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Evaluation of the Physicomechanical and Antibacterial Properties of *Glycyrrhiza Glabra a*nd *Piper Nigrum* Modified Glass Ionomer Cement

Raffat Aziz, Shaukat Khalid, Muhammad Khawaja Hammad Uddin, Mah Zul Kaif, Affan Ahmad

ABSTRACT

Objective: To investigate the relationship between concentration of *Glycyrrhiza glabra* (GG) and *Piper nigrum* (PN) on the inhibition zone of *S. mutan, Lactobacili* and *E.coli* and their effect on the physicomechanical properties of conventional Glass Ionomer Cement (GIC).

Study Design and Setting: This experimental study tested the antibacterial activity and microhardness of modified-GIC at Baqai Medical University and NED University Karachi, Pakistan.

Methodology: The herbs were added to GIC in 4-16% concentration separately and in combination. Sample characterization was done by Fourier Transform Infrared Spectroscopy (FTIR) in 4000-700 cm^{-1} range. The antibacterial efficacy was tested in-vitro using disc diffusion method on brain heart infusion (BHI) agar plates. Microhardness was tested by Vickers microhardness tester. The pH was measured using digital pH meter and the data was analyzed using Anova test on SPSS software to compare the inhibition zones and a Post-hoc Tukey's test was conducted.

Results: An increase was observed in the inhibitory zone of group 2 (GIC + GG), group 3 (GIC + PN) and group 4 (GIC+GG+PN) as compared to that of conventional GIC (control group) and the increase was more pronounced for GG as compared to PN. The modified groups showed increase in pH at all intervals. Microhardness of modified groups was equal to control group indicating that the addition of herbs into conventional GIC did not impact its physicomechanical properties.

Conclusions: The tests revealed improved antibacterial activity of herb-modified GIC without significant changes in the physicomechanical properties.

Keywords: Antibacterial, Glass Ionomer cement, *Glycyrrhiza glabra*, Microhardness, Physicomechanical, *Piper nigrum*.

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INTRODUCTION

Dental caries affects around 75% of the world population according to WHO. A large population of school-going children (60-90%) and most adults are affected by it. It is a complex oral disease with multi-factorial etiology which includes bacteria, diet, oral hygiene habits, immunity of host and disruption in oral micro-ecological equilibrium of tooth plaque. Although a number of oral *Streptococci* strains have the ability to produce tooth plaque biofilms and cause degradation, S*treptococcus mutans (S. mutans)* plays a key role in the initiation of caries process.[1]

Various restorative materials and anti-caries agents have been developed for the treatment of this disease. Acid-producing bacteria cause demineralization of tooth structure at the restoration-tooth interface leading to secondary caries and hence failure of restoration which is why materials which stop surface colonization and bacterial development are preferred for use in restorative applications. About 50% of restorations fail within 10 years primarily due to the development of secondary caries. Therefore, good bactericidal characteristics are essential for restorative materials. This leads to the search of materials with improved bactericidal

properties.[2]

Glass ionomer cement (GIC) also known as "glass polyalkenoate cement", is an acid-base cement which have gained popularity due to its ability to bond to tooth structure chemically and fluoride leaching properties.[3, 4] This ability of GIC to release fluoride and its low pH prior to hardening may be the cause of the antibacterial activity of GIC powder. GIC have become the most popular water-based cements in recent years for its use in procedures such as fissure and sealant restorations, cementation of dental crown and bridges, placement of orthodontic brackets, etc. Although GIC leaches 10 ppm of fluoride in the first 48 hours after application, this is still considered minimal for obtaining the necessary bactericidal effects and research shows that bacteria remain viable under GIC restorations for up to two years.[5, 6] By including more antimicrobial agents into GIC, therapeutic advantages maybe achieved. Incorporating antibacterial compounds into restorative materials unfortunately frequently leads to changes in the material's mechanical and physical properties over time.

Various plants and herbs are known to have the ability to combat cariogenic and periodontal disease causing bacteria without leading to development of any bacterial resistance. Studies have been able to demonstrate the effectiveness of several of these plants and to better understand their mechanisms of action with the use of scientific approaches and advanced methods. Despite the fact that some of them, like in mouthwash or toothpaste, have showed efficacy against cariogenic salivary flora, there is not enough information present in the literature for the addition of natural antibacterial agents to GIC.

Two herbs used in this study are *Glycyrrhiza glabra (GG)* and *Piper nigrum (PN).* GG is a sweet herb produced from the root of *liquorice* generally considered as safe herbal medication. Titerpene glycoside glycyrrhizin, primary active component of this herb, has anti-ulcer, anticancer, antidiabetic, anti-inflammatory and antibacterial properties.[7, 8]

PN is commonly known as white pepper. It can be used to treat rheumatism, muscle discomfort, colds, flu, and fever. It is applied locally to treat some skin conditions and soothe throat inflammation externally. Its antibacterial and anti-mutagenic properties have been evaluated. It slows down bacterial growth rate and alkaloid compounds in its extract can damage bacterial DNA and inhibit the production of bacterial cell walls.[9, 10]

Although these two powerful herbs have therapeutic and antibacterial properties, no literature exists on the use of these in conventional GIC restorative material and their effect on *S. mutans, E.coli* and Lactobacili. These microbes were selected due to their role in caries activity. Therefore this study aims to investigate the relationship between concentration of both herbs on the growth inhibition zone of *S. mutan, Lactobacili* and *E.coli* and their effect on the physical and mechanical properties of conventional GIC by incorporating GG and PN separately as well as in combination into the conventional GIC powder in 4%, 8%, 12% and 16% concentrations. The hypothesis of the present study is that there is significant difference in the antibacterial properties of Conventional GIC and modified GIC.

METHODOLOGY

This experimental research study was conducted at Baqai Medical University from July 2022 - December 2023. After approval of project (ERC No. BMU-EC/01-2023) from institutional Ethics Review Committee, the herbs (GG and PN) were bought from the local market of Karachi and identified from PCSIR voucher number FMRC/Herb./0180/23and FMRC/Herb./0181/23. The GIC was purchased from the dental store of Baqai Dental College (BDC), Baqai Medical University. The herbs were powdered manually by grinding in mortar and pestle for 10 minutes. The powdered herbs were added w/w into the powder of conventional GIC in 4%, 8%, 12% and 16 % concentration separately as well as in combination. Weighing balance (KERNALS-220-4 SALFORD SCIENTIFIC.UK) was used for weight by weight (w/w) measurement of powdered herbs and GIC in grams. Conventional GIC was used as control group (CG). The powder liquid ratio for conventional GIC is 1:1 and the same was used for the preparation of all samples. Cylindrical samples of conventional GIC and modified GIC were made using S.S and Teflon molds. The sample size was calculated using Mead's resource equation which is:

E= Total number of variable – Total number of groups

where total no of variables = number of variables (4) x No of groups (4)

Fig. 1: (A). Mixing pad with GIC **(B).** Mixing of material **(C) .** Sample Preparation with S.S and Teflon Mold

(A) **(B)** **(C)**

E = (4 x 4) – 4 = 12 samples for each test and a total of 192 samples required for all tests. The samples were divided into following groups:

G_1: Conventional GIC (GC Gold Label 2) (control), **G_2:** Conventional GIC + *Glycyrrhiza glabra* , **G_3:** Conventional GIC + *Piper nigrum and* **G_4:** Conventional GIC + *Glycyrrhiza glabra + Piper nigrum*

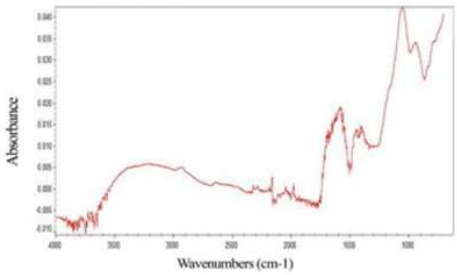
The molecular interactions of GIC and powdered herbs was analyzed using Fourier Transform Infrared Spectroscopy (FTIR, Thermo Scientific Nicolet iD7 ATR, USA) and Ominic (version 9.0) software with the wavelength range of 4000-700 cm^{-1} at Research Laboratory of Baqai Institute of Pharmaceutical Sciences. The sample discs were ground in mortar and pestle individually to make powder. The powder was then placed centrally on the platform to measure the transmittance.

The antibacterial efficacy of GIC and modified GIC was tested in vitro using the conventional disc diffusion method on brain heart infusion (BHI) agar plates at Baqai Institute of Pharmaceutical Sciences. Strains of *S. mutans*, *E.coli*, and *Lactobacillus* were employed for this investigation. There were nine agar plates used. Sterile instruments were used to make the cement discs (6 mm diameter, 4 mm thickness) which were then sterilized by UV radiation (CAMAG UV Cabinet, CAMAG Germany) at 254nm for 60 minutes. To ensure a uniform dispersion of the inoculum, the surface of every agar plate was swabbed three times using a sterile swab. A spectrophotometer was used to verify the check of Mueller–Hinton agar plates (Sigma Aldrich, MO, USA) seeded with 1.8× 108 cfu/mL (0.5 OD600) of the test bacteria. Sterile agar punchers were used to create three wells measuring 6 × 4 mm in diameter in each plate. The specimens were placed into the wells and the plates were then placed into an aerobic incubator at 37°C for 24 hours to check for the presence of inhibition zones after a 24-hour incubation period. A digital caliper was used to measure the inhibition zones surrounding the samples.

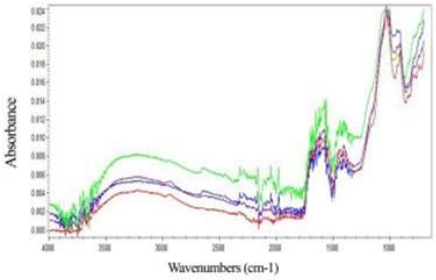
The pH was measured using Accumet Research 10 microprocessor pH meter (Fisher Scientific UK) at Baqai Institute of Pharmaceutical Sciences. The sample discs were powdered and dissolved individually in 5ml distilled water. The electrode was washed and submerged in the solution and the measurement was recorded. The electrode was washed thoroughly after each analysis. The pH was recorded after 1 day, 7 days and 28 days.

Microhardness of the samples was tested by Vickers microhardness tester HMV- G-31 Brand Shimazu Display Type Digital (Japan) at Nadirshaw Eduljee Dinshaw (NED) University Karachi, Pakistan. Twelve samples having 6mm thickness and 4mm in diameter from each group were tested. The finishing of samples was done using sequential grit paper (coarser to finer) mounted on a Rotor-3 finishing machine followed by polishing using diamond polishing paste on automatic lapping and polishing unit. The samples

Fig 2.1: Absorbance peaks of conventional GIC (group 1) and GIC+GG (group 2

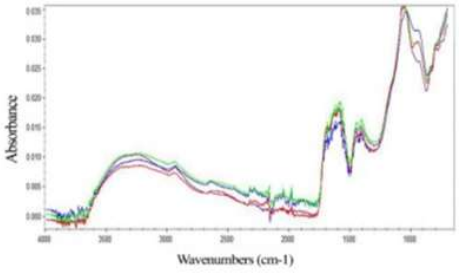


Group1: GIC

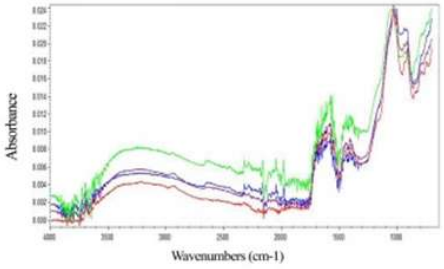


Group2: GIC+*GG* (4-16%)

Fig 2.2: Absorbance peaks of GIC + PN (group 3) and GIC+GG+ PN (group 4)



Group3: GIC+ *PN* (4-16%)



Group4: GIC + *GG* + *PN* (4-16%)

were positioned and a load (P) of 300gm was applied for 15 seconds. The following formula was used:

Vickers Hardness number = KP/L (value of K is 1.854).

Where P is load applied, L is average diagonal length of sample and K is constant.

Data was analyzed using SPSS software (version 20). Mean and standard deviation (SD) was calculated for all groups. Analysis of variance (Anova) was used to compare the inhibition zones. Post-hoc Tukey's test was done for the comparison of variations in zones of inhibition of all experimental groups. A *p*-value < 0.05 was considered significant.

RESULT

Characterization of samples was done using an ATR-FTIR spectrophotometer. Fig 2.1 shows the peaks of control group. From the many peaks, the major ones are ë 1 ?- ë 5, with values of 1068, 1365, 1456, 1637 and 1740 cm^{-1}. The GG-modified GIC had multiple significant peaks including ë 1 with 1060 cm^{-1}, ë2 with 1360 cm^{-1}, ë3 with 1460 cm^{-1}, ë4 with 1650, and ë5 with 1755 cm^{-1}. The FTIR analysis of PN-modified GIC (4-16%) is shown in Fig 2.2 below. The peaks of GIC modified by the combination of both GG and PN are also displayed in Fig 2.2. From the several peaks, ë5 with 1,050 cm1, 1,360 cm1, 1,450 cm1, 1,630 cm^{-1}, and 1,745 cm^{-1} were significant. Peak shift is observed with increase in herb content. This demonstrates that the powder created has significant bio-active characteristics.

There was an increase observed in the inhibitory zone of group 2 (GIC + GG), group 3 (GIC + PN) and group 4 (GIC+GG+PN) as compared to the inhibitory zone of conventional GIC (control group). This indicates that GG and PN enhanced the antibacterial activity of GIC significantly separately and in combination. Incorporation of GG into conventional GIC enhanced the antibacterial activity more as compared to the addition of PN into GIC I.e a statistically significant difference was observed in both group 1 and group 2 when compared with group 3, i.e. ($p < 0.05$). As per the findings of this investigation, the combination of GG and PN with conventional GIC resulted in a greater mean inhibitory zone when compared with group 3 (GIC + PN). The ANOVA results indicate that there was a statistically significant antibacterial effect of herb-modified GIC against *S. mutans*, *L. bacilli*, and *E. coli* as compared to unmodified GIC.

A quick way to test material's physical properties is by evaluating its microhardness. It is the most typical strength parameter used to describe dental cements. The microhardness values of the groups was significantly different from each other but more or less equal to control group with the highest value among the modified groups being 69.123 + 2.9847 of group 3 (GIC+ PN) and the lowest being 61.173 + 22.0267 of group 2 (GIC + GG). The microhardness of conventional GIC (group 1) is 71.600 + .00. This demonstrates that the difference between the microhardness values of conventional and modified groups was insignificant and addition of herbs into conventional GIC did not impact the physicomechanical properties of GIC. The mean hardness value and SD of each group is given below in table 2.

The acidic pH of GIC during setting is due to the acrylic acid component of GIC. The herb-modified groups of GIC showed increase in pH at all intervals (after 1 day, 7 days and 28 days). The pH values of all the groups was close to neutral at 7 and 28 days interval indicating stabilization of ions in cement. After 7 days, a significant increase in pH of all modified groups was observed. The pH values of control group and herb-modified groups after 1 day, 7 days and 28 days of soaking in distilled water are given below in table 3. The pH value of control group was lower as compared to the pH values of modified groups reaching a stable value of 5.8. This maybe due to anti-oxidative properties of GG and PN which also caused increase in anitibacterial and anticariogenic properties of modified GIC.[11]

DISCUSSION

The growing concern of development of bacterial resistance to modern antibiotics has provided the opportunity to study natural herbs for their potential use against infectious diseases.[12] The herbs, GG and PN have a proven history of safety, efficacy, have previously been used in CaOH, ZnO eugenol, AgNPs, ZnPO cement, mouth-rinses and chlorhexidine and the absence of any recorded detrimental side effects in both traditional and modern medical literature. Although, the incorporation of natural herbs in restorative materials has been proven beneficial, it is also known to compromise the physical and mechanical properties of restorative material.[13-15] Although, GIC contains fluoride, it is unable to achieve and maintain the effective level of antibacterial effect after a certain period.[16] Therefore, this study investigates the therapeutic, antibacterial and the

Table 1. Control and Group vise Mean SD Distribution with Significant *p*-value

Variables	N	Mean	SD	Min- Max	Test Statistics	*p* value
Control group 1	12	5.4100	0.346	5.24-5.57	5.048	0.004
Group 2	12	5.8592	0.154	5.69-6.02		
Group 3	12	5.6825	0.316	5.51-5.85		
Group 4	12	5.7105	0.298	5.54-5.87		

Table 2. Statistics of Vickers Microhardness tests

	Group	Mean	SD	N
4-16%	Group 1	71.600	.0000	12
	Group 2	61.173	22.0267	12
	Group 3	69.123	2.9847	12
	Group 4	66.277	2.9610	12
	Total			48

Table 3. Number of samples against tested time intervals

pH measurement GIC GC Gold Label 2 (control)			
Date	After1day	After 7 days	After 28 days
8/2/23	5.02	5.83	5.74
pH measurement Grp I GIC +*Glycyrrhiza glabra*			
GIC+GG	After 1 day	After 7 days	After 28 days
4%	5.63	5.83	6.07
8%	5.68	6.0	6.09
12%	5.73	5.97	6.07
16%	5.64	5.87	6.03
pH measurement Grp 3 GIC +*Piper nigrum*			
GIC+Pn	After 1 day	After 7 days	After 28 days
4%	5.50	5.60	5.61
8%	5.56	5.61	6.09
12%	5.67	5.87	6.05
16%	5.07	5.23	5.89
pH measurement Grp 4 GIC+*Glycyrrhiza glabra*+*Piper nigrum*			
GIC+GG+Pn	After 1 day	After7 days	After 28 days
4%	5.85	6.20	6.21
8%	5.72	6.01	6.09
12%	5.50	6.00	6.00
16%	4.95	5.89	5.90

microhardness of herb-modified GIC.

The FTIR gives information about the chemical changes in the material in the form of any new bands in the absorption spectrum that result after any modification to the material. The analysis of GG and PN has identified different compounds including monoterpenes, linolene, O-cymene, coumarins, phenols, etc. The lipophilic molecule was assumed as the trigger for all these terpenoids' potential membrane disruptions. Phenols and coumarins have strong antibacterial effect against both gram-positive and gram-negative bacteria by causing protein denaturation and modifying the permeability of bacterial cell membrane.[17] The analysis showed carboxylic acid's OH group and the C=O stretching vibrations in the carboxylic group peak at 1635 cm^{-1} and 1706 cm^{-1}, respectively.

GlC is the one of the most commonly used material in dentistry owing to its excellent biocompatibility, known antibacterial activity and its ability to chemically bond to tooth surface. However, Several studies conducted has led to the conclusion that fluoride release takes place mainly in the first 24 - 48 hours but decreases and stabilizes with the passage of time as a result the antibacterial activity of GIC diminishes as the time passes resulting in secondary caries formation. The antiviral and anticarcinogenic properties of liquorice root were studied by Badr et al. (2011). One study reported that GG extract showed great efficacy in the antibacterial test against *S. mutans*. Similarly, Tripathi et al. (2022) studied the antimicrobial activity of PN in concentration against *E. coli* and *S. mutans*. Therefore these herbs were selected for this study and the results of this study indicate a significant difference in the inhibition zone of control group and experimental groups. This suggests that the addition of GG and PN in conventional GIC significantly slowed the bacterial growth. These findings are supported by the study conducted by Khere etal., 2019; Paulraj and Nagar, 2020.[18, 19] The comparison between the different percentages used (4%, 8%, 12% and 16%) showed similar zone of inhibition suggesting that there was no significant difference among groups ($p > 0.05$). These results are contradictory to Hajipour et al. (2012) who reported that the antimicrobial activity was dependent upon the concentration of antibacterial agent incorporated.[20] Although multiple researches suggest that the antimicrobial activity increases with increase in concentration, the findings of our study are consistent with the results of study reported by Tripathi (2022) which suggest no difference in response to multiple dosage forms.[21] The current investigation demonstrates the enhanced antibacterial efficacy of the *Glycyrrhiza glabra* and *Piper nigrum* modified GIC against strains of *E. coli*, *S. mutans*, and *L. bacilli*.

Microhardness is the most typical parameter of strength for dental cements.[22] The mean microhardness values of the modified and unmodified GIC as shown in table 1 are 71 of conventional GIC (control group), 69.05 of GG + GIC, 68.1 of PN + GIC and 65.1 of GG + PN + GIC. These results suggest improvement of antibacterial properties of conventional GIC with the addition of GG and PN without any significant impact on the physicomechanical properties of the material.

GlC has low pH during the initial setting phase which can irritate the pulp.[23] The pH values of all the groups tested was close to neutral ranging from 5.0 - 6.0 after 7-28 days. This highlights the ability of the material to inhibit bacterial growth as well as stabilization of ions over the period of 7-28 days.[24] The pH of control group was lower than the pH of herb-modified groups ranging from 4.99-5.02 at day 1, climbing to 5.83 after 7 days and reaching a stable value of 5.74 after 28 days. Group 4 having 4% concentration of herbs in combination (conventional GIC+ GG+PN) achieved the highest final pH value of 6.11. The antioxidative characteristics of GG and PN, which are a result of the phenolic and flavonoid antioxidants contained in PN and polyphenolic components of GG, are responsible for this rise in pH of GIC. In comparison to traditional GIC alone, the herb-modified GIC groups showed improved antibacterial and anticariogenic activities because of these antioxidants. Researches suggest that this pH could go as high as 7.[25] The pH of the modified groups increased rapidly in the first three days making it more biocompatible since it would diminish the toxicity of the intense acidity during the initial setting reaction.

The current study's findings demonstrate that, even with the

varying percentages (4, 8, 12 and16%), herbs (GG and PN) in combination with GIC exhibit higher antibacterial efficacy against *S. mutans*, *L. bacilli*, and *E. coli* while maintaining the physicomechanical properties of GIC. Based on the findings of the current investigation and other relevant studies, the modification of GIC with herbs may have potential clinical importance in preventive dentistry due to its demonstrated effects on bacterial growth inhibition and mechanical reinforcement as well as its capacity to release possible bioactive compounds and growth factors. However, the study has several limitations as well such as the study was done in-vitro, to fully understand the potential of herbs in dentistry, in vivo studies and clinical trials should be done. This investigation has used only one brand of GIC and results may differ with other brands. This study did not determine the duration of antibacterial effects of modified GIC against *S. mutans*, *Lactobacili* and *E. coli* and the antibacterial activity on other biofilm models involved in dental caries and gum disorders was not examined in the present investigation.

CONCLUSION

Based on the results obtained in this investigation, the addition of GG and PN significantly improved the antibacterial activity of herb-modified GIC without producing any significant changes in the physicomechanical properties.

Authors Contribution:
Raffat Aziz: Conceptualization, lab work and manuscript writing
Shaukat Khalid: Organization of data and provided supervision
Muhammad Khawaja Hammad Uddin: Data analysis and Review of manuscript as Co-supervisor
Mah Zul Kaif: Manuscript writing, editing and proofreading
Affan Ahmad: Provided support for statistical analysis as Co-supervisor

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