

Original Article

## Comparative Analysis of Antimicrobial Properties and Compressive Strength of Traditional and Thyme-Enhanced Glass Ionomer Cement

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### ABSTRACT

The antibacterial properties of restorative materials play an important role in minimizing the occurrence of recurrent caries. This study focuses on evaluating and comparing the antimicrobial effect and compressive strength of thyme-modified glass ionomer cement with conventional glass ionomer cement. Thyme extract was derived from dried thyme leaves and incorporated into the traditional GIC formulation. The modified GIC was prepared by blending the extract with the powder and liquid components in three different ratios: 2:1:1, 3:1:2, and 3:2:1, designated as group I, group II, and group III, respectively, while group IV served as the control (unmodified GIC). The antibacterial potential of both modified and unmodified GIC was evaluated against standard strains of *Streptococcus mutans* and *Lactobacillus* using the MIC assay. The samples were incubated under appropriate conditions for different durations (1, 2, 3, and 4 h). Compressive strength was determined using cylindrical molds according to ISO 9917-1:2007 standards, with the maximum force the specimen could endure before fracture recorded in MPa. The findings showed that all modified groups exhibited significantly higher antimicrobial activity against *S. mutans* without compromising strength compared to the control group ( $P > 0.05$ ). However, when tested against *Lactobacillus*, no statistically significant difference was observed between the modified and control groups ( $p > 0.05$ ). These results indicate that thyme-modified glass ionomer cement possesses superior antimicrobial properties compared to conventional glass ionomer cement.

**Keywords:** Secondary caries, Antimicrobial, Thyme extract, GIC, Strength, Restoration

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### Introduction

Dental cavities develop due to the demineralization of the tooth's hard structure caused by acids of microbial origin, along with other factors affecting tooth integrity. The oral cavity hosts various bacterial species, with *Lactobacilli* and *Streptococcus mutans* being recognized as primary contributors to tooth decay. Glass ionomer cement (GIC) is a widely used restorative material [1]. The International Organization for Standardization (ISO) classifies it as "glass

polyalkenoate cement," although "glass ionomer" remains a commonly accepted term in dentistry [2]. In recent years, GICs have become the most frequently used water-based cement for final cementation in procedures such as crowns, bridges, orthodontic braces, and minimally invasive restorations. Their popularity is attributed to biocompatibility, prolonged fluoride ion release, and strong adhesion to enamel and dentin [3]. However, GICs have limited antibacterial properties and suboptimal physical and mechanical

characteristics, which may contribute to caries recurrence after restoration. Additionally, their lower strength is a significant drawback. This has led to the need for modifications in GIC formulations to improve their performance as direct filling materials. The addition of antimicrobial agents to GICs has been investigated for its potential therapeutic benefits [4]. Studies have examined the controlled and rapid release of antimicrobial substances such as antibiotics, silver ions, zinc ions, iodine, and chlorhexidine, the widely recognized gold-standard antibacterial agent [5]. Several in vitro studies indicate that incorporating chlorhexidine into GIC enhances its biological properties. However, the inclusion of antimicrobial agents in restorative materials often affects their mechanical and physical durability over time. If the dosage or release of these agents is not properly controlled, their antibacterial effects may be short-lived and could pose risks to surrounding tissues [6]. This limitation has likely prevented the widespread commercial adoption of GICs modified with chlorhexidine and other antimicrobial agents.

For centuries, plants have played a crucial role in disease prevention and treatment, a practice that continued until the emergence of chemistry in the 16th century. Phytomedicine, a form of herbal therapy, utilizes various plant-derived components such as extracts for medicinal and health-enhancing purposes and is considered one of the least toxic treatment options [7]. According to the World Health Organization, nearly 80% of the global population relies on traditional herbal medicine to meet essential healthcare needs, including compounds like flavonoids, phenols, and saponins [8].

Thyme is a small perennial shrub that exhibits both horizontal and vertical growth, rarely exceeding a height of 40 cm. As it matures, its stems become woody [9]. The leaves are generally small, measuring between 2.5 to 5 mm, and their shape and surface texture vary across different varieties. *Thymus vulgaris* (*T. vulgaris*) is a highly aromatic plant with over one hundred known varieties worldwide, extensively used in both culinary and medicinal applications. The *Thymus* genus includes several medicinally valuable species, primarily due to the therapeutic properties of its essential oil, commonly known as thyme oil. These species are recognized for their biological and pharmacological benefits, largely attributed to their main active component, thymol [10].

Thyme essential oil is distinguished by its high concentration of bioactive compounds such as thymol, carvacrol, p-cymene, and  $\gamma$ -terpinene. Studies have shown that thymol and carvacrol exhibit strong

bactericidal and bacteriostatic properties, along with significant antioxidant activity [11]. While thyme extract has demonstrated effectiveness against caries in the salivary environment when incorporated into oral care products like toothpaste and mouthwash, limited research exists on its integration into GIC for restorative applications. Given the increased risk of recurrent caries following restorative procedures, careful selection of direct filling materials is essential [12]. An ideal restorative material should provide broad-spectrum antibacterial protection.

Our research team possesses extensive experience and expertise, contributing to high-quality scientific publications [13-28]. This study aimed to enhance the antimicrobial properties of GIC by incorporating thyme leaf extract. With this objective in mind, this study was designed to compare and evaluate the antimicrobial activity and compressive strength of thyme-modified GIC against conventional glass ionomer cement. The null hypothesis proposed that no significant differences would be observed between conventional GIC and its thyme-modified counterpart.

## Materials and Methods

### *Preparation of thyme leaf extract*

Thyme leaves were subjected to a drying process for five days. Before use, all glassware was thoroughly cleaned with distilled water and subsequently dried in a hot air oven. To prepare the extract, 0.5 grams of thyme leaves were combined with one hundred milliliters of distilled water in a beaker. The mixture was stirred and then heated in a water bath with the beaker covered for 10 minutes until the volume was reduced to 5 ml, yielding a concentrated thyme leaf extract. The solution was then passed through filter paper to separate the solid residues, and the filtrate was collected in a conical flask. The obtained extract was stored for further use in subsequent procedures.

### *Test pathogens and inoculum preparation*

The antimicrobial properties of the thyme-modified Glass ionomer cement were tested against the pathogens *S. mutans* and *Lactobacillus acidophilus*. The bacterial strains were provided by the Department of Microbiology. Using a sterile loop, the pure cultures of *S. mutans* and *Lactobacillus acidophilus* were cultured on Mueller Hinton agar. Afterward, the bacteria were transferred into tubes containing 5 milliliters of sterile Mueller Hinton broth and incubated at 37 °C for 24 hours. Once incubation was completed, the bacterial suspension was adjusted to 0.5 McFarland scale, corresponding to  $1.5 \times 10^8$  colony-forming units (CFU).

### Specimen preparation

In this study, type II glass ionomer cement (GIC) from GC Corporation was utilized. Thyme extract was incorporated after mixing the liquid and powder components of conventional GIC at various concentrations, and the mixtures were categorized accordingly (**Table 1**). Within a minute, the prepared specimens were placed into cylindrical wells using a sterile cement carrier, and the upper surface of the cement layer was leveled with a sterile glass slide. The completed cement was poured into cylindrical molds with dimensions of 2 mm in thickness and 6 mm in diameter. Once the cement was set, the disc-shaped specimens were removed from the molds. The exact dimensions of each specimen were recorded using calipers. For each group, 12 samples were prepared: six for *S. mutans* and six for *Lactobacillus*. These bacterial strains were used to assess the antimicrobial efficacy of the modified GIC. To measure compressive strength, cylindrical molds with a diameter of 4.0 mm and a height of 6.0 mm were used, and the test was conducted following the guidelines in ISO 9917-1:2007. After molding, the specimens were leveled, removed an hour later, and stored in deionized water for 24 hours before the compressive strength assessment.

**Table 1.** Grouping

Groups	Description (P-powder, E-extract of thyme, L-Liquid, GIC-glass ionomer cement)
I	P <sup>GIC</sup> : E: L <sup>GIC</sup> = 2:1:1
II	P <sup>GIC</sup> : E: L <sup>GIC</sup> = 3:1:2
III	P <sup>GIC</sup> : E: L <sup>GIC</sup> = 3:2:1
IV	Control group-conventional unmodified GIC

### Minimal inhibitory concentration (MIC) assay

The antimicrobial activity of both unmodified and modified GIC was tested using standard strains of *S. mutans* and *Lactobacillus*. MHA broth was prepared, and sterilized, and 200 microliters were added to each of the four wells. To each well, 50 µL of bacterial suspension (containing *S. mutans* and *Lactobacillus acidophilus*) was added, with a concentration of  $5 \times 10^5$  CFU/ml. The first 3 wells were filled with 3 different concentrations of modified GIC (2:1:1), (3:1:2), and (3:2:1), while the fourth well served as the control, containing conventional GIC. The wells were incubated under appropriate conditions for various time intervals (1 hour, 2 hours, 3 hours, 4 hours). At each interval, the percentage of dead cells was determined using an ELISA reader, with measurements taken at a wavelength of five hundred forty nanometers.

### Compressive strength evaluation

Specimens exhibiting deformation or containing voids were excluded from the study. The diameter of each specimen was measured with a digital micrometer gauge. The samples were then placed vertically in the Universal Testing Machine (Instron, ElectroPuls®, E3000). Compression load was applied along the specimen's longitudinal axis at a crosshead speed of 0.5 millimeters per minute until fracture occurred. The results were recorded following the corresponding graph.

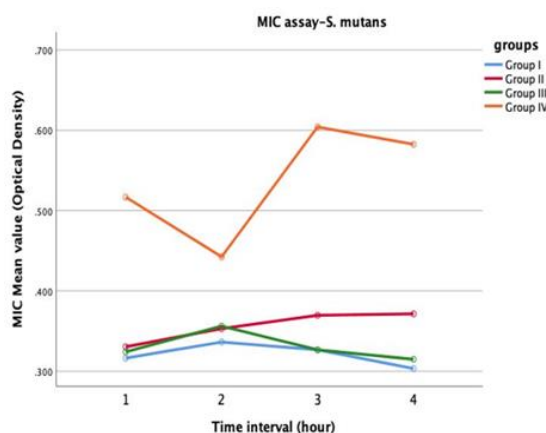
### Statistical analysis

The collected data were entered into an Excel spreadsheet and analyzed using SPSS version 24.0 (IBM Corporation). Descriptive statistics and repeated measures ANOVA were employed to determine the mean MIC values. For compressive strength, a one-way analysis of variance (ANOVA) was used to compare the groups, followed by pairwise comparisons with Tukey's post hoc test. A significance level of  $P \leq 0.05$  and 95% confidence intervals were applied.

## Results and Discussion

### Antimicrobial efficacy against *S. mutans*

In this study, repeated measures ANOVA was used to assess the antibacterial activity of modified and unmodified GIC against *S. mutans*. The results revealed that the three thyme-modified groups outperformed the control group (Group IV), showing statistically significant differences (**Figure 1**). Tukey's HSD multiple comparison tests indicated an important difference between group IV and each of the modified groups ( $P < 0.05$ ) (**Table 2**).



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

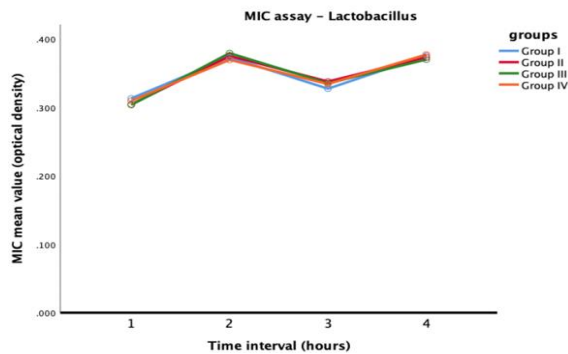
**Table 2.** Pairwise comparison of antimicrobial efficacy on *S. mutans* between four groups

Pairwise comparison	Mean difference	SE	95% CI		P-value
			Lower	Upper	
Group I vs Group II	0.035 <sup>+</sup>	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 <sup>+</sup>	0.006	0.198	0.233	0.00*
Group II vs Group III	0.025 <sup>+</sup>	0.006	0.008	0.043	0.003*
Group II vs Group IV	0.180 <sup>+</sup>	0.006	0.162	0.197	0.00*
Group III vs Group IV	0.206 <sup>+</sup>	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 is 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.

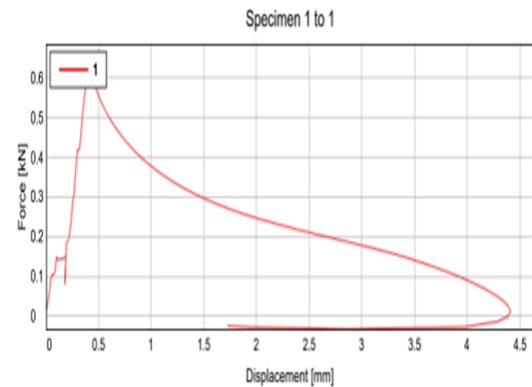
**Table 3.** Pairwise comparison of antimicrobial efficacy on *Lactobacillus* between four groups

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 samples, and the resulting data were recorded on a linear graph (**Figure 3**). To compare compressive strength across groups, a one-way analysis of variance (ANOVA) was conducted, revealing an important difference between the groups, with an F-value of 718.17 and a p-value of 0.000 ( $P < 0.05$ ) (**Table 4**). Tukey's post hoc test for pairwise comparison showed no important difference between group IV and groups I and II ( $P > 0.05$ ), indicating that groups I (2:1:1) and II (3:1:2) were as effective as group IV. However, a significant difference was observed between group III and group IV ( $P < 0.05$ ) (**Table 5**), with group IV (conventional GIC) demonstrating higher compressive strength.



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

**Table 4.** Comparison between groups for compressive strength evaluation

Group	n	Mean $\pm$ SD	SE	95% CI		df	F-value	P-value
				Lower	Upper			
Group 1	12	169.92 $\pm$ 1.5770	0.455	168.92	170.92	3	718.17	0.000*
Group 2	12	168.44 $\pm$ 2.30	0.664	166.97	169.90			
Group 3	12	94.25 $\pm$ 9.14	2.63	88.44	100.6			
Group 4 (control)	12	170.65 $\pm$ 1.92	0.55	169.43	171.87			

\*Significant at 0.05, the 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 <sup>+</sup>	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 <sup>+</sup>	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 <sup>+</sup>	1.99	71.08	81.71	0.00*

\*Significant difference at P = 0.05, <sup>+</sup>significant difference value P < 0.05, P-value was derived from Tukey Post hoc test.

The microbial community of dental plaque undergoes continuous changes due to the complex factors contributing to caries formation. As the microflora produces acids, these changes can disrupt the balance between the tooth's mineral content and the plaque's microbial environment, promoting the growth of acid-tolerant, acid-producing, and pathogenic bacteria [29]. Dental caries result from the early loss of minerals from the tooth surface due to organic acids. The presence of fluoride ions, in combination with calcium and phosphate ions, has the potential to remineralize these early lesions and reverse demineralization [30]. Glass ionomer cements (GICs) are widely used in dental practice for both restorative and preventive purposes due to their unique properties. This has led to various modifications of conventional GICs to enhance their physical and antibacterial properties, without compromising their chemical bonding to enamel and dentin. Research indicates that incorporating antimicrobial agents into restorative materials offers therapeutic benefits but may often reduce their physical and mechanical strength. Therefore, this study was conducted to evaluate both the antimicrobial and physical properties of thyme-modified GIC.

*T. vulgaris*, a widely recognized aromatic plant with approximately 100 species globally, is commonly utilized for its medicinal benefits. The primary constituent of thyme oil is thymol, known for its antimicrobial properties, making it effective in dental applications. When blended with other essential oils, thymol helps in reducing tooth decay by inhibiting the growth of oral pathogens. It is also one of the antibacterial components found in Listerine [31]. Apart from its medicinal uses, thyme is highly nutritious, with its leaves being a rich source of essential vitamins and minerals. Additionally, thyme extract contains bioactive compounds like phenols, protein amino acids, and enzymes, which function as reducing and stabilizing agents [32], making it a suitable choice for this study.

Numerous studies have affirmed the antimicrobial efficacy of thyme oil. For instance, Hosseini *et al.* [33] discovered that thyme essential oil, when added to chitosan-based films, exhibited the most potent antibacterial effects against both gram-positive bacteria like *Listeria monocytogenes* and

*Staphylococcus aureus* and gram-negative bacteria such as *Salmonella enteritidis*. While most prior research has focused on thyme-based mouthwashes and toothpaste, there is limited investigation into its use in restorative materials. In one study, Abdel Hameed *et al.* [32] demonstrated that thyme extract mouthwash effectively reduced the total bacterial count in children's saliva, outperforming even a strong antiseptic like chlorhexidine [34]. Thyme was selected for this study due to its powerful antibacterial properties, which include inhibiting bacterial growth, decreasing lactic acid production, and reducing cellular glucose uptake (CGU). Although the exact mechanism of action remains unclear, some evidence suggests that thymol's biocidal effects may stem from membrane disruption [35].

The findings of this study demonstrated that thyme-modified glass ionomer cement exhibited enhanced antimicrobial properties, particularly when tested against *S. mutans*, the primary pathogen responsible for dental caries. This result aligns with previous studies, such as the one by Ashour *et al.* [31], which showed that thyme extracts combined with copper nanoparticles (TVE-CuNPs) and incorporated into GIC increased antimicrobial effectiveness [36]. Similarly, Jana Sedlářiková *et al.* [11] found that thyme essential oil displayed antimicrobial activity even at its lowest concentration. Another study by Hatim *et al.* [37] confirmed thyme's antibacterial properties, while Thosar *et al.* [38] observed significant inhibition zones for zinc oxide-thyme (ZoT) against *E. faecalis*. The strong antibacterial activity is primarily attributed to the high concentrations of p-cymene (29.1%) and thymol (38.1%) in thyme, which are known to combat oral infections effectively [39]. Thymol, a major phenolic compound in thyme, disrupts the outer membranes of Gram-negative bacteria and increases ATP permeability in their cytoplasmic membranes [40], a mechanism likely responsible for the antimicrobial effects observed in this study. Additionally, carvacrol, another active component of thyme, has been proven to combat *S. mutans* and *C. albicans* [41]. Studies have consistently shown that thymol exhibits strong antibacterial effects against *S. mutans*, *C. albicans*, *P. gingivalis*, and *A. actinomycetemcomitans* [41]. A recent study by Lapinska *et al.* [42] indicated that composite resins with 2 µL of thyme essential oil demonstrated superior antimicrobial properties against *S. mutans* and *C. albicans*, reinforcing the strong antibacterial action of thyme against *S. mutans*, as shown in the present study. All thyme-modified GIC groups outperformed the conventional GIC group in this regard.

Regarding *Lactobacillus*, this study showed similar antibacterial activity between the modified and unmodified GIC groups, with no statistically significant difference ( $P > 0.05$ ). This suggests that both types of GIC displayed comparable antimicrobial effects. The inclusion of antibacterial agents should not impair the properties of the restorative material. It has been noted that the hydrophilic nature of GIC differs from that of essential oils, leading to phase separation in experimental conditions. The differing solubility of water in polyacrylic acid aqueous solutions and essential oils causes them to be immiscible, leading to uneven distribution of the essential oils within the GIC liquid. For this reason, thyme extract was prepared from dried leaves for this study to ensure better incorporation.

Compressive strength is a key property to examine in dental cement, especially considering that most masticatory forces are compressive. According to ISO 9917 (2007), the minimum compressive strength is set at 50 MPa for base/lining materials and one hundred MPa for restorative materials. Therefore, evaluating compressive strength remains essential when modifying GICs. In the present study, pairwise comparison revealed no significant difference in compressive strength between group I and group II when compared to group IV (control), aligning with the findings of Farret *et al.* [43], who suggested that the addition of antibacterial materials at specific concentrations did not affect the compressive strength of GICs. However, when comparing group III with group IV, important differences were noted, with group IV (control) exhibiting the highest compressive strength. This result supports previous studies indicating a reduction in compressive strength because of the incorporation of antimicrobial agents. Higher concentrations of plant extract likely weakened the material by disrupting the crosslinking of GIC, as demonstrated in Sanders *et al.* study [44]. Additionally, research by Porter *et al.* revealed that adding thyme oil at concentrations of 5% and 10% to traditional GIC significantly reduced its compressive strength. In this study, the lower compressive strength observed in group III may be attributed to the higher concentration of thyme extract. Previous studies also note that essential oils reduce compressive strength by interfering with the chemical bonding of the polyalkenoate matrix and glass, which in turn disrupts the setting reaction of the material. Therefore, in this study, essential oils were not used. The results suggest that a lower concentration of thyme extract can enhance antimicrobial properties without compromising compressive strength.

Thyme-modified GIC may offer clinical benefits, as it can inhibit the growth of *S. mutans* and *Lactobacillus*, preventing the progression of dental caries and the failure of restorations. It could be particularly useful for patients with deep dentinal caries, early childhood caries, rampant caries, or those with high caries risk. However, the current study did not account for intraoral factors such as masticatory stress, moisture, and variations in operator technique. Therefore, further research is needed to assess the long-term stability of this material.

## Conclusion

Thyme, known for its high nutritional content and rich supply of essential minerals and vitamins, presents a safe and promising option as a novel restorative material. The findings of this study demonstrated that a lower concentration of thyme extract can improve antimicrobial effectiveness without negatively affecting compressive strength. This makes it a valuable addition to restorative dentistry, particularly in preventing secondary caries.

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**Conflict of Interest:** None

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**Ethics Statement:** None

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