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# EVALUATION OF ANTIMICROBIAL ACTIVITY AND TENSILE STRENGTH OF GLASS IONOMER CEMENT MODIFIED WITH PLANT EXTRACT MIXTURE

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## *ABSTRACT*

**Aim:** is to assess antimicrobial activity and diametral tensile strength (DTS) of glass ionomer cement (GIC) modified with plant extract mixture (PE).

**Material and Methods:** conventional GIC was utilized. It is treated with PE. Alcoholic extract of Aloe Vera (AVE) was prepared. Using a Soxhlet extractor, an alcoholic extract of Salvadora persica(SPE) was produced. The PE was incorporated to the glass ionomer liquid (GIL).Forty Samples were fabricated using 6 mm diameter and 3 mm thick split-Teflon molds. Samples were divided into two main groups (n=20): group I (antimicrobial test) and group II (diametral tensile strength test). Each group was divided into Four subgroups (n=5) according to liquid composition into: subgroup (A): control subgroup, subgroup (B): 1.5GIL: 1PE, subgroup(C): 1GIL:1PE and subgroup (D): 1GIL:1.5PE. Antimicrobial activity against Streptococcus mutants (SM) was appraised by the agar well diffusion assay method. Universal testing machine was utilized to determine (DTS).

**Results**: One way ANOVA test for quantitative data between the four groups followed by post hoc LSD analysis between each two groups were used. The highest antimicrobial activity was in subgroup ID (1GIL:1.5 PE) and the lowest value was in control subgroup. The highest DTS was in subgroup IIC (1GIL:1PE) and lowest was in control subgroup.

**Conclusions:** Plant extract significantly improved the antimicrobial activity of GIC against SM. The DTS of GIC was improved within limits by addition of PE only up to (1 GIL: 1 PE).

**KEYWORDS**: Glass ionomer cement, Aloe Vera, Salvadora Persica, Antimicrobial activity, Diametral tensile strength.

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## INTRODUCTION

The GICs are luting and restorative materials that have thermal compatibility with enamel, bio-compatible material and chemical adhesion to tooth structure.[1,2] But in relative to modern resin composite materials, the main disadvantages of GIC are their relatively low wear, brittleness, and fracture resistances.[3,4]

Although the GIC has some antibacterial properties, the therapeutic benefit might need to be increased by adding bactericides. A number of antimicrobial compounds were searched to use as additives within GIC. These materials should to be chosen carefully to inhibit the growth of cariogenic bacteria while not being harmful to the pulp or gingiva cells.[5, 6]

Regrettably, the addition of antibacterial agents to restorative materials changes their qualities over time. Additionally, if the dosage is not appropriately regulated, the addition of antibacterial medicines may be both temporarily useful and harmful to the surrounding tissues; so Plant natural extract is the most recent direction for improving the mechanical and physical properties of dental restoratives.[7] Herbal products may contain naturally occurring bioactive components that are both minimally harmful and highly effective. [8]

Aloe Vera and SP are two examples of plants that exhibit beneficial activities. Miswaks were made by SP. Using miswaks for oral hygiene has many benefits: it acts chemically due to its distinct chemical composition and mechanically through the friction that occurs between the plant fibers and the tooth surface and [9] it has antibacterial activity against oral pathogens that cause dental caries. [10, 11] The antibacterial and strengths of GIC were significantly altered by the addition of salvadora persica extract (SPE).[12, 13]

One plant that is a member of the Liliaceae family is Aloe Vera. Aloe Vera showed antibacterial efficacy against lactobacillus and mutans streptococci.[14, 15] The incorporation of AV in GIC seems to increase the antibacterial activity of GIC.[16]

One of the most crucial elements in determining the clinical efficacy of dental materials is mechanical strength. An easy method for determining the strength of brittle materials is the DTS test. Bioactive materials should have acceptable DTS.[17]

Hence, this study will be carried out to assess antimicrobial activity and DTS of GIC modified with PE.

The null hypothesis of this study suggests that addition of PE of AV and SP to GIC will not enhance either the antimicrobial effect or its DTS compared to conventional GIC.

## MATERIAL AND METHODS

NOVA GLASS-L Conventional glass ionomer luting cement (IMICRYL,Turkey with batch no. 22F899-1783) was used. It was modified by PE. Forty samples were prepared. Samples were divided into 2 main groups (n=20) according to test type: group I (Antimicrobial test) and group II (Indirect tensile strength test). Each group was divided into Four subgroups (n=5) according to liquid composition into: subgroup (A): control subgroup, subgroup (B):1.5GIL: 1PE, subgroup(C):1GIL:1PE and subgroup (D):1GIL:1.5PE.

### Preparation of the two plant extract mixture (PE):

For preparation of SPE; Stems of SP were washed, heat dried, and ground into powder that was put inside the soxhelt extractor (LAB-LINE 5000 MULTI-UNIT EXTRACTION HEATER soxhelt, USA) with70% ethanol (as a solvent). After repeated cycles inside the soxhelt extractor, a thick extract was obtained.[13]

Aloe Vera leaves' serrated edges and bottom were sliced and peeled. The interior gel was broken up into tiny bits, mixed with 70% ethanol, and then transferred into a closed container to be filtered.[18]

Then two extracts were proportioned according to weight (1 AV: 1 SP) to prepare PE.

**Chemical analysis of plant extracts mixture:**

Diethylether was used to dilute the PE to examine its chemical composition. A gas chromatography GC (Agilent Technologies 7890A) interfaced with a mass-selective detector MS (Agilent 5975C, Germany) was utilized in the analysis. The analysis was carried out at Beni Suef, Egypt's Faculty of Postgraduated Studies for Advanced Sciences (PSAS). [19]

**Preparation of GIC and extract combination:**

The GIL was mixed with PE in different weight ratios. The ratios of mixing liquid were 1.5GIL: 1PE, 1GIL: 1PE and 1GIL: 1.5 PE.

**Specimen's preparation:**

A total of 40 GIC discs were fabricated. The specimens were divided into group I (Antimicrobial test) and group II (Indirect tensile strength test). Each group was divided into four subgroups (n=5) based on liquid composition into: subgroup (A): control subgroup, subgroup (B): 1.5GIL: 1 PE, subgroup(C): 1GIL: 1PE and subgroup (D): 1GIL: 1.5 PE. The glass ionomer powder was added as partitions to the liquid with sterile spatula within 20-25 seconds. Six mm diameter and three mm thick split-Teflon molds were filled to fabricate samples.

**Antimicrobial agar diffusion assay test:**

Standard strain streptococcus mutants (ATCC 25175) were utilized to determine the antimicrobial efficacy of modified glass ionomer. For the culturing, brain heart infusion broth was utilized. Four wells with a diameter of 6 x 3 mm were made in five agar plates by sterile agar punchers. Following by the insertion of specimens into the wells, the plates were incubated aerobically at 37˚C for 48 hours. The sizes of inhibitory zones surrounding the specimens were determined using a digital micrometer caliper (Fisher scientific caliper, U.S.A.). The measurements were made from the center of the disc to a clearly visible edge at a location around the circumference of the zone. To find the diameter of the zone according to CLSI (clinical and laboratory standards institute), multiply the measurement by two. [13, 20]

**Diametral tensile strength test:**

The DTS test was run utilizing universal testing equipment (Instron, USA). The specimens were placed edgewise. A load was applied at a speed of 0.5 mm/minute till broken of the samples into two halves in the middle. At failure, the maximum load was noted and the DTS was estimated. [21]

## RESULTS

**1- Antimicrobial test results:**

Regarding the comparison of the inhibition zone of the antimicrobial test between the 4 subgroups, the mean ± SD in the four subgroups were 7.8±0.8, 13.2±0.8, 15.2±0.8 and 21.2±0.8 respectively. Significant differences were existed between the four subgroups as the highest value was in subgroup(I) D, followed by subgroup(I)C, then subgroup(I)B and the lowest value was in subgroup (I)A.

TABLE (1) Mean and standard deviation of inhibition zone (mm) of antimicrobial test between of all subgroups:

| | Control (I) A | 1.5GIL: 1 PE (I)B | 1 GIL: 1 PE (I)C | 1GIL: 1.5 PE (I)D | P value |
|---|---|---|---|---|---|
| | N=5 | N=5 | N=5 | N=5 | |
| Antimicrobial test | $(7.8\pm0.8)^a$ | $(13.2\pm0.8)^b$ | $(15.2\pm0.8)^c$ | $(21.2\pm0.8)^d$ | <0.001* |

***One way ANOVA test for quantitative data between the 4 groups followed by post hoc LSD analysis between each 2 subgroups.***
****: Significant level at P value < 0.05***

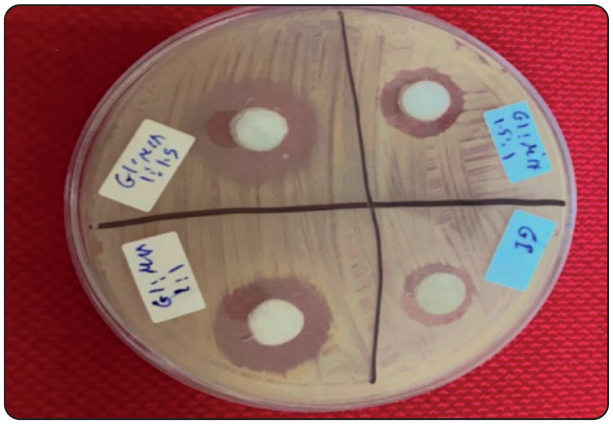

Fig. (1) Antimicrobial activity Test of herbal modified glass ionomer aganist streptococcus mutatans

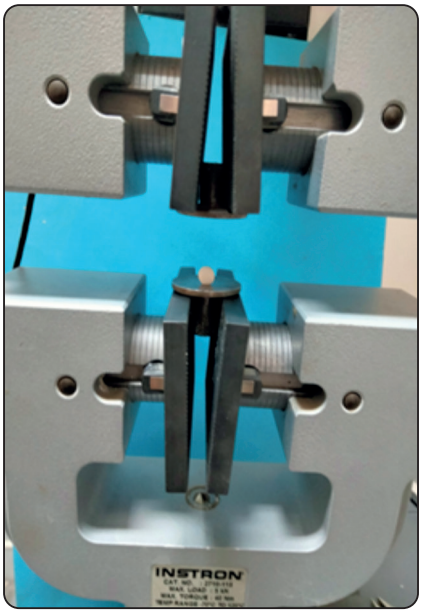


Fig. (2): Diametral tensile strength test for herbal modified glass ionomer using universal testing machinne

**2- Diametral tensile strength results:**

Regarding the comparison of DTS between the 4 subgroups, the mean ± SD in the four subgroups were 4.1±0.8, 5.2±2.1, 7.6±2.1 and 5.9±0.8 respectively. There were significant differences between the subgroups as subgroup (II) C showed a significant increase in comparison with other subgroups.

TABLE (2) Means and standard deviation of DTS (MPa) of all subgroups:

| | Control (II)A | 1.5GIL: 1PE (II)B | 1 GIL: 1 PE (II)C | 1 GIL: 1.5 PE (II)D | P value |
|---|---|---|---|---|---|
| | N=5 | N=5 | N=5 | N=5 | |
| **Diametral tensile strength** | (4.1±0.8)[a] | (5.2±2.1)[a] | (7.6±2.1)[b] | (5.9±0.8)[a, b] | ***0.024**** |

***One way ANOVA test for quantitative data between the 4 groups followed by post hoc LSD analysis between each 2 subgroups.***

****: Significant level at P value < 0.05***

## DISCUSSION

The primary cause of dental caries is streptococcus mutans. Dental caries is caused by this bacterium when it breaks down carbohydrates into organic acid. This process demineralizes and denatures the tooth material. [22] Streptococcus mutans (ATCC 25175) was chosen for this study due to its significant impact on the prevalence of tooth decay.

Because conventional GIC, also known as man-made dentin, has some advantages over other GICs, it was chosen for this investigation. GIC has chemical adhesion to the tooth structure, biological compatibility, fluoride-releasing properties, and Low bactericide capacity which can act against microorganisms to a certain extent. [23] But may need to be augmented by bactericides to optimize its therapeutic benefit. [24] The use of antibacterial compounds

in GIC has been shown in numerous trials to provide a variety of therapeutic effects; however it also commonly results in compromised mechanical and physical qualities. Herbal extracts showed the advantage of having beneficial effects without running the danger of bacterial resistance.[25]

In this study, SPE and AVE were prepared. Glass ionomer liquid was combined with this extract in three different weight ratios (1.5 GIL: 1PE, 1 GIL: 1PE, and 1 GIL: 1.5 PE) in order to modify conventional GIC. Regarding the antimicrobial and indirect tensile strength characteristics, the modified GIC were assessed and contrasted with a traditional GIC (Control).

In the present study, Antimicrobial activity was evaluated using the agar well diffusion assay method against SM. The disk-diffusion assay is simple, inexpensive, and capable of testing a large variety of bacteria and antimicrobial agents.[26]

According to the findings of agar diffusion tests conducted against SM, the modified GIC containing varying amounts of plant extract significantly inhibited the development of the bacteria in comparison to the control group. The effect became more noticeable as the concentration of the extract combination increased, as demonstrated in subgroup ID (1GIL: 1.5PE), which displayed the statistically highest inhibition zone. Subgroup IC, subgroup IB, and subgroup IA had the lowest value. The kind of antimicrobial agent that was added to GICs and its rate of release from the specimen's surface layer both had a substantial impact on the inhibition of bacterial growth.

The distinct phytochemical components in each of the two integrated plant extracts may be responsible for the antibacterial action. GC/MS was used in this investigation to identify the volatile and semi-volatile chemicals in the plant extract combination.[19] 12 chemicals were found when the plant extract mixture was analyzed using GC/MS. Antimicrobial action was demonstrated by octanal, benzothiophene, phenols, naphthalene, benzoic acid, and 1-hexadecene.[27, 28, 29]

Since many clinical failures are caused by tensile stresses, the DTS is an essential criterion in determining the success of any dental cement. Since it is impossible to evaluate the tensile strength of brittle materials directly, such as GIC, the DTS test was accepted by the British Standards Institution in 1981. [21]Because of its relative simplicity and repeatable results, the dialemter tensile strength test is frequently utilized. It is also the most often used technique for determining the tensile strength of friable materials since it does not involve the challenges that come with doing a flexural tensile strength test. Prior to the mechanical testing (24-hour protocol), the specimens were stored to guarantee that the test was conducted using the materials at their final strength. [30]

Wilson and Mclean state that the diametral tensile strength of standard luting material is 6.4–10.9 after 24 hours. [3]In this test, the control subgroup's mean diametral tensile strength is (4.1±0.8). These findings are consistent with (Zhen Chun Li) [31] which found that the mechanical characteristics of luting cements varied significantly. This discrepancy could be the result of several factors, including the operator, material type, and material manipulation.

Results of DTS test showed significant difference between IIA, IIB, IIC and IID subgroups. Surprisingly, the 1GIL: 1PE subgroup IIC showed a significant improvement in the DTS values from the control and the other modified subgroups. This could be explained due to the presence of silica in Salvadora persica (one of the plant extract mixture). (Lihua et al.) [32] and (Tjandrawinata et al.) [33] Demonstrated how adding silica fillers strengthens traditional GIC because silica has the ability to the matrix by chemical bonding and hence strengthening the GIC.

Furthermore, it was believed that by adding benzoic acid and cyclopentanecarboxylic acid—

which were found in the extract by GC/MS analysis to GIL, the degree of cross-linking would also increase along with the formation of polysalt bridges, strengthening the mechanical qualities of the cement (Singer et al).[34]

In extract, there is also phenolic compound that have high molecular weight with unique properties. Active crosslinks are produced when the carboxyl group of GIC and the hydroxyl phenolic group of the extract interact. These crosslinks may be the cause of improvement of diametral tensile strength. [35]

Another possibility is that the extract may have an impact on the quantity of unreacted particles of the powder in the matrix, which could act as fillers to reinforce the matrix and keep the cement from cracking. [33]

However after that concentration (1GIL: 1PE) the diametral tensile strength began to negatively affected. That might be due to increase the amount of unreacted powder to limit that affect the reaction of modified glass ionomer powder and liquid.

The null hypothesis of this study is rejected as the addition of the of the plant extract mixture of Aloe Vera and miswak to glass ionomer cement enhanced the antibacterial effect as well as the tensile strength of the glass ionomer cement.

## CONCLUSION

Within the limitations of the current study, it can be concluded that:

1. Plant extract significantly improved the antimicrobial activity of GIC against SM, and the higher the concentration of the plant extract in the GIC, the higher was its antimicrobial activity.
2. The DTS of GIC was improved within limits by addition of PE only up to 1 GIL: 1 PE.
3. The optimum mix providing effective antimicrobial activity while still having highest tensile strength was 1 GIL: 1 PE.

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