* strain. The inhibition zones formed by groups I, II, and III are represented in Figure 4.

Antibacterial Efficacy against *S. mutans*

Antimicrobial efficacy on *S. mutants* between the three groups was tabulated and shown in Table 1. Group I when compared with group II showed effective antimicrobial efficacy without statistical significant difference between the groups (p value > 0.05), whereas control group showed least antimicrobial efficacy with the difference being statistically significant (p value < 0.05) (Table 2 and Fig. 5)

Antibacterial Efficacy against *Lactobacillus*

Antimicrobial efficacy on *Lactobacillus* between the three groups was tabulated and shown in Table 3. Group I when compared to group II showed highest mean diameter of inhibition zone against *Lactobacillus*, without statistically significant difference between the groups (p value > 0.05), while the control group (group III) failed to inhibit growth which showed least antimicrobial efficacy with the difference being statistically significant (p value < 0.05). (Table 4 and Fig. 6)

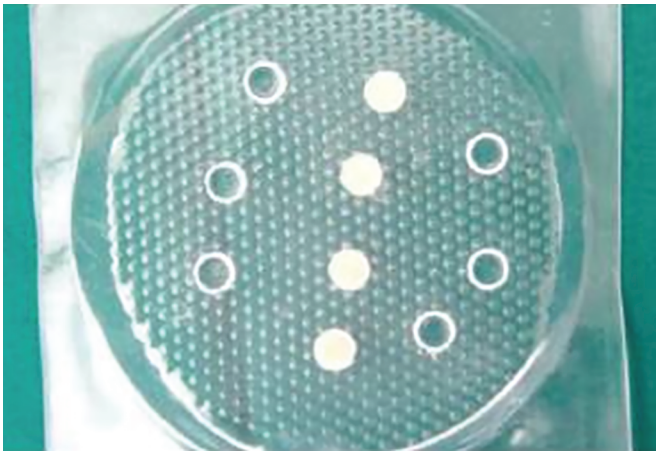

Fig. 2: Cylindrical moulds of 6 × 2 mm

Discussion

Dental caries is the most widespread dental disease in pediatric age-group. Dental caries is initiated mainly by two groups of bacteria *S. mutans* and *Lactobacilli*. These bacteria cause carbohydrates mainly sucrose which are sticky in nature to form organic acid which in turn demineralizes and denatures the tooth substance leading to dental caries or cavity. Glucans, which facilitate the attachment of bacteria to the tooth surface, is synthesized by *S. mutans* with the help of glucosyltransferase (GTF). If dental caries is not managed in time, it leads to pain, infection, and in the later stages extraction of teeth which has a direct bearing on child's esthetics and functional occlusion.

Streptococcus mutans (ATCC 25175) has a profound effect on the incidence of dental decay in the human population; hence, it

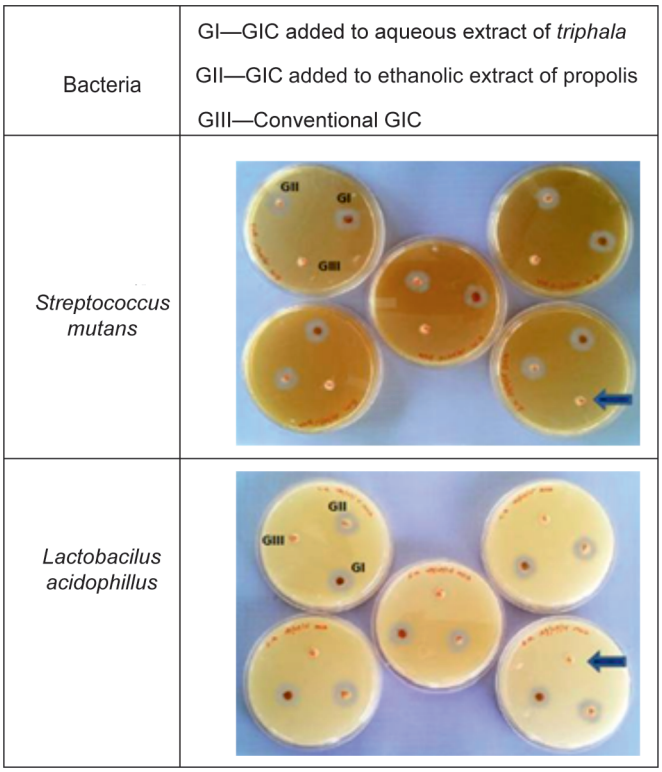

Fig. 4: Zone of inhibition against *S. mutans* and *Lactobacillus* for group I, group II and group III

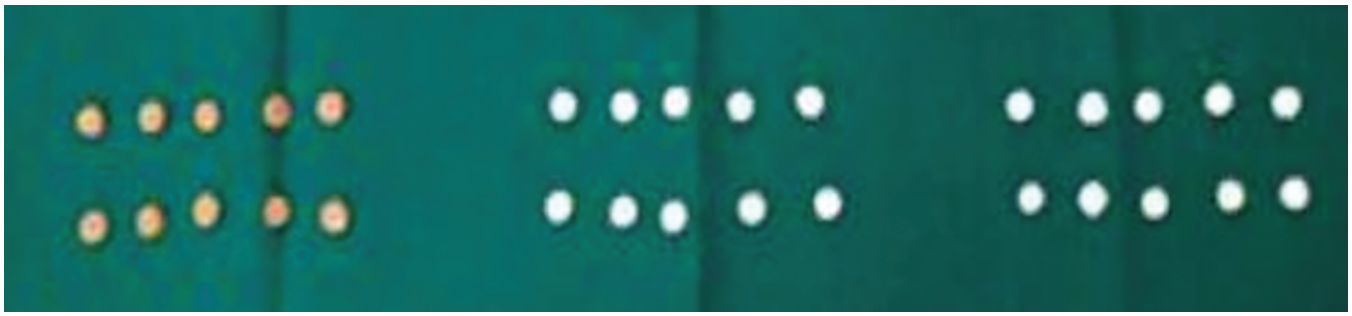

Fig. 3: 30 specimens

Table 1: Comparison of antimicrobial efficacy on *Streptococcus mutants* between the three groups—group I (GIC with aqueous extract of *triphala*), group II (GIC with ethanolic extract of propolis) and control group (plain GIC)

Variables	*n*	*Mean*	*S.D.*	*Median*	*Min–Max*	*Test statistics*	*p value*
Group I	5	11.60	0.41	11.50	11.00–12.00	9.795	0.007*
Group II	5	11.80	1.15	12.00	10.00–13.00		
Control group	5	05.50	0.50	05.50	05.00–06.00		

p value derived from Kruskal–Wallis test; *Significant at $p < 0.05$

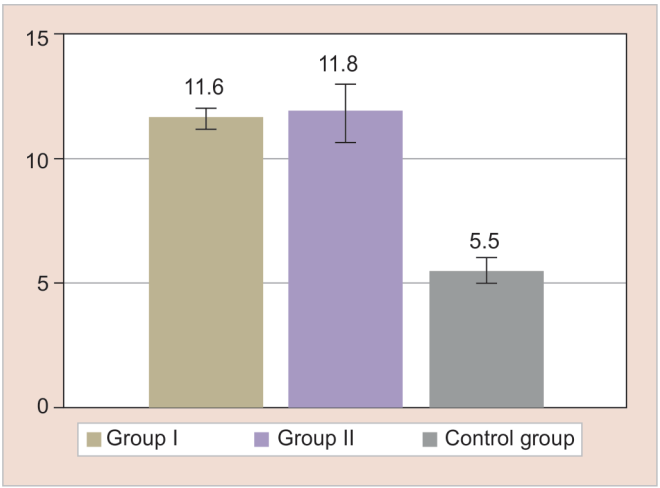

Fig. 5: Antimicrobial efficacy on *Streptococcus mutants* between the three groups—group I (GIC with aqueous extract of *triphala*), group II (GIC with ethanolic extract of propolis) and control group (Plain GIC). *Note*: The error bar represents the standard deviation of the mean

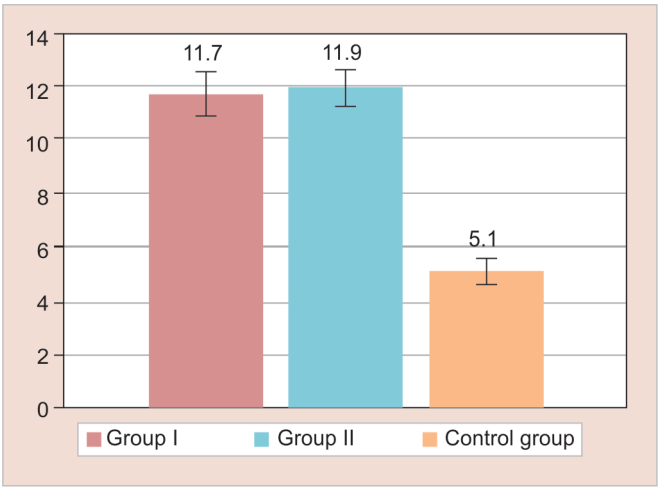

Fig. 6: Antimicrobial efficacy on *Lactobacillus* between the three groups—group I (GIC with aqueous extract of *triphala*), group II (GIC with ethanolic extract of propolis) and control group (Plain GIC). *Note*: The error bar represents the standard deviation of the mean

Table 2: Pairwise comparison of antimicrobial efficacy on *Streptococcus mutants* between the three groups—group I (GIC with aqueous extract of *triphala*), group II (GIC with ethanolic extract of propolis) and control group (plain GIC)

Variables	*N*	*Test statistics*	*p value*
Group I v/s group II	5	−1.400	1.000
Group I v/s control group	5	2.426	0.046*
Group II v/s control group	5	2.925	0.010*

Pairwise comparison done by Dunn–Bonferroni test; *Significant at $p < 0.05$

was selected for the study. *Lactobacillus* are highly acidogenic from carbohydrates and are acid tolerant, and they are often cultured from established carious lesions.[17]

Conventional GIC

Among the widespread restorative materials used in dentistry, the conventional GICs were selected in this study due to their major advantages of adhesion to tooth structure, fluoride uptakes, and release which can inhibit caries, furthermore the variety of the clinical application of GICs.[16]

Nakajo et al. concluded *S. mutans* on the surface of GIC fillings was less than on composite resin fillings.[18] Hoszek et al. said that conventional GICs have low bactericide potential which can act against microorganisms to the certain extent. Therefore, the ability of GICs in complete elimination of the plaque proliferation, caries development, and periodontal disease in few patients is still questionable.[19] Also, following insertion of GICs in to the cavity, it has been proved that approximate release of fluoride is around 10 ppm which is still considered low for attaining the desired antibacterial effects.[16,20] Hence, GIC was not able to hold its acidity and fluoride ion after a particular point of time. Yap et al.[21] stated that even with the presence of fluoride content in GIC, the expected efficient antimicrobial property is yet difficult to achieve; hence, the motto of this present study was to improve the antimicrobial characteristics of GIC; therefore, the modification using *triphala* and propolis was done.

Antibacterial Efficacy of GIC Containing Aqueous Extract of *Triphala*

In the present study, group I had showed the more inhibition of 12.5 mm, whereas conventional GIC shows inhibition of 5 mm. This confirms the earlier studies that the inhibition of GIC against caries formation is solely due to fluoride release and/or acidity,[22] and the inhibition of microorganisms by conventional GICs in cavities is not reliable.[23] The antimicrobial efficacy of GIC with *triphala* may be due to the following reasons, which is proven by numerous literatures:

In the main ingredient of *Triphala*, *Terminalia chebula* acts as a anticaries agent,[24,25] as it prevents sucrose-induced adherence, thereby eliminating the virulence of cavity-inducing organisms. According to Biradar et al., *triphala* can retard the growth of bacteria[26] and also Jagtap and Karkera reported that extracts of main ingredient in *triphala*, i.e., *Terminalia chebula*, prevents the growth and adherence of *S. mutans*.[27]

Also, another ingredient *Terminalia bellerica* (in *Triphala*), which contains tannic acid, can be adsorbed to the surface of the bacterial cells, resulting in protein denaturation and bacterial cell death.[28] Kau et al. reported that tannic acid is bacteriostatic or bactericidal to few pathogens.[29] It may be a reason for the present study in the enhancement of antimicrobial activity. The

Table 3: Comparison of antimicrobial efficacy on *Lactobacillus* between the three groups—group I (GIC with aqueous extract of *triphala*), group II (GIC with ethanolic extract of propolis) and control group (plain GIC)

Variables	*n*	*Mean*	*S.D.*	*Median*	*Min–Max*	*Test statistics*	*p value*
Group I	5	11.70	0.83	11.50	11.00–13.00	9.639	0.008*
Group II	5	11.90	0.65	12.00	11.00–12.50		
Control group	5	05.10	0.74	05.00	04.00–06.00		

p value derived from Kruskal–Wallis test; *Significant at $p < 0.05$

Table 4: Pairwise comparison of antimicrobial efficacy on *Lactobacillus* between the three groups—group I (GIC with aqueous extract of *triphala*), group II (GIC with ethanolic extract of propolis) and control group (plain GIC)

Variables	*n*	*Test statistics*	*p value*
Group I v/s group II	5	−0.356	1.000
Group I v/s control group	5	2.493	0.038*
Group II v/s control group	5	2.849	0.013*

Pairwise comparison done by Dunn-Bonferroni test; *Significant at $p < 0.05$

study conducted by Srinagesh et al. suggested that the anti-oral streptococci efficacy of *triphala* and chlorhexidine are quite similar and comparable.[7] Also, similar results were obtained by Jagadish et al.[30] and Prajapathi and Raol.[31] Another study done by Prabhakar et al. proved the statistical significance, with *triphala* being highly antibacterial when compared with chlorhexidine.[32] The antibacterial efficacy of type IX GIC had the least antimicrobial efficacy which was in accordance with our results.[33] This can be reason for the *triphala*-modified GIC to show superior antimicrobial activity.

Antibacterial Efficacy of GIC Containing Ethanolic Extract of Propolis

In the present study, group II had showed the superior inhibition of 13.0 mm when compared to the other two groups. The antimicrobial activity of GIC added propolis is mainly due to two mechanisms of action associated with propolis, (1) anti-microbial activity and (2) inhibits glucosyl transferase activity.[34]

The superior microbicidal compounds present in EEP is galangin and caffeic acids, which play a role in inhibition of bacterial growth and proliferation.[35,36] Next is the flavonoids that causes alteration in permeability of microorganisms. A previous literature has revealed that the components present in the propolis have an inability to bind to DNA, resulting in the inhibition of bacterial RNA-polymerase. Hence, these components, galangin, caffeic acids, and flavonoid, are considered to be bacterial enzymes inhibitors[35,37–40] which was a major reason in causing antimicrobial efficacy when mixed with GIC.

Viable bacteria were found to be less in the GIC containing 50% EEP when compared to 25% EEP. Hence, 50% EEP was used in the present study.[41] Also his study proved that distinct antibacterial activity of propolis containing GIC against *S. mutans* which is in accordance with our results. Also, another study done by Ophori et al. concluded that EEP is highly antibacterial; hence, it is suggested to treat dental caries.[42] A study done by Erdem et al. proved that addition of Ethanolic extract of propolis to GIC will increase the antimicrobial efficacy without altering its properties.[42] Hence, the promising results were obtained when ethanolic extract of propolis were added with GIC.[43]

Clinical Significance

Atraumatic restorative treatment (ART) has been developed for treatment of caries in parts of world with limited access to dental treatment facilities, where demineralized soft carious lesion are excavated and it is restored with suitable adhesive restorative materials. As dental hand instruments solely cannot help in removal of entire carious lesion, there may be chances of remnant cariogenic bacteria which can survive in depth underneath the restorations. Consequently, when the restoration (GIC) is not capable to arrest the carious progression, the restoration will result in failure. As researchers proved that few ART restorations fail because of secondary caries development over a period of 6 years, there lies a need for improvement in filling materials which can overcome the problem resulting in success rate of ART. Hence, the GIC containing propolis and *triphala* will be beneficial to prevent secondary caries formation and would be used as a promising material for restoration. Further extensive research is required in regard to physical, mechanical properties and bonding effects of GIC which can promote a novel natural bioactive restorative material.

Conclusion

The results of this present study proved that modified GIC with *triphala* and propolis had a maximum zone of inhibition proving its higher antimicrobial efficacy against *S. mutans* and *Lactobacillus* when compared to conventional GIC. Therefore, propolis and *triphala* added GIC can be a better replacement for restoring of the dental cavities. Future investigations are required to know about its physical and mechanical properties.

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