Original Article

COMPARATIVE EVALUATION OF ANTIMICROBIAL ACTIVITY AND COMPRESSIVE STRENGTH OF CONVENTIONAL AND THYME-MODIFIED GLASS IONOMER CEMENT

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https://doi.org/10.51847/FrmCSw6TqP

ABSTRACT

Recurrent caries can be prevented in large part by the antibacterial activity of restorative materials. This study aims at evaluating and comparing the antimicrobial activity and compressive strength of thyme-modified Glass ionomer cement with conventional Glass ionomer cement. The thyme extract was prepared from the dried thyme leaves. By combining an extract with the powder and liquid of traditional GIC, modified GIC was created in Powder^{GIC}, Extract, Liquid^{GIC} ratio of 2:1:1, 3:1:2 and 3:2:1 and labeled as Group I, Group II, Group III respectively, and Group IV as control (unmodified GIC). The antibacterial effectiveness of modified and unmodified (control)GIC was examined using standard strains of S. mutans and Lactobacillus.MIC assay was done for all the groups; the incubation was done under suitable conditions for varied time intervals (1h, 2h, 3h, 4h). Using cylindrical molds, compressive strength was assessed in accordance with ISO 9917-1:2007. To get the compressive strength values in MPa, the greatest force that the specimen could withstand before it fractured was noted. The results proved that, against S.mutans, all modified groups showed the highest antimicrobial activity without compromising strength when compared with the control group ($p>0.05$)and against Lactobacillus and no statistically significant difference between modified and control groups ($p>0.05$) was seen. Thus, thyme-modified glass ionomer cement has enhanced antimicrobial activity when compared to conventional glass ionomer cement.

Key words: Thyme extract, Antimicrobial, GIC, Strength, Restoration, Secondary caries.

Introduction

Cavities are related to the demineralization of the hard structure of the teeth by acids of microbial origin as well as several other factors that affect the tooth structure. The oral cavity contains many types of bacteria, of which Streptococcus mutans and Lactobacilli are believed to cause tooth decay. Restorative cement which includes glass ionomer cement (GICs) is a class of substances [1]. The International Organization for Standardization (ISO) refers to them as "glass polyalkenoate cement," although the phrase "glass ionomer" is acknowledged as an inconsequential term that is frequently employed in dentistry [2]. In recent years, GICs are considered the most commonly employed water-based cement for the concluding cementation of dental crowns, bridges, Braces, and non-traumatic restorations. Glass ionomer is a popular material due to its biocompatibility, fluoride ion release over an extended period, and ability to bond to enamel and dentin [3]. But due to minimal antibacterial effect and lack of physical and mechanical characteristics, recurrence of caries has been reported after restoration, on the other hand, decreased strength of the restorative material was a major drawback. Hence there is a need for use of direct filling materials, thereby modification of GIC came into existence. The addition of different antimicrobial agents to GICs may have therapeutic advantages [4]. Research has focused on the rapid or decreased release of antimicrobial agents such as antibiotics, zinc ions, silver ions, iodine, and most commonly chlorhexidine, the gold standard antibacterial agent [5]. Numerous in vitro investigations supported the idea that when GIC is combined with CHX, its biological qualities are improved. Unfortunately, adding antibacterial agents to restorative materials usually affects their physical and mechanical characteristics over time. If the dose or release is not well managed, they may also have short-term efficacy and be hazardous to nearby tissues [6]. This is likely the cause of the lack of use of GICs in manufacturing when combined with chlorhexidine and other antimicrobials.

Plants were utilized for prevention and therapy for ailments as years pass by, until the invention of chemistry in the sixteen century. A herbal medication known as phytomedicine uses various plant components like extracts as a medicinal and health-improving agent that are considered the least toxic [7]. Based on the World Health Organization, up to eighty percent of people worldwide have a dependency on traditional medicine (herbal) as a form of

fundamental medical needs, including flavonoids, phenols, and saponins [8]. Thyme is a little perennial shrub that tends to grow both horizontally and vertically. Rarely does it grow taller than 40 cm. When they get older, the stems turn woody [9]. Thyme leaves are fairly small, usually 2.5 to 5 mm long, and vary widely by variety in terms of form and hair coverage. Thymus vulgaris (T. vulgaris), a large fragrant plant with over 100 varieties worldwide, is extensively utilized in both culinary and medicinal recipes. Due to the numerous therapeutic benefits of its essential oils, commonly referred to as thyme oil, the genus Thymus contains significant medicinal herbs that come highly recommended. Because of their pharmacological and biological characteristics, thymus species are regarded as therapeutic plants. Its primary constituent, thymol, is what gives it its properties [10]. A high concentration of physiologically active substances, including thymol, carvacrol, p-cymene, and -terpinene, is a distinguishing feature of thyme essential oil. It has been demonstrated that carvacrol and thymol possess strong bacteriostatic and bactericidal effects in addition to having a high antioxidant capacity [11]. There is insufficient evidence in the literature for the addition of thyme extract to GIC for restorative purposes, even though thyme extract is effective against salivary environment-induced caries in human mouthwash or toothpaste. Because of the increased likelihood of recurrent caries following restorative therapy, the use of direct filling materials must be done with extreme caution [12], we need a restorative material that can inhibit a broad spectrum of bacteria. Our team has a wealth of knowledge and research expertise, which has resulted in publications of the highest caliber [13-30]. Therefore, the study's objective was to improve the antibacterial capabilities of GICs by modifying them with Thyme leaf extracts. Hence, keeping this in mind the present study was planned to compare and evaluate the antimicrobial activity and compressive strength of thyme-modified GIC with that of conventional Glass ionomer cement where the null hypothesis stated that there is no difference between conventional GIC and thyme-modified GIC.

Materials and Methods

Preparation of thyme leaves extract

Thyme leaves were dried for 5 days. The glassware was adequately washed using distilled water and dried in a hot air oven prior. In the beaker, 0.5 g of Thyme leaves are added to 100 ml of distilled water. The mixture is mixed and boiled in a water bath by covering the beaker for 10 minutes up to the level of 5ml, hence the concentrated thyme leaves extract was prepared. The solution is filtered using filter paper and the filtrate is collected in a separate conical flask. This extract is preserved for further procedure.

Test pathogens and inoculum preparation

The antimicrobial activity of the synthesized modified Glass ionomer cement with thyme leaves was tested against pathogenic microorganisms *Streptococcus mutans* and *Lactobacillus acidophilus*. The bacterial strains were obtained from the Department Of microbiology. Using a sterile complete loop of each pure culture, the facultative strains of S. mutans and Lactobacillus acidophilus were fully grown on Mueller Hinton Agar. Individually injected in tubes holding 5 mL of sterile Mueller Hinton broth, the bacteria were subcultured in the proper culture conditions and incubated at 37 degrees Celsius for 24 hours. Once that was done, the suspension was changed to 0.5 Mcfarland scale = 1.5 × 108 colony-forming unit (CFU).

Specimen preparation

The type II GIC (GC Corporation) was used in the present study. Thyme extract was added after combining the liquid and powder components of conventional GIC in different concentrations and grouped **(Table 1)**. In less than a minute, the prepared specimens were placed in the cylindrical wells using the sterile cement carrier, and the cement layer's upper surface was leveled using a sterile glass slide. The completed cement was then poured into cylindrical molds with a thickness of 2 mm and a diameter of 6 mm. After the cement ignited, the specimens in disc shape were taken out of the mold. We measured and noted the precise specimen dimensions with calipers. Twelve samples were prepared for each group, six for S.mutans and the other six for Lactobacillus. Strains of S.mutans and Lactobacillus were used, and how well the tested groups counteracted bacteria. For the measurement of compressive strength, the cylindrical molds with dimensions of 4.0 mm in diameter and 6.0 mm in height, and compressive strength were assessed in accordance with ISO 9917-1:2007. For each group, twelve specimens were prepared. To create a smooth surface, materials were then added to the mold and leveled. After being removed from the mold an hour later, the samples were kept in deionized water for 24 hours for the evaluation of compressive strength.

Table 1. Grouping

Groups	Description(P-Powder,E-Extract tof thyme, L-Liquid,GIC-Glass ionomer cement)
I	P^{GIC}: E: L^{GIC} = 2:1:1
II	P^{GIC}: E: L^{GIC} = 3:1:2
III	P^{GIC}: E: L^{GIC} = 3:2:1
IV	Control group-conventional unmodified GIC

Minimal inhibitory concentration (MIC) assay

The antibacterial effectiveness of modified and unmodified GIC was evaluated using standard strains of S. mutans and Lactobacillus.MHA broth was prepared, and sterilized and 200 µL was added to all four wells. Bacterial suspensions of 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 (2:1:1),(3:1:2), and (3:2:1) and the fourth well is considered 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.

Compressive strength evaluation

Specimens that were deformed or had voids were discarded. Each specimen's diameter was measured using a digital micrometer gauge. The samples were then positioned vertically using the Universal Testing Machine (Instron, ElectroPuls®, E3000). At a crosshead speed of 0.5 mm/min, compression load was applied to the specimen's long axes until fracture, and readings were recorded as per the graph.

Statistical analysis

Data entry into an Excel spreadsheet, followed by statistical analysis using SPSS version 24.0, was done with the collected data (IBM corporation). Descriptive analysis and repeated measure ANOVA were used to calculate the mean MIC values. For compressive strength, the groups were compared by the use of one-way analysis of variance (ANOVA), and the groups were compared pairwise using Tukey's post hoc test at the significance level $P \leq 0.05$ with 95% confidence intervals.

Results and Discussion

Antimicrobial efficacy against S.mutans

Repeated measure ANOVA is used in this study to test the antibacterial effect of modified and unmodified GIC, against *S.mutans*, first, three thyme-modified groups performed better and also showed statistically significant results when compared with Group IV (control) **(Figure 1)**. Multiple comparison Tukey HSD It was determined by a pairwise comparison test that there was a significant difference between Group IV and any of the other three groups($p<0.05$) **(Table 2)**.

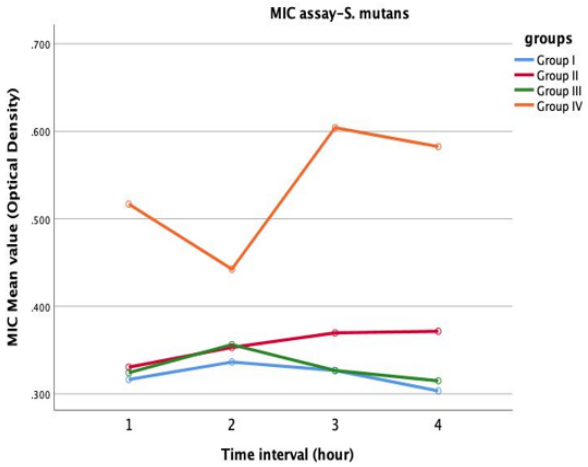

Figure 1. Antimicrobial efficacy on *S.mutans* between four groups.

Table 2. Pairwise comparison of antimicrobial efficacy on *Streptococcus mutans* between four groups

Pairwise comparison	Mean difference	SE	95% CI		P-value
			Lower	Upper	
Group I vs Group II	0.035+	0.006	0.018	0.052	0.00*
Group I vs Group III	0.009	0.006	0.007	0.027	0.417
Group I vs Group IV	0.215+	0.006	0.198	0.233	0.00*
Group II vs Group III	0.025+	0.006	0.008	0.043	0.003*
Group II vs Group IV	0.180+	0.006	0.162	0.197	0.00*
Group III vs Group IV	0.206+	0.006	0.188	0.223	0.00*

+ Mean difference is significant, P value was significant at 0.05,P value was derived from Multiple comparison Tukey HSD Test.

Antimicrobial efficacy against lactobacillus

Antimicrobial activity against *lactobacillus*, both modified and control groups, showed similar activity proving there were no statistically significant results between conventional GIC and modified GIC. The repeated measure ANOVA linear chart was shown in **Figure 2**. The pairwise comparison shows there was no statistically significant difference when comparing Group IV with other groups ($p>0.05$) **(Table 3)**. This proves there was an almost equal antibacterial activity for thyme-modified and conventional groups against *Lactobacillus*.

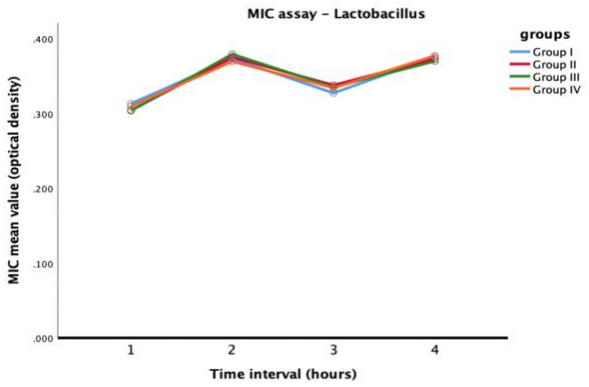

Figure 2. Antimicrobial efficacy on *Lactobacillus* between four groups.

Table 3. Pairwise comparison of antimicrobial efficacy on *Lactobacillus* between four groups

Pairwise comparison	Mean difference	SE	95% CI		P-value
			Lower	Upper	
Group I vs Group II	0.00025	0.0019	-0.005	0.0051	0.99
Group I vs Group III	0.00029	0.0019	-0.005	0.0056	0.99
Group I vs Group IV	0.00029	0.0019	-0.005	0.0050	0.99
Group II vs Group III	0.00054	0.0019	-0.004	0.0058	0.99
Group II vs Group IV	0.00004	0.0019	-0.005	0.0053	1.00
Group III vs Group IV	0.00058	0.0019	-0.004	0.0059	0.99

Compressive strength evaluation

The compression load was applied to the specimens and the linear graph values were recorded **(Figure 3)**. One-way analysis of variance (ANOVA) was used to analyze the compressive strength between the groups, and it was discovered that there was a statistically significant difference between the groups with an F value of 718.17 and a p-value of 0.000 (p0.05) **(Table 4)**. The pairwise comparison was done using Tukey's post hoc test, where there was no statistically significant difference between Group IV when compared with group I & II (p>0.05) proving that 2:1:1(Group I) and 3:1:2 (Group II) and Group IV were equally effective, but when comparing Group III with Group IV there was a significant difference (p<0.05) **(Table 5)** where Group IV(conventional group)has performed with increased compressive strength.

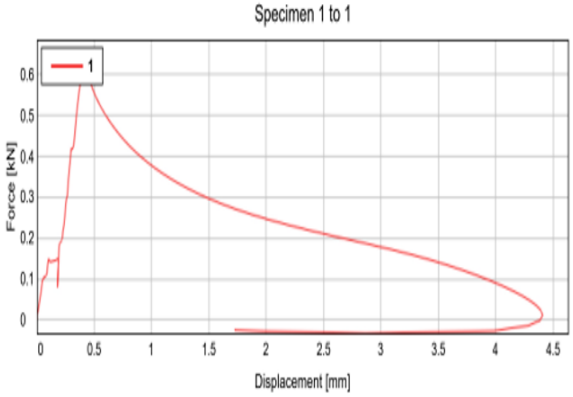

Figure 3. The linear graph of compressive strength of thyme leaves modified GIC

Table 4. Comparison between groups for compressive strength evaluation

Group	n	Mean ± SD	SE	95% CI		df	F value	P-value
				Lower	Upper			
Group 1	12	169.92±1.577	0.455	168.92	170.92	3	718.17	0.000*
Group 2	12	168.44±2.30	0.664	166.97	169.90			
Group 3	12	94.25±9.14	2.63	88.44	100.6			
Group 4 (control)	12	170.65±1.92	0.55	169.43	171.87			

*Significant at 0.05, P value was derived by one way ANOVA

Table 5. Pairwise comparison for evaluation of compressive strength

Pairwise comparison	Mean difference	SE	95% CI		P-value
			Lower	Upper	
Group I vs Group II	1.48	1.99	-3.83	6.79	0.87
Group I vs Group III	75.66^{+}	1.99	70.35	80.98	0.00*
Group I vs Group IV	0.73	1.99	-6.04	4.58	0.98
Group II vs Group III	74.18^{+}	1.99	68.86	79.49	0.00*

Group II vs Group IV	2.21	1.99	-3.09	7.53	0.68
Group III vs Group IV	76.40^{+}	1.99	71.08	81.71	0.00*

*significant difference at p=0.05, $^{+}$significant difference value p<0.05, P value was derived from Tukey Post hoc test.

The resident microflora of the tooth plaque undergoes dynamic alterations as a result of the multifactorial process that causes caries to develop. With the creation of acids by the microflora, these alterations can cause an imbalance between the mineral phase of the tooth and the microbial ecology of plaque, which in turn encourages the growth of bacteria that are acid-tolerant, acidogenic, as well as pathogenicity [31]. Dental caries are brought on by the early demineralization of tooth hard tissue by organic acids. Fluoride ions, when present with calcium and phosphate ions, may be able to remineralize early caries lesions, potentially reversing this demineralization [32]. Due to their special characteristics, GICs are employed in dentistry for restorative and preventive purposes. These uses prompted numerous changes to be made to standard GICs in order to improve their physical and/or antibacterial qualities without impairing their chemical adherence to enamel and dentin. According to several studies, combining antimicrobial agents with restorative materials has many therapeutic advantages but usually compromises their physical and mechanical qualities, hence this study was done to test both antimicrobial and physical properties of thyme-modified GIC.

Thymus vulgaris L. (T. vulgaris) a significant aromatic plant with around 100 species in the world is widely used for medicinal purposes. Thyme oil's main component is thymol. Thyme oil exhibits antimicrobial action and has been effective in dental practice. When combined with other essential oils, thymol can reduce tooth decay by helping to stop the growth of oral pathogens in the mouth. Thymol is one of the essential oils in Listerine that has antibacterial properties [33]. Thyme has high nutritional value because, in addition to being safe, its leaves are said to be a rich source of important minerals and vitamins. Additionally, thyme extract contains phytochemicals including phenol and protein amino acids, as well as enzymes that act as reducing and stabilizing agents [34], hence thyme was chosen in the present study.

Several studies have confirmed that thyme oil has antimicrobial properties. When thyme and clove essential oils were added to chitosan-based films, Hosseini *et al.* [35] found that the thyme essential oil had the strongest antibacterial effects against the Gram-positive bacteria Listeria monocytogenes and S. aureus as well as the Gram-negative Salmonella enteritidis. Previous studies were mainly related to thyme-based mouthwash and toothpaste but there is very less study related to restorative material. Rabab G. Abdel Hameed *et al*. results proved that the thyme extract mouthwash was successful as an antimicrobial agent. It significantly reduced the total bacterial count in the saliva of children when compared to a potent antiseptic like Chlorhexidine [36]. Thyme was utilized in this current investigation because of its potent antibacterial properties, which include growth inhibition, a reduction in lactic acid generation, and a reduction in cellular glucose uptake (CGU). Although the precise mechanism of action is uncertain, some evidence suggests that membrane rupture is what gives thymol its biocidal properties [37].

The results of the present study proved thyme-modified glass ionomer cement proved to have increased antimicrobial efficacy especially when the bacterial strain was tested against *S.mutans* which is the main causative organism for dental caries, this is in accordance with the following studies, the study was done by Amal Adnan Ashour *et al*. where it was proved that the Thymus vulgaris extract, when biosynthesized with copper nanoparticles(TVE-CuNPs) and combined with GIC, showed enhanced antimicrobial efficiency [38]. Another study by Jana Sedlaríkova *et al*. results proved that antimicrobial effects were seen even at the lowest concentration of thyme essential oil [11]. The study done by Nadira A Hatim *et al*., the antibacterial test showed thyme acts as antibacterial material [39]. In a study done by Nilima *et al*. in 2016, the zones of inhibition were highest for ZoT (zinc oxide thyme), against E Faecalis [40]. This antibacterial activity is mainly due to the high concentration of p-cymene (29.1%) and thymol (38.1%) present in thyme which is found to have high antibacterial effects against oral infections [41]. Thymol, the primary phenolic found in thyme, is known to break down Gram-negative bacteria's outer membrane and increase their cytoplasmic membrane's ATP permeability [42], this can be a reason for the present study to show an antimicrobial effect. Also, Carvacrol, another component of thyme oil, has been shown to have antibacterial efficacy against *S. mutans* and *C. albicans*. [43], Studies have shown that thymol has strong antibacterial properties against *S.mutans*, *C.albicans*, *P. gingivalis*, and *A. actinomycetemcomitan*s, [43]. A recent study done by Barbara Lapinska *et al*. PID seen that Composite resin containing 2 µL of thyme essential oil showed the best antimicrobial properties against *S. mutans* and *C. albicans* [44], this proves that thyme has strong antibacterial properties against *S.mutans* which was proved in this current study where all the thyme modified GIC groups performed better when compared to the conventional group. The current study demonstrates similar activity for the modified and unmodified groups in the case of Lactobacillus, demonstrating that the two types of glass ionomer cement exhibited comparable antibacterial activity with no statistically significant difference (p>0.05). The properties of the restorative material should not be compromised by antibacterial agents. It has been found that the hydrophilic properties of GIC are significantly different from those of essential oils. Phase separation between polyacrylic acid aqueous solutions and essential oils occurs due to differences in water solubility in experimental

tests, making both liquids immiscible and causing an uneven distribution of the essential oils in the GIC liquid. For this reason, the extract was made from the dried leaves for this study.

Since most masticatory forces are compressive and compressive strength is the strength value that is most frequently used to describe dental cement, compressive strength is one of the characteristics that need to be examined the most. According to ISO 9917 (2007), the specified minimum compressive strength is 50 MPa for base/lining materials and 100 MPa for restorations. Hence, it remains crucial to assess the compressive strength during changing the GIC. In this current study, the Pairwise comparison revealed that there was an insignificant difference between Group I, and Group II when compared with Group IV(control) which abides by the studies of Farret *et al*. [45], who mentioned that involvement of anit-bacterial materials at specified concentrations did not influence the compressive strength properties of GIC.In the present study when comparing Group III with Group IV, significant results were found, where Group IV (control group) gave the highest compressive strength, this in turn proved by other findings that the reduction in compressive strength due to the addition of antimicrobial agent, this can be explained as an increase in the concentration of plant extract weakened the material by interfering of antimicrobials with the crosslinking of GIC where study done by Sanders *et al*. [46] proved the same. Also, according to Porter GC *et al*., adding thyme oil at 5 and 10%to traditional GIC considerably decreased its compressive strength. Hence in this present study the lower compressive strength in Group III could be mainly due to the higher concentration of extract. Also from the previous study it was noted that adding essential oil decreases compressive strength due to the inability to chemically attach to the polyalkenoate matrix and glass which can disturb the setting reaction of the material thereby affecting the compressive strength therefore in this present study essential oil was not used. With the above findings, it can be said that a lower concentration of thyme extract can enhance antimicrobial properties without compromising compressive strength.

Hence, thyme-modified GIC can be helpful clinically because it can stop the growth of S. mutans and Lactobacillus, which can stop caries from progressing and prevent restoration failure. It can be applied clinically to individuals with deep dentinal caries, early childhood caries, rampant caries, and high caries indices. Unfortunately, the present study does not take into account intraoral variables including typical masticatory stress, wetness, and operator discrepancies. Thus, additional research is required to assess the material's long-term stability.

Conclusion

As thyme has great nutritional value and is considered a rich source of important minerals and vitamins, it can be a safe, promising novel restorative material. The results proved that a lower concentration of thyme extract can enhance antimicrobial properties without compromising compressive strength. Therefore it can be applied in restorative dentistry to prevent secondary caries.

Acknowledgments: None

Conflict of interest: None

Financial support: None

Ethics statement: None

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