Evaluation of Antimicrobial activity and physical properties of Cinnamon modified Glass ionomer cement.

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ABSTRACT:

Aim: The aim of the study is to evaluate antimicrobial activity and physical properties of cinnamon modified GIC.

Background: Dental caries can be defined as a biofilm-mediated, sugar-driven, multifactorial, dynamic disease that results in phasic demineralization and remineralization of dental hard tissues. GIC is the most used type of restoration in the primary dentition. Cinnamon extract has potent antimicrobial activity against numerous pathogenic microbes like E. coli, C. jejuni, S. enteritidis, and S. aureus that can be effectively capable of suppressing the growth of bacterial development. Hence the research on cinnamon modified GIC was done in this present study.

Materials and methods: The cinnamon extract was prepared. The powder and liquid of conventional GIC was mixed followed by the addition of the prepared extract with three different concentrations. Standard strains S.mutans and Lactobacillus were used to test the antimicrobial efficacy of modified and unmodified (control)GIC. MIC assay was done where the first three wells were used with three different concentrations of modified GIC (1:1),(1:2),(2:1) respectively and the fourth well was kept as control (Conventional unmodified GIC).The incubation is done under suitable conditions for varied time intervals (1h, 2h, 3h, 4h). Compressive strength was evaluated according to ISO 9917- 1:2007 using cylindrical molds. The maximum force applied when the specimen fractures was recorded to calculate the compressive strength values in MPa.

Results: The antimicrobial activity of Cinnamon modified GIC was carried out by MIC assay where the better antimicrobial efficacy against s.mutans showed at 1:2 concentration second hourly when compared to other concentration, where in case of Lactobacillus,conventional (control) group performed better when compared with other groups and there was no significant changes in terms of compressive strength.

Conclusion: The antimicrobial activity against S.mutans was found to be better at 1:2 concentration in second hour intervals, and against Lactobacillus conventional GIC was found better when compared to other groups. There was no change in compressive strength of cinnamon modified GIC.

Key words: Cinnamon, modified GIC,Caries,Dental material,antimicrobial,strength

INTRODUCTION:

Glass-ionomer cement (GIC) materials have been in clinical use for the past 50 years(1). They have undergone several modifications to yield different categories of these materials. Although all GICs share the same generic properties, some small changes between commercial products may occur. They are used in lining, bonding, sealing, luting or restoring a tooth(2). In general, GICs are best useful for reasons of adhesion to tooth structure, fluoride release and being tooth-coloured although their sensitivity to moisture, inherent opacity, long-term wear and strength are not as adequate as desired(3). They are used in situations where their lower physical properties like adequate remaining tooth structure to support the material and inability to bear heavy occlusal loading has no play in prognosis(3). The last decades have shown that the usage of these materials is being extended. However, they are likely to retain their specific niches of clinical application. Dental caries can be defined as a biofilm-mediated, sugar-driven, multifactorial, dynamic disease that results in phasic demineralization and remineralization of dental hard tissues(4). Dental caries is unlinked to age and affects both in primary and permanent dentitions. The initial demineralization enamel decay followed by pulp and weakening of crown structure, leads to loss of crown structure(5). In case of periodontitis, the exposed root surfaces are more prone for damage because of less enamel. The balance between protective and pathological factors influences the initiation and progression of caries. The recurrent caries is the most common reason for replacement of all types of restorations in general dental practice(6). Marked variations in the clinical diagnosis of the lesions have been reported. The prevention of recurrent lesions by the use of fluoride-releasing restorative materials has been successful(7). As recurrent carious lesions are localized and limited, alternative treatments to restoration replacement like crowns are suggested, which is not practical in all cases. The unwanted loss of tooth structure is not advisable(8). The lack of the hermetic seal in the restored tooth causes persistence of cariogenic bacteria that leads to recurrent caries, leading to failure of restoration(9). One possible solution to overcome this is to use dental materials with a good hermetic seal and with antibacterial properties(10). Glass ionomer cement (GIC) is the most used type of restoration in the primary dentition. Therapeutic benefit for this may therefore be achieved by combining antibacterial agents with GIC. However, the incorporation of antibacterial agents in restorative materials frequently interferes with its physical and chemical properties(11). Studying the physical properties combined with the antimicrobial effect after adding those agents is a valuable approach.

The therapeutic procedures used in the treatment of caries cannot always eliminate all the microorganisms in total but leaves some in the residual tissue(12)s. Restoration with low hermetic seal, the persisting cariogenic bacteria, can cause recurrent caries, leading to failure of restoration. One possible solution to overcome this problem is to use dental materials with both bacteriostatic and bactericidal properties.

Conventional glass ionomer cements, introduced in 1972, by Wilson and Kent, is a tooth colored and chemically adhesive material, with a therapeutic action of fluoride releasing property which is anti cariogenicity in nature, and is being widely used in dentistry(13). The ability of glass ionomer cement to release fluoride continuously over an extended period of time, results in an anticariogenic potential showing a reduction in caries adjacent to the restoration(14).

The minimal intervention approach for managing dental caries, which gained importance in the last decade is Atraumatic Restorative Treatment (ART)(15). ART has been developed for treatment of caries in places with limited access to dental treatment facilities, where demineralized tooth tissues are removed using hand instruments and the cavity is restored with adhesive restorative materials(16). However, ART dental hand instruments cannot remove carious dentin as effectively as rotary burs, and cariogenic bacteria can survive incarceration under GIC restorations for up to two years(17). Consequently, cavities treated by ART may have residual infected dentin, and if GIC is unable to arrest the carious process, the restoration will fail. Research has shown that few ART restorations fail because of secondary caries development over a period of six years(18). Since its introduction, major improvements have been made in the first ASPA Glass Ionomer Cement, but certain limitations still remain(19). The improvement of filling materials, to overcome the problems caused by incomplete removal of infected dentin, will be beneficial for further increasing the success rate of ART.

Several attempts in developing GIC with enhanced antibacterial effects by addition of bactericides, such as, chlorhexidine hydrochloride, cetyl pyridinium chloride, cetrimide, and benzalkonium chloride have been reported in the literature(20). The most appropriate choice of antibacterial agents to combine with GIC would be antiseptic agents that have proven to be useful in clinical dentistry, and are the ones that do not disturb the physical properties(21). Cationic disinfectants have been investigated both in-vitro and in-vivo for their antibacterial effects against various microorganisms(22). Literature reveals that only chlorhexidine has been widely incorporated in GIC and all the studies have shown an increase in the antibacterial effects in vitro but the physical properties still remain questionable.

Cinnamon has also been used as a neuroprotective agent and health-promoting agent for the treatment of diseases such as inflammation, gastrointestinal disorders and urinary infections(23). Another potential medical use of cinnamon would be with regards to its antimicrobial properties, especially antibacterial activity(24). Cinnamon is one of the plant products that is used daily by many people worldwide. It primarily contains vital oils and derivatives, such as cinnamaldehyde, cinnamic acid, and cinnamate. It has been utilized as traditional medicine and its effect as an antibacterial was documented. This activity is due to the presence of products such as phytochemicals and cinnamaldehyde that represent its main component. Cinnamon extract has been utilized for numerous years due to its huge restorative significance(25). This extract has potent antimicrobial activity against numerous pathogenic

microbes like Escherichia coli, Campylobacter Jejuni, Salmonella enteritidis, and Staphylococcus aureus that can be effectively capable to suppress the growth of bacterial development(26).Plant-derived products are among such medicinal agents, and because of their varied components, they are regarded as excellent sources of bioactive compounds. Essential oils extracted from diverse components of medicinal plants, such as cinnamon, clove, thyme, tea tree, and eugenol oils, can thus provide a variety of therapeutic benefits, including antibacterial properties. According to prior research, Thymus vulgaris has powerful antibacterial properties against a variety of oral infections, including Streptococcus pyogenes, S. mutans, Candida albicans, Aggregatibacter actinomycetemcomitans, and Porphyromonas gingivalis. This action is mostly due to the presence of the hydroxyl group, which interacts with the bacterial cytoplasmic membrane, causing it to tear and allow cellular contents to leak.Cinnamon bark and cinnamon leaf essential oils have also been shown to have potent antimicrobial effects against a variety of fungal and bacterial strains, as well as oral germs This is due to the well-known characteristics of cinnamaldehyde, which enhances cell surface hydrophobicity, lowers S. mutans aggregation, and inhibits glycolytic enzymes required for acid generation and caries development(27). As a result, the purpose of this study was to investigate and compare the effects of adding cinnamon and thyme oils to the liquid phase of high-viscous GIC on its antimicrobial properties, compressive strength, and fluoride-releasing capacity, as well as to determine the optimal concentrations of each of the two antimicrobials to incorporate into this novel form of the material(28). The addition of cinnamon oil and thyme oil into traditional GICs confers an antibacterial action against S. mutans and C. albicans while having no effect on the compressive strength and fluoride release capacities of them. Our team has extensive knowledge and research experience that has translated into high quality publications (29–45) . The aim of the study is to evaluate the antimicrobial activity and compressive strength of cinnamon modified GIC.

MATERIALS AND METHODS:

Preparation of cinnamon extract:

The cinnamon leaves were dried for 5 days. The glasswares were properly washed, rinsed with distilled water and dried in a hot air oven at 70 degree celsius before use. In the beaker, 1g of leaves were measured and added to 100mL of distilled water. The mixture was boiled using a heating mantle at 60-70 degree celsius for 15 minutes . The solution is filtered using Whatman No: 1 filter paper and the obtained 80 mL filtrate is collected in a separate conical flask. This filtered extract was further condensed to 5 mL at 60-70 degree celsius.

Groups	Description
I (1:1)	P^{GIC}: E: L^{GIC} = 2:1:1
II (1:2)	P^{GIC}: E: L^{GIC} = 3:1:2
III (2:1)	P^{GIC}: E: L^{GIC} = 3:2:1
IV	Control group –conventional GIC

Bacterial Strain and Inoculum Preparation:

Streptococcus mutans and Lactobacillus acidophilus bacterial strains were obtained from the Department Of microbiology, Saveetha Dental college and Hospitals. A sterile complete loop of each pure culture

was taken, and the facultative strains of *S.mutans* and Lactobacillus acidophilus were fully grown on Mueller Hinton Agar. The microorganisms were subcultured in appropriate culture media and it was inoculated individually in tubes containing 5 mL of sterile Mueller Hinton broth and incubated at 37 degree celsius for 24 h. The suspension was then adjusted to 0.5 Mcfarland scale = 1.5 × 108 colony-forming unit (CFU).

Specimen preparation for antimicrobial testing :

After mixing the powder and liquid of conventional GIC, the plant extract was incorporated. The final obtained cement was placed into cylindrical molds measuring diameter of 6 mm and 2 mm in thickness 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 off the cement, the disk-shaped specimens were removed from the mold. The precise specimen was measured using calipers and recorded. Six specimens were prepared in each group, three for s.mutans and other three specimens for lactobacillus. For monitoring the antibacterial effects of the tested groups, standard strains *S.mutans and Lactobacillus* were used.

MIC Assay:

Standard strains *S. mutans and Lactobacillus* were used to test the antimicrobial efficacy of modified and unmodified GIC. MHA broth was prepared, sterilized and 200 µL was added to all four wells. Bacterial suspensions about 50 µL (*S. mutans* and Lactobacillus acidophilus) were added to all 4 wells in the range of 5×10^5 CFU/ml. The first three wells contain three different concentrations of GIC (1:1),(1:2),(2:1) and the fourth well is considered as the control (Conventional GIC). The incubation is done under suitable conditions for varied time intervals (1h, 2h, 3h, 4h). Using an ELISA reader, the percentage of dead cells is calculated at a wavelength of 540 nm at regular time intervals.

Specimen preparation for compressive strength evaluation:

Compressive strength was evaluated according to ISO 9917- 1:2007 using cylindrical molds (4.0 mm diameter × 6.0 mm height). Six specimens were prepared for each group. Then materials were placed into the mold and leveled to obtain a smooth surface. One hour later specimens were removed from the mold, and stored in deionised water for 24 h. Malformed specimens or those with voids were discarded. The diameter of each specimen was checked using a digital micrometer gauge. The specimens were then placed in vertical position in a Zwick universal testing machine (Zwick Zmart Pro, ZwickRoell GmbH & Co. KG, Ulm, Germany). Compressive load was applied on the long axis of the specimens at a crosshead speed of 0.5 mm/min until fracture. The maximum force applied when the specimen fractures was recorded to calculate the compressive strength values in MPa.

Statistical analysis:

The data collected were entered in the microsoft excel sheet following which statistical analyses were carried out using SPSS version 24.0 (IBM corporation). The mean MIC values were calculated using descriptive analysis.Normality test Shapiro–Wilk was used to examine whether or not the variables follow a normal distribution. All quantitative variables showed parametric distribution;

therefore, for both antimicrobial and compressive strength testing One-way analysis of variance (ANOVA) was used for comparison between the groups and Tukey's post hoc test was used for pairwise comparison between the groups. The significance level was set at $P \leq 0.05$.

RESULTS:

Table 1: Mean MIC values of S.mutans

S.mutans				
Conc	**1h**	**2h**	**3h**	**4h**
01:01	0.407	0.368	0.407	0.517
01:02	0.342	0.263	0.342	0.459
02:01	0.315	0.372	0.315	0.314
control	0.332	0.418	0.332	0.381

Antimicrobial activity against S.Mutans:

The antimicrobial activity against s.mutans,mean values of cinnamon modified GIC are tabulated in table 1, at 1:2 concentration in the second hour interval proved to have better results when compared to other concentrations with the mean value 0.263.

Table 2: Mean MIC values of Lactobacillus

Lactobacillus				
Conc	**1h**	**2h**	**3h**	**4h**
01:01	0.569	0.602	0.569	0.627
01:02	0.422	0.464	0.422	0.464
02:01	0.398	0.409	0.398	0.449
control	0.315	0.412	0.315	0.24

Antimicrobial activity against Lactobacillus:

The antimicrobial activity against lactobacillus, mean values of cinnamon modified GIC are tabulated in table 2, control group (conventional GIC) with the least mean value of 0.24 four hourly had a better antimicrobial efficacy when compared to other other groups.

The maximum force applied when the specimen fractures was recorded to calculate the compressive strength values in MPa.There was no change in compressive strength of cinnamon modified GIC in all proportions.

DISCUSSION:

Glass ionomer cement has gained wide acceptance and popularity, mainly due to its ease of handling characteristics, chemical bonding to tooth structure, and fluoride release.(46) However, according to Mazzaoui et al(47), the amount of fluoride released from GIC is not sufficient to show an antibacterial effect against caries-causing pathogens which can give rise to secondary caries. In addition, Yap et (48)al showed that GIC does not promote an efficient antibacterial effect despite the presence of fluoride. The cariostatic effect of GIC is still doubtful(49). Clinical studies have shown that residual bacteria located under a GIC restoration are viable for up to 2years.Minimum Inhibitory Concentration (MIC) assays determine the lowest concentration of an antimicrobial agent that prevents visible growth of a microorganism(50,51). A lower optical density value indicates that less drug is required for inhibiting growth of the organism.(52)(53)The antimicrobial activity of Cinnamon modified GIC has been found out by MIC assay. The activity of the S.mutans and Lactobacillus with a periodicity of 4 hour intervals were recorded and displayed in figures (Figure 1 and 2).The results proved that the at 1:2 concentration in the second hour interval

proved to have better results when compared to other concentrations with the mean value 0.263. The incorporation of 3% Nano particles Cinnamon powder in the orthodontic composite resin enhanced its antimicrobial properties without compromising the shear bond strength of orthodontic brackets.The FTIR measurements indicated no any chemical reaction between cinnamon powder and the composite, before and after curing.(54). According to Yaseen et al, the antimicrobial activity of Cinnamon is due to the presence of several compounds such as cinnamaldehyde(50.5% of Cinnamon bark), eugenol (4.7%), benzoic acid, benzaldehyde and cinnamic acid as the effect of these compounds is responsible for its antimicrobial property.(45–48). These compounds are known to be either bactericidal or bacteriostatic agents, depending upon concentration used.

According to Bhanushali et al, Cinnamon bark oil has antibacterial properties against L. acidophilus. Incorporation of 2% v/v cinnamon bark oil did not adversely affect the compressive strength of GIC.(55).The antimicrobial activity of Cinnamon is due to the presence of several compounds such as cinnamaldehyde(50.5% of Cinnamon bark), eugenol (4.7%), benzoic acid, benzaldehyde and cinnamic acid as the effect of these compounds is responsible for its antimicrobial property. Matan et al concluded that cinnamon bark oil is not harmful when consumed with food products and it inhibits the growth of yeast, molds, and bacteria(56), Jatan et al Cinnamaldehyde was the major component of cinnamon bark oil which is responsible for its antibacterial effect. It renders antibacterial effects by damaging the cell wall of the bacteria. Varalakshmi et al found cinnamon bark extract had inhibited both Gram-positive and Gram-negative bacteria indicating broad spectrum inhibitory effect. MIC assay of cinnamon modified GIC on Lactobacillus are shown in figure 2, where the control group (conventional GIC) with the least mean value of 0.24 four hourly had a better antimicrobial efficacy when compared to other other groups.

According to Sherief et al, it could be extrapolated that the addition of cinnamon oil at 5% v/v into conventional GIC might confer strong inhibitory effects against both S. mutans and C. albicans and enhance its fluoride releasing ability without jeopardizing its compressive strength(26).According to Gupta et al, cinnamon oil was found to be a much better antagonistic agent, exhibiting broad range of antimicrobial activity against the microbes causing dental caries than clove oil and chlorhexidine.(57) Hence, it represents an alternative source of natural antimicrobial substances for use in chemotherapeutic agents.

The compressive strength of the GIC is analyzed by using the Zwick universal testing machine. The GIC molds were held in the Zwick universal testing machine and the results proved that the cinnamon modified GIC did not significantly affect the compressive strength of GIC.Hence, cinnamon containing GIC can be beneficial clinically as S.mutans is the organism responsible for the carious lesion and it is the main organism causing secondary caries. By incorporating this bacteriostatic agent to GIC, the progress of caries and failure of restorations can be prevented by inhibiting the growth of S.mutans. Clinically, it can be used in the cases of deep dentinal caries, early childhood caries, rampant caries, and patients with high caries index. However, intraoral variables,

such as normal masticatory stress, moisture, and operator inconsistencies, are not taken into consideration in the present study. Therefore, further studies are necessary to test the long-term stability of this material.

CONCLUSION:

The antimicrobial activity of the cinnamon modified GIC is better than that of the conventional GIC in case of S.mutans organism, whereas the conventional group had a better antimicrobial activity against Lactobacillus. The cinnamon modified GIC did not significantly affect the compressive strength of GIC.Hence Cinnamon containing GIC can be considered as alternative restorative material,further studies are necessary to test the long-term stability of this material.

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CONFLICT OF INTEREST:

The authors declare no potential conflict of interest.

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Figure 1:

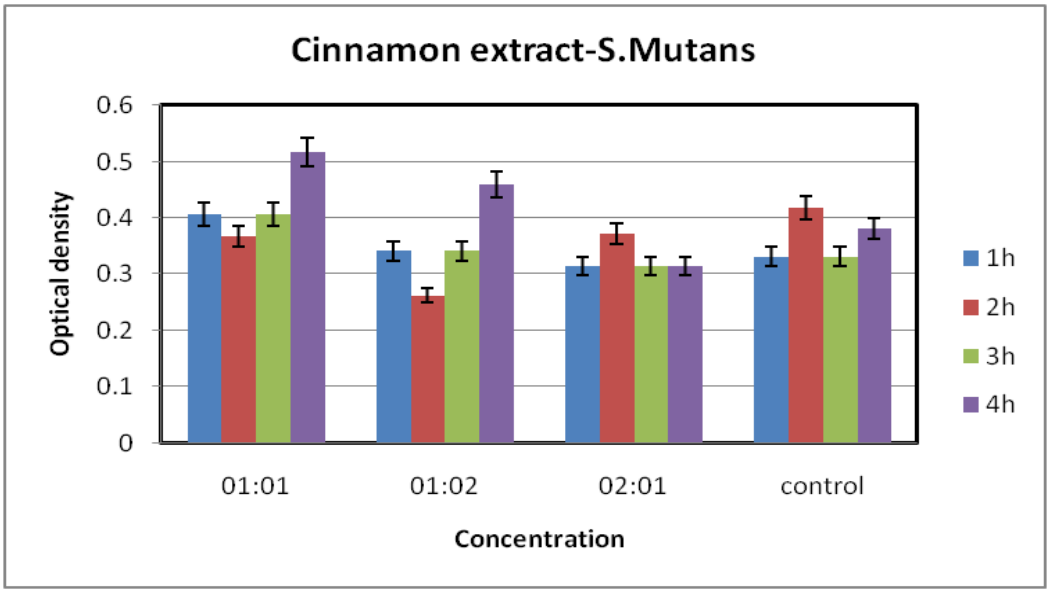
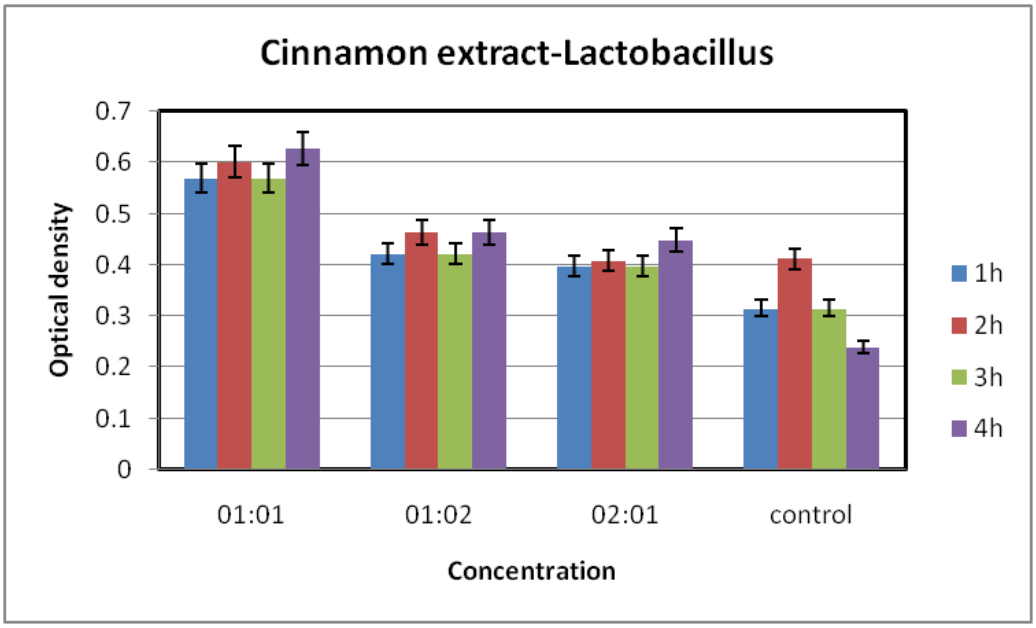


Figure 2:


Cinnamon extract-Lactobacillus
Optical density
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
01:01
01:02
02:01
control
Concentration
1h
2h
3h
4h