# Evaluation of the physico-mechanical properties of a new antimicrobial-modified glass ionomer cement

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## Dedication

*I would like to dedicate this thesis to the memory of my father, to my mother who is always been my role-model for hard work, to my siblings, to my beloved children; Ali and Laila and to my husband who has been proud and supportive of my work, and who has shared the journey for completing this thesis.*

*Lamia Singer*

## Table of Contents

## List of Abbreviations

| | |
|---|---|
| GIC | Glass ionomer cement |
| CHX | Chlorhexidine |
| *O. europaea* | *Olea europaea* |
| *S. persica* | *Salvadora persica* |
| *F. carcia* | *Ficus carcia* |
| GC/MS | Gas chromatography/Mass spectrometry |
| *S.mutans* | *Streptococcus mutans* |
| *M.luteus* | *Micrococcus luteus* |
| CS | Compressive strength |
| FS | Flexural strength |
| $W_{sp}$ | Water sorption |
| $W_{sol}$ | Water solubility |
| FS | Flexural strength |
| F | Force |
| r | Radius |
| ANOVA | Analysis of variance |
| IQR | Interquartile range |
| M | Mean or Median value |

## 1. Abstract

**Objective**: The use of natural plant extracts for the control of dental and periodontal pathogens is considered a promising alternative to synthetic chemicals owing to their claimed minimal harmful effects and maximum efficacy. Therefore, this study aimed to prepare an extract mixture of three plants to enhance the antimicrobial activity of a conventional glass ionomer cement (GIC). **Materials and methods:** An alcoholic extract mixture of olive leaves *(Olea europaea)*, fig leaves (*Ficus carica)* leaves and roots of miswak (*Salvadora persica)* was prepared using a glass Soxhlet extractor. The prepared extract mixture was proportioned to the water used for preparation of a freeze-dried glass ionomer cement at three different extracts: water mass ratios (groups; 1:2, 1:1, and 2:1). Chlorhexidine diacetate (0.5 %) modified glass ionomer cement was prepared for comparison. Gas chromatography-mass spectrometry was used to chemically analyse the extract mixture. The control and modified materials were evaluated with regard to: antimicrobial activity, compressive strength, flexural strength, water sorption, solubility, shear bond strength and film thickness. Statistical analysis was performed using Minitab 17.3.1 for Microsoft Windows. **Results:** The extract mixture was significantly effective against *Streptococcus mutans* and also against *Micrococcus luteus* but only with the highest concentration group (2:1). Compressive strength and flexural strength results revealed that the 2:1 group recorded the highest values among all the other tested groups. Furthermore, there were no statistically significant differences between all the groups with regard to the percentage of water sorption, however for water solubility the 2:1 plant-modified group was significantly different from all of the other groups. Shear bond strength results showed insignificant difference between the control group and each of the CHX-GIC and the three plant modified groups 1:2, 1:1, 2:1. Failure mode analysis revealed the predominance of mixed and cohesive failures. All the tested groups yielded a film thickness of less than 25 µm film thickness. **Conclusion**: Natural plant extracts can be a promising agent for enhancing the antimicrobial activity and other properties of GIC without adversely affecting its performance.

**Key words: Water-based cements, Phytomedicine, Herbal extracts, Antimicrobial activity.**

# 2. Introduction and Aims with References

## 2.1 Introduction

Glass polyalkenoate cements, commonly known as glass-ionomers (GICs), are a class of dental materials that are made of calcium or strontium alumino-fluoro-silicate glass powder (base) combined with a water soluble polyacrylic acid. Introduction of glass-ionomer cements (GICs) has provided the dentist with a biocompatible, esthetic self-adhesive material. They are commonly used in atraumatic restorative treatments and as liners, bases, fissure sealants and as bonding agents for orthodontic brackets [1-4].

It has been claimed that GICs inhibit caries initiation or progression through their inherent ability to release and reuptake fluoride ion although, there is no clear scientific evidence to fully support this caries inhibitory potential. Many investigations have evaluated the possibility of incorporation of a direct antimicrobial agent into GICs for innovating an esthetically pleasing, self-adhesive dental material that can arrest residual caries, decrease the incidence of recurrent caries and provide a tight seal under restorations [5]. Attempts involved the usage of different antimicrobial compound such as antibiotics, metal ions and oxides, iodine, and most commonly chlorhexidine which is considered the gold standard for antibacterial applications [6-8].

Moreover, there has been an approach adopted by numerous studies for exploring the activity of natural plant substances against microorganisms involved in the etiology of oral and dental diseases. Findings showed that many plant parts or extracts had potentials to be used in dentistry due to their activity against cariogenic bacteria and those bacteria associated with periodontal diseases [9, 10].

*Olea europaea (O. europaea), Salvadora persica (S. persica),* and *Ficus carcia (F. carcia) are* of those plants that have shown satisfactory therapeutic properties. Chemical analysis of the extracts of these plants revealed the presence of saponins, tannins, silica, resin, trimethylamine, alkaloids, and phenols [11-14]. Each of these components has a definite and unique pharmacological activity that can be directly linked to its chemistry [15, 16].

Taking into consideration the smart behavior of glass-ionomer (GI) cements in which ions can freely travel in and out offering it the opportunity to be used as a template for soluble

antimicrobials [17]. Besides the data that natural medicinal plant extracts could be a source of biologically active compounds and many of which have been used for the development of new pharmaceutical products [10]. Therefore, this study aimed to modify a conventional GIC with natural plant extracts to enhance its antimicrobial activity without altering its physical and mechanical performances.

## 2.2 Aim of the Study

This current study aimed to:

1. Prepare GICs using three different plant extract mixtures and chlorhexidine diacetate at different concentrations to achieve improved antimicrobial effects of these modified GICs.
2. Perform antimicrobial testing.
3. Analyse the extract mixture chemically.
4. Perform physical and mechanical material testing with respect to:
   a. Compressive strength.
   b. Flexural strength.
   c. Bond strength and failure mode analysis.
   d. Water sorption and solubility.
   e. Film thicknesses.

## 2.3 References

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# 3. Publications

## 3.1 Study 1

Evaluation of the antimicrobial activity and compressive strength of a dental cement modified using plant extract mixture

Lamia Singer, Gabriele Bierbaum, Katja Kehl, Christoph Bourauel

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BIOMATERIALS SYNTHESIS AND CHARACTERIZATION

Original Research

# Evaluation of the antimicrobial activity and compressive strength of a dental cement modified using plant extract mixture

Lamia Singer[1] · Gabriele Bierbaum[2] · Katja Kehl[2] · Christoph Bourauel[3]

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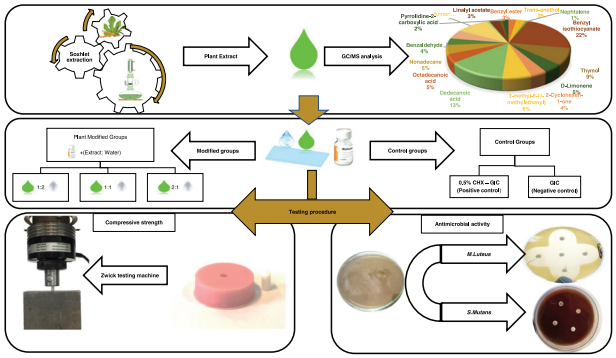
## Abstract

Literature lacks sufficient data regarding addition of natural antibacterial agents to glass ionomer cement (GICs). Hence, the aim of the study was to increase the antimicrobial properties of GICs through its modification with mixture of plant extracts to be evaluated along with an 0.5% chlorohexidine-modified GIC (CHX-GIC) with regard to biological and compressive strength properties. Conventional GIC (freeze-dried version) and CHX were used. Alcoholic extract of *Salvadora persica, Olea europaea*, and *Ficus carcia* leaves were prepared using a Soxhlet extractor for 12 h. The plant extract mixture (PE) was added in three different proportions to the water used for preparation of the dental cement (Group 1:1 PE, 2:1 PE, and 1:2 PE). Specimens were then prepared and tested against the unmodified GIC (control) and the 0.5% CHX-GIC. Chemical analysis of the extract mixture was performed using Gas chromatography–mass spectrometry. Antimicrobial activity was evaluated using agar diffusion assay against *Micrococcus luteus* and *Streptoccocus mutans*. Compressive strength was evaluated according to ISO 9917-1:2007 using a Zwick testing machine at a crosshead speed of 0.5 mm/min. Antimicrobial activity against *Streptoccocus mutans* was significantly increased for all the extract-modified materials compared to the unmodified cement, and the highest concentration was comparable to the CHX-GIC mixture. The activity against *Micrococcus luteus* was also significantly increased, but only for the material with the highest extract concentration, and here the CHX-GIC group showed statistically the highest antimicrobial activity. Compressive strength results revealed that there was no statistically significant difference between the different mixtures and the control except for the highest tested concentration that showed the highest mean values. The plant extracts (PEs) enhanced the antimicrobial activity against *S. mutans* and also against *M. luteus* in the higher concentration while compressive strength was improved by addition of the PE at higher concentrations.

## Graphical Abstract

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## 1 Introduction

Glass ionomer cements (GICs) belong to a group of materials known as acid-base cements. The proper name for them, according to the International Organization for Standardization (ISO) is "glass polyalkenoate cement", but the term "glass ionomer" is recognized as an acceptable trivial name, and is widely used within the dental community [1].

Over the past years, GICs have been the most commonly used water-based cements for final cementation of dental crowns, bridges, orthodontic brackets and atraumatic restorative treatment [2]. There are several properties that make glass ionomer a material of choice among which are their ability to bond adhesively to enamel and dentin, their biocompatibility and their ability to release fluoride ions over a prolonged period of time. Furthermore, GICs were shown to be rechargeable with fluoride ions [3, 4].

Literature has shown that microorganisms have been found to be viable for at least a period of 2 years under the GIC. Moreover, in spite of the fact that studies have shown that GICs release ~10 ppm of fluoride during the first 48 h following insertion into the cavity, this is still regarded low for achieving the desired antibacterial effects [5].

Therapeutic benefits may be gained by reinforcing GICs with additional antibacterial agents. Studies have been focusing on release or slow release of antibacterial agents such as antibiotics, zinc ions, silver ions, iodine, and most commonly chlorhexidine that is considered the gold standard for antibacterial applications. Several in vitro studies confirmed the enhancement of the biological properties of GIC when being incorporated with CHX [6–8].

Unfortunately, the incorporation of antibacterial agents in restorative materials frequently results in changes in the physical and mechanical properties of the restorative material over time, and might have short-term effectiveness and toxicity to surrounding tissues if the dose or release is not properly controlled. This is probably the reason why the combination of chlorhexidine and other antimicrobials with GICs is not yet employed in production [9, 10].

Many years ago, up to the advent of iatrochemistry in the 16th century, plants were used for treatment and prophylaxis in order to cure or alleviate illnesses. Phytomedicine can be defined as the herbal medicine that utilize different plant parts or extracts as a therapeutic or health-promoting agent. Herbal extracts showed an advantage of having beneficial effects without the risk of developing bacterial resistance [11, 12]. According to the World Health Organization, as many as 80% of the world's people depend on traditional medicine (herbal) for their primary healthcare needs [13].

Among the effective medicinal plants are *Olea europaea, Ficus carcia*, and *Salvadora persica. Olea europaea (O. europaea)* is cultivated on a large scale in the Arabian Peninsula, India, and Asia, but the Mediterranean region is the main area of agricultural production [14, 15]. Recently, studies concerned with the medicinal properties of olive products have been focusing on olive polyphenols that have shown in animal and in vitro studies an antioxidant and an antimicrobial property [15].

*Ficus Carcia (F. carcia)* belongs to the mulberry tree family (Moraceae) which is one of the oldest harvested fruits in the world [16, 17]. Phytochemical studies on *F. carica* leaves revealed the presence of numerous bioactive compounds such as phenols, flavonoids, tannins, alkaloids, and saponins. Owing to these compounds *F. carcia* was reported to have antioxidant, anti-inflammatory, antiviral, and antibacterial activities [18, 19].

*Salvadora persica (S. persica)*, of the family Salvadoraceae, is an evergreen shrub, with a short trunk 4–6 m tall, smooth green leaves and white bark. *S. persica* has a wide geographic distribution ranging from India, Nepal in the east through Pakistan and Egypt in the west, and from Central Africa to Southwestern Africa [20]. Miswak, a chewing stick is prepared from *S. persica* roots or stems. It is used as an toothbrush stick for oral hygiene and in treatment of gum inflammation. It has been shown that an extract of miswak *(S. persica)* possesses different antimicrobial and antifungal properties due to the presence of trimethylamine, chlorides, fluoride, silica (Si02), sulfur, saponins, flavonoids, and phenols [20–22].

Literature lacks sufficient data regarding addition of natural antibacterial agents to GICs, despite that some of them have shown effective results against cariogenic salivary flora as mouthwashes or toothpaste. Hence, the aim of the study was to increase the antimicrobial properties of GICs through its modification by mixture of *S. persica, F. carcia* and *O. europaea* extracts to be evaluated along with an 0.5% CHX-modified GIC with regard to biological and compressive strength properties.

## 2 Materials and methods

### 2.1 Preparation of the three-plant extract mixture

*S. persica, F. carcia*, and *O. europaea* plants were used to prepare the extracts. Each of these plant parts was separately washed, dried, ground into powder, and added to the thimble of a Soxhlet extractor. Each extraction process was performed using ethyl alcohol (70%) alcohol for several hours. The extraction products from each plant was then filtered, proportioned to prepare a mixture of them all. The plant mixture was then placed at 37 °C in a rotary evaporator (Buchi Rotavapor R-300, Essen, Germany) to remove the ethanol leaving a crude mix that was stored in the fridge in a closed flask at 4 °C until use [10].

**Table 1** Specimens' grouping

| Group name | Description |
|---|---|
| 1—Control | Conventional, unmodified GIC. |
| Plant extract-modified groups (PE):<br>2—2:1 PE<br>3—1:2 PE<br>4—2:1 PE | The prepared plant extract (PE) was added to the water used for the preparation of GIC in three different extract to water mass ratios. |
| 5—CHX-GIC | 0.5% CHX diacetate (w/w) (Merck KGaA, Darmstadt, Germany) was added to the powder of GIC to be mixed with distilled water. |

## 2.2 Preparation of GIC, extract and CHX combinations and specimen grouping

Conventional, freeze-dried (powder/water) GIC, hand mix version (Medicem aqua, Promedica GmbH, Neumunster, Germany, Lot 1849261) was used.

The tested groups were prepared by either modifying the water used for preparation of GIC with different concentrations of the PE mixture or by adding CHX to the powder of GIC. These modified groups were then compared with a non-modified GIC as a control group (Table 1).

For the five groups, all specimens were mixed at a temperature of 23 ± 1 °C and a relative humidity of 50 ± 10% as per the powder/water ratio prescribed by the manufacturer (1:2). Freshly mixed specimens were prepared for each testing procedure.

## 2.3 Chemical analysis of plant extract mixture (GC/MS)

The analysis was done at the Agriculture Research Center, Giza, Egypt using a gas chromatography (GC) (Agilent Technologies 7890A) interfaced with a mass-selective detector (MS) (MSD, Agilent 7000). One milliliter of the PE was diluted in diethylether and injected to analyse its chemical constituents. The GC was equipped with a polar Agilent HP-5ms (5%-phenyl methyl poly siloxane) and a capillary column (30 m, 0.25 mm inner diameter, and 0.25 µm film thickness). The injector and detector temperatures were set at 200 °C and 250 °C respectively. The carrier gas was helium and delivered at a linear velocity of 1 ml/min. Mass spectra were obtained at 70 eV ionization potential, acquisition mass range of 50–800 *m*/*z* in positive mode, and an interface temperature of 250 °C. The quantification of all identified components was investigated using a percent relative peak area. A tentative identification of the compounds was performed based on the comparison of their relative retention time and mass spectra with those of the of the authentic compounds and by computer matching with NIST and WILEY library as well as by comparison of the fragmentation pattern of the mass spectral data with those reported in literature [23].

## 2.4 Agar well diffusion assay

Two gram-positive bacterial strains were used in the current study, *Streptococcus mutans* (DSMZ 20523) and *Micrococcus luteus* (DSMZ 4698).

MH agar plates were inoculated with suspensions of the indicator strains, adjusted to a 0.5 McFarland standard, equivalent to an *E. coli* suspension between $1 \times 10^8$ and $2 \times 10^8$ CFU/ml. After removing the suspension by pipetting, plates were dried for 20 min.

### 2.4.1 Specimens' preparation for antimicrobial testing

The powder and liquid of GIC for each group were mixed with sterile spatulas according to the manufacturer instructions. Nine Petri dishes were used for each bacterial strain. Four wells (5 mm diameter) were prepared in each plate using a sterile cork borer so that each plate could receive the freshly mixed, unset control and modified groups with the different concentrations. Seven specimens were prepared for each group. For monitoring the antibacterial effect of the tested groups, the plates were incubated (Heraeus GmbH & Co. KG, Hanau, Germany) at 37 ± 1 °C for 48 h to allow the microorganisms to grow, and then the diameters of the circular inhibition zones around the samples were measured by using a digital micrometer [5, 24].

## 2.5 Compressive strength

Compressive strength was evaluated according to ISO 9917-1:2007 using cylindrical molds (4.0 mm diameter × 6.0 mm height). Ten specimens were prepared for each group, powder and liquid were mixed according to the manufacturer's instructions (1:2). Then materials were packed into the mold between polyester strips and thick glass plates on both sides to obtain a smooth surface. One hour later specimens were removed from the mold, grinded with silicon carbide paper and stored in deionised water for 24 h. Malformed specimens

or those with voids were discarded. The diameter of each specimen was checked using a digital micrometer gauge (Digimatic, Mitutoyo Europe GmbH, Neuss, Germany). The specimens were then placed in vertical position in a Zwick universal testing machine (Zwick Zmart Pro, ZwickRoell GmbH & Co. KG, Ulm, Germany). Compressive load was applied on the long axis of the specimens at a crosshead speed of 0.5 mm/min until fracture. The maximum force applied when the specimen fractures was recorded to calculate the compressive strength values in MPa [25].

### 2.6 Statistical analysis

All variables are numerical data presented as mean and standard deviation (SD). Normality test Shapiro–Wilk was used to examine whether or not the variables follow a normal distribution. All quantitative variables showed parametric distribution; therefore, One-way analysis of variance (ANOVA) was used for comparison between the groups. Tukey's post hoc test was used for pairwise comparison between the groups when ANOVA test is significant. The significance level was set at $P \leq 0.05$. Statistical analysis was performed using Minitab 17.1.0 for Microsoft Windows.

## 3 Results

### 3.1 Chemical analysis of plant extract mixture

Gas chromatography–mass spectrometry (GC/MS) revealed the presence of 38 volatile and semi-volatile compounds as summarized in (Table 2).

**Table 2** Results of gas chromatography–mass spectrometry analysis

| | Retention time (min) | Compounds | % Area |
|---|---|---|---|
| 1 | 3.448 | 6,7-Dimethyl-4-hydroxycoumarin | 4.78 |
| 2 | 3.907 | 6-Methylchromanone | 0.87 |
| 3 | 4.199 | 3,4,5-Trimethoxycinnamic acid | 0.86 |
| 4 | 4.572 | o-Cymene | 0.63 |
| 5 | 4.633 | α-Pinene | 11.01 |
| 6 | 5.092 | 7-Methoxy-3-(4-methoxyphenyl)coumarin | 1.99 |
| 7 | 5.724 | Limonene | 2.53 |
| 8 | 6.232 | Terpinolene | 1.77 |
| 9 | 6.741 | Myrtenol | 2.46 |
| 10 | 7.163 | p-Mentha-3,8-diene | 1.63 |
| 11 | 7.364 | α-Thujenal | 3.73 |
| 12 | 8.184 | Bornyl acetate | 1.41 |
| 13 | 8.93 | α-Terpineol | 3.02 |
| 14 | 9.25 | α-Selinene | 4.34 |
| 15 | 9.406 | δ-Guaiene | 0.94 |
| 16 | 9.964 | Humulene | 1.18 |
| 17 | 10.099 | Longifolene | 4.2 |
| 18 | 10.23 | γ-Gurjunene | 1.76 |
| 19 | 10.956 | *cis*-Sesquisabinene hydrate | 0.99 |
| 20 | 11.161 | Farnesol | 1.45 |
| 21 | 11.342 | Himbaccol | 2.65 |
| 22 | 13.441 | β-Santalol | 2.28 |
| 23 | 13.667 | Lanceol, *cis* | 4.65 |
| 24 | 13.888 | α-Terpinyl acetate | 2.77 |
| 25 | 13.966 | 3,6,3′,4′-Tetrahydroxyflavone | 2.01 |
| 26 | 14.512 | Kaur-16-ene | 1.41 |
| 27 | 14.815 | Squalene | 4.33 |
| 28 | 14.922 | Ledol | 15.51 |
| 29 | 15.11 | 7,3′,4′,5′-Tetramethoxyflavanone | 2.22 |
| 30 | 15.398 | Quercetin 3′-methyl ether | 2 |
| 31 | 16.566 | p-Cresol, 2,2′-methylenebis(4-methyl-6-tert-butylphenol) | 1.76 |
| 32 | 17.657 | Apigenin 8-C-glucoside | 0.65 |
| 33 | 18.006 | 2′-Hydroxy-2,4,4′,5-tetramethoxychalcone | 0.9 |
| 34 | 18.309 | Juniperol | 1.29 |
| 35 | 18.752 | Isovitexin | 0.17 |
| 36 | 19.814 | 6,2′,3′-Trimethoxyflavone | 1.39 |
| 37 | 22.546 | 7-Hydroxychromanone | 0.94 |
| 38 | 22.878 | 4-Hydroxy-7-methoxy-3-(4-methoxyphenyl) coumarin | 1.56 |

### 3.2 Agar well diffusion assay for antimicrobial activity

#### 3.2.1 Antimicrobial activity against *S. mutans*

The variables showed parametric distribution and thus one-way ANOVA was used to test the antibacterial effect of the plants' extract against *S. mutans* followed by Tukey's post hoc for pairwise comparison between the tested groups (Fig. 1). An ANOVA indicated that there was a statistically significant antibacterial effect of the extract against *S. mutans*, $F\ (4,\ 30) = 63.23$, $P$ value $< 0001$.

Post hoc comparison using Tukey's test indicated that the mean values of the groups CHX-GIC and 2:1 PE (20.2, 20.4 respectively) were statistically significantly higher than the mean values of the remaining groups. Furthermore, there was statistically insignificant difference between the mean values of the groups 1:2 PE (17.6) and 1:1 PE (17.7) though they were statistically significantly higher than the mean of the control group (14.8).

#### 3.2.2 Antimicrobial activity against *M. luteus*

The variables showed parametric distribution and thus one-way ANOVA was used to test the antibacterial effect of the plants' extract against *M. luteus* followed by Tukey's post hoc test for pairwise comparison between the tested groups (Fig. 2). An ANOVA indicated that there was statistically significant antibacterial effect of the extract against *M. luteus*, $F\ (4,\ 30) = 109.87$, $P$ value $< 0001$.

Post hoc comparisons using Tukey's test indicated that the mean value of the group CHX-GIC (27.7) is statistically significantly the highest among the tested groups, followed by the mean value of the group 2:1 PE (25.6) which was

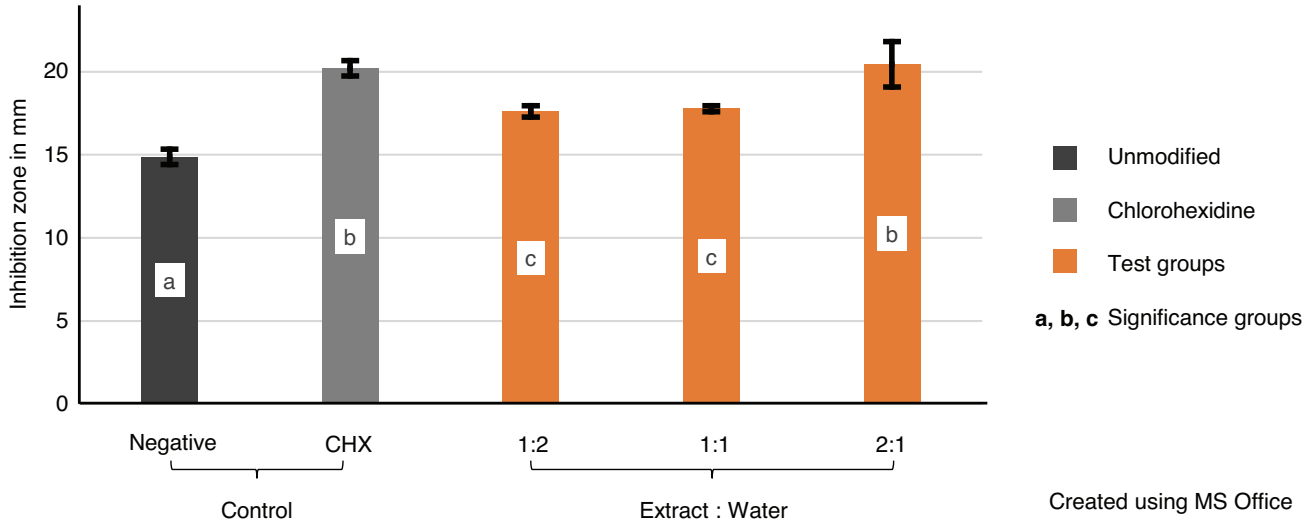


**Fig. 1** Mean values of inhibition zones (mm) showing intergroup comparison against *Streptococcus mutans* and 95% confidence interval of tested groups. Groups that do not share a letter are significantly different

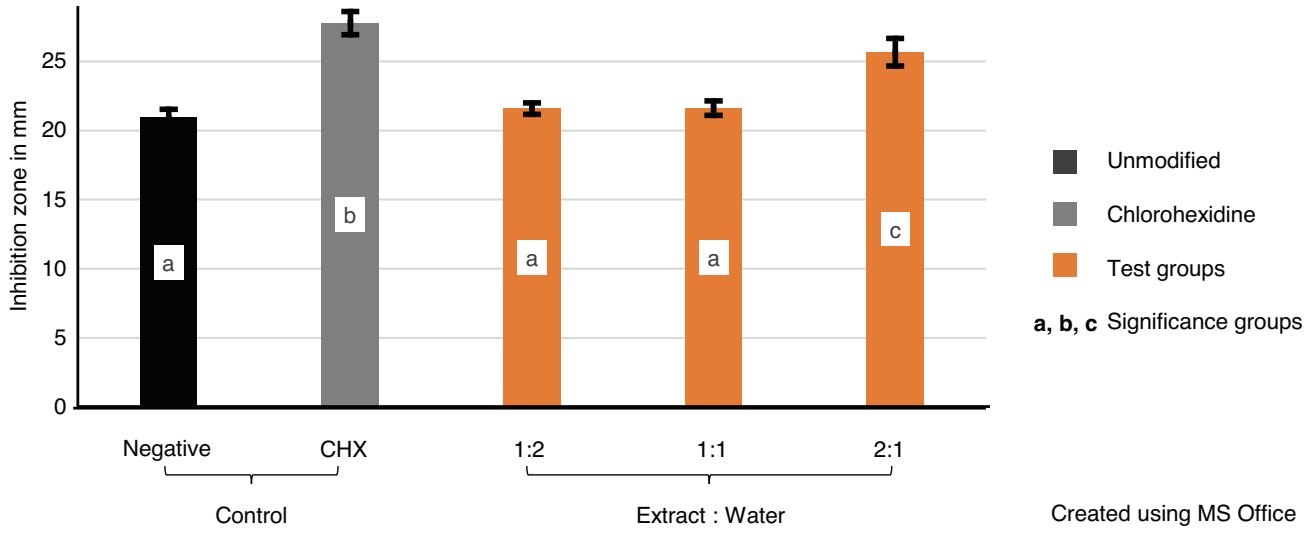


**Fig. 2** Mean values of inhibition zones (mm) showing intergroup comparison against *Micrococcus luteus* and 95% confidence interval of tested groups. Groups that do not share a letter are significantly different

statistically significantly higher than the mean values of the groups control, 1:2 PE and 1:1 PE (20.9, 21.5, and 21.6 respectively).

### 3.3 Compressive strength

The variables showed parametric distribution and thus one-way ANOVA was used to test the compressive strength of the plants' extract followed by Tukey's post hoc test for pairwise comparison between the tested groups (Fig. 3). An ANOVA indicated that there was a statistically significant effect on the compressive strength, $F\ (4,\ 45) = 13.94$, $P$ value $< 0001$.

Post hoc comparisons using Tukey's test indicated that the mean value of the compressive strength of the group 2:1 PE (86.2) was statistically significantly higher than the mean values of all tested groups. Moreover, there was statistically insignificant difference between the mean values of the groups control, CHX-GIC, 1:2 PE and 1:1 PE (63.8, 63, 60.6, and 64.6 respectively).

## 4 Discussion

Numerous studies revealed that incorporation of antibacterial agents in restorative materials has many

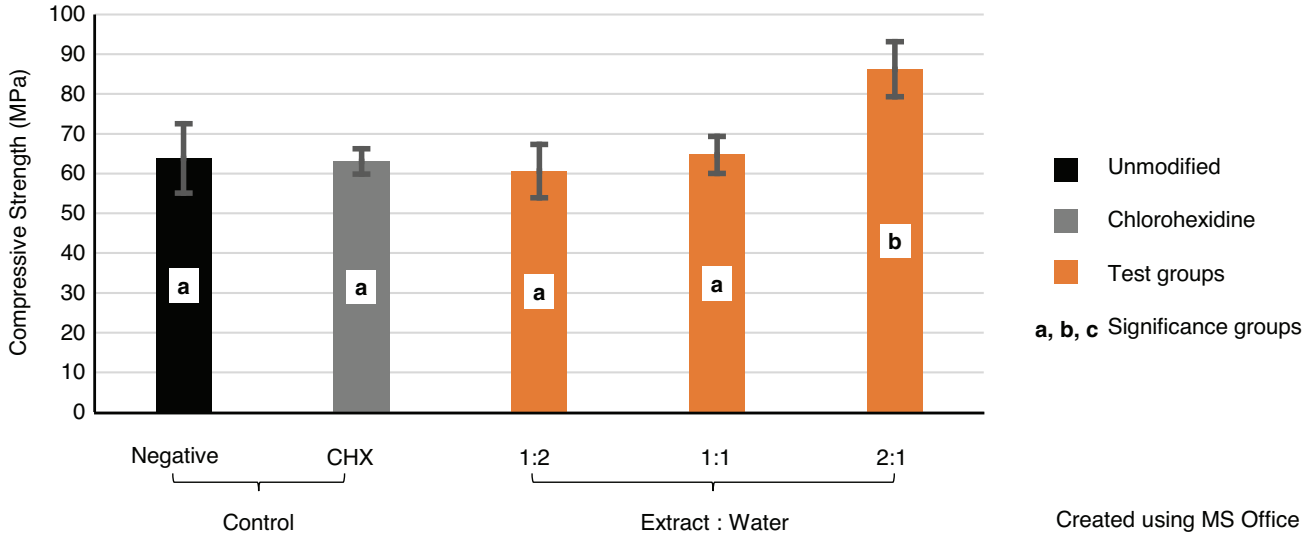


**Fig. 3** Mean compressive strength values (MPa) showing intergroup comparison and 95% confidence interval of tested groups. Groups that do not share a letter are significantly different

therapeutic benefits, but frequently results in impaired physical and mechanical properties. These limitations provoke the need to develop some innovative strategies that can act against dental caries without altering the material properties. One of such strategies is to explore the abundantly available medicinal plants in nature that have proven a profound antimicrobial activity [8, 26].

In the present study, *Salvadora persica, Ficus carcia*, and *Olea europaea* were extracted using alcohol to prepare extract mixtures of the three plants. This extract mixture was used to modify a conventional freeze-dried GIC by adding it to the water used for the final mixture at three different volume ratios of extract to water (1:1 PE, 1:2 PE, 2:1 PE). The extract-modified materials were evaluated and compared with a conventional GIC (Control) and 0.5% CHX-modified GIC (CHX-GIC) with regard to the antimicrobial and compressive strength properties.

Chlorhexidine is a broadband antimicrobial agent with a bactericidal and bacteriostatic effect [27]. The antibacterial effect of CHX is concentration dependent, whereas high concentrations of CHX might negatively affect the physical and mechanical properties of GIC [28, 29]. 0.5% of CHX (w/w) was chosen in the current study to be added to GIC powder, based on studies reporting that this percentage might be the best option for incorporation of CHX into GIC, since antibacterial activity increased and the physical–mechanical properties were not compromised [30–32].

Antimicrobial activity was assessed using the agar well diffusion method against *S. mutans* and *M. luteus*. These microorganisms were chosen because *S. mutans* has been identified as the major causative agent of dental caries, playing a main role in carbohydrate fermentation and acid production [33], whereas M. *luteus* is a very sensitive indicator strain for the release of antibacterial compounds. *M. luteus* was also the most predominant opportunistic pathogen among all isolates from the teeth and gums of children belonging to age group 7–16 years [34].

The agar disc diffusion method, developed in 1940, is one of the most common methods used in many laboratories for routine antimicrobial susceptibility testing. Although not all fastidious bacteria can be tested accurately by this method, standardization has been made to test bacteria like *M. luteus* or Haemophilus influenzae using specific culture media and incubation parameters. Nevertheless, disk-diffusion assay offers many advantages over other methods including simplicity, low cost, the ability to test enormous numbers of microorganisms and antimicrobial agents [35].

Results of agar diffusion assays against *S. mutans* showed that PEs with the different concentrations had a significant effect on inhibition of bacterial growth compared to the control group. Such an effect was more pronounced with increasing the concentration of the extract as in group 2:1 PE that showed the statistically highest inhibition zones compared to groups 1:1 PE and 1:2 PE; see Fig. 1.

These results are in accordance with Ribeiro and Erickson [36] and Botelho [37], who reported that the antimicrobial activity was dependent upon the concentration of the disinfectant added. However, the results contradict Jedrychowski et al. [28] who indicated a no effects of dose response. Moreover, the 2:1 PE cement showed comparable results to CHX-GIC cement with both being significantly the highest among all other tested mixtures.

For *M. luteus*, mean inhibition zones of 2:1 PE plant modified group were significantly higher than the control

group. However, CHX-GIC group was significantly the highest followed by 2:1 PE group compared to the other tested groups, see Fig. 2. This can be explained on the basis that *M. luteus* is very sensitive to chlorhexidine which can be efficiently taken up by the bacteria according to Wendel et al. [38].

The antimicrobial activity might be attributed to the different phytochemical constituents in each of the three incorporated PE. Identification of volatile and semi-volatile compounds in the PE in the current study was made using a combination of two analytical techniques; GC and mass spectrometry. GC can separate compounds with high resolution, but it cannot identify them. Mass spectrometry can provide detailed structural information on most compounds to be accurately identified and quantified after their separation [23].

GC/MS analysis of the extract mixture revealed the presence of 38 compounds including; monoterpenes hydrocarbons (α-pinene, limonene, 0-cymene), monoterpene alcohols (linalool, α-terpineol), and sesquiterpene (Himbaccol, α-Selinene, ledol, Juniperol). All of these terpenoids are thought to cause membrane disruption that is triggered by the lipophilic compounds [39].

Coumarins (6,7-Dimethyl-4-hydroxycoumarin, 7-Methoxy-3-(4-methoxyphenyl) coumarin, 2′-Hydroxy-2,4,4′,5-tetramethoxychalcone coumarin,), Trimethoxycinnamic acid and Phenols (7-Hydroxychromanone and p-Cresol) were also identified. Studies have reported that coumarins as well as phenols exhibited strong antibacterial activity against both Gram-positive and Gram-negative strains by damaging the bacterial cell membrane causing denaturation of protein and affecting cell membrane permeability [40, 41].

Moreover, flavonoids and saponin have been detected at various percentages in the PE. Flavonoids (Isovitexin, 2′-Hydroxy-2,4,4′,5-tetramethoxychalcone, Quercetin 3′-methyl ether and 7,3′,4′,5′-Tetramethoxyflavanone, Apigenin 8-C-glucoside) antimicrobial efficacy is due to inhibition of nucleic acid synthesis and alteration of cytoplasmic membrane function [42]. Whereas, saponin (Squalene, Kaur-16-ene) causes leakage of proteins and certain enzymes from the bacterial cells [43].

Chlorohexidine's mechanism of action was explained by the release of positively charged cationic molecules through the dissociation of CHX salt. These cationic molecules bind to the negatively charged bacterial cell walls where, at low concentrations, the result is bacteriostatic while at high concentrations, membrane disruption occurs resulting in cell death [44].

The clinical success of a material is defined by its ability to withstand the stresses and strains induced during mastication and function. The most commonly used strength value to characterize dental cements is the compressive strength. The minimum compressive strength required according to ISO 9917 (2007) is 50 MPa for base/lining and 100 MPa for restorations. Therefore, it was important to evaluate the compressive strength when modifying the GIC [25].

Compressive strength test was performed after 24 h storage as it is recommended to compare the mechanical properties of GIC between periods of 1 and 24 h or more because their final setting is achieved after 24 h, and they usually present lower strength values during the first hours [45].

Results of compressive strength tests showed insignificant difference between all of the control, 1:1 PE, 1:2 PE, and CHX-GIC groups; see Fig. 3. Such findings are in accordance with Farret et al. [46], Marti et al. [47], and Jaidka et al. [32], who stated that incorporation of antimicrobial agents at certain concentrations did not affect the compressive strength properties of GIC.

However, the current study based on its conditions and findings contradicts with Cefaly et al. [48], Ewoldsen et al. [49], and Sanders et al. [50]. The reduction in compressive strength with antimicrobials in the former studies was attributed to the alteration of the powder/liquid ratio of the mixture and/or interference of antimicrobials with the cross-linking of GIC that occurs by the coordination of $Al^{3+}$ and $Ca^{2+}$with the COOH groups on the acidic polymers, thus decreasing the mechanical properties. Moreover, the majority of antimicrobial agents are added in the form of powders that easily absorb water, decreasing the compressive strength of the GIC. This explanation does not comply with the present study as the PE was added in the form of liquid and CHX powder was added in a very small percentage that did not seem to cause such a problem [49, 50].

Surprisingly, the 2:1 PE group showed a significant improvement in the compressive strength values from the control and the other modified groups; see Fig. 3. This could be explained on the basis of the phytochemical analysis of PEs that revealed the presence of silica in *Salvadora persica* [51]. Lihua et al. [52] and Tjandrawinata et al. [53] proved that addition of silica fillers improves the compressive strength of conventional GIC through the ability of silica to adhere to the matrix by chemical bonding and hence reinforcing the GIC.

Moreover, Cinnamic and bornyl acetic carboxylic acids were identified by GC/MS in the extract. It was assumed that by adding these acids, to glass ionomer liquids, the degree of cross-linking increases together with polysalt bridge formation and subsequently the mechanical properties of the set cement. This was in accordance with Prentice et al. [54], who showed that increasing the concentration of polyacrylic acid with another carboxylic acid considerably reduced the pH, which improves the release of ions from the surface of the glass ionomer powder and increases the rate of cross-linking.

Further studies with respect to other bacterial strains, shear bond strength, and applicability in dental practice are in progress.

## 5 Conclusion

- PEs enhanced the antimicrobial activity of GIC against *Streptococcus mutans,* while their effect against *Micrococcus luteus* was only pronounced at high extract concentrations.
- The compressive strength of GIC was improved by the addition of high concentration of PEs.

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### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.



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## 3.2 Study 2

Evaluation of the flexural strength, water sorption, and solubility of a glass ionomer dental cement modified using phytomedicine

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*Article*

# Evaluation of the Flexural Strength, Water Sorption, and Solubility of a Glass Ionomer Dental Cement Modified Using Phytomedicine

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**Abstract:** Objectives: Various medicinal plant parts and extracts have been proven to be sources of biologically active compounds, many of which have been incorporated in the production of new pharmaceutical compounds. Thus, the aim of this study was to increase the antimicrobial properties of a glass ionomer cement (GIC) through its modification with a mixture of plant extracts, which were evaluated along with a 0.5% chlorohexidine-modified GIC (CHX-GIC) with regard to the water sorption, solubility, and flexural strength. Methods: *Salvadora persica, Olea europaea*, and *Ficus carcia* leaves were prepared for extraction with ethyll alcohol using a Soxhlet extractor for 12 h. The plant extract mixture (PE) was added in three different concentrations to the water used for preparation of a conventional freeze-dried GIC (groups 1:1, 2:1, and 1:2). Specimens were then mixed according to the manufacturer's instructions and tested against the unmodified GIC (control) and a GIC modified with 0.5% chlorhexidine. Water sorption and solubility were evaluated after 7 days of immersion in distilled water. Flexural strength was evaluated in a three-point bending test after 24 h using a universal material testing machine at a crosshead speed of 1 mm/min. One-way analysis of variance (ANOVA) was used for comparison between the groups. Tukey's post hoc test was used for pairwise comparison when the ANOVA test was significant. Results: There were no statistically significant differences between the control (M = 20.5%), CHX-GIC (M = 19.6%), 1:1 (M = 20.0%), 1:2 (M = 19.5%), and 2:1 (19.7%) groups with regard to the percentage of water sorption, while for water solubility the 2:1 (M = −0.39%) plant-modified group was significantly different from all of the other groups. Flexural strength test results showed that the 2:1 group (M = 26.1 MPa) recorded significantly higher mean values compared to all other tested groups. Conclusion and clinical relevance: The plant extracts did not negatively affect the water sorption and solubility of the GIC, while the flexural strength was improved by the addition of the plant extract at higher concentrations.

**Keywords:** conservative dentistry; glass ionomer cement; *Salvadora persica*; *Olea europaea*; *Ficus carcia*

## 1. Introduction

Glass ionomer cements (GICs) belong to the class of materials known as acid-based cements. They are based mainly on three constituents, namely a water-soluble acid, ion-leachable (basic) glass, and water. GICs are commonly presented as an aqueous solution of polymeric acid and a fine glass powder, which are mixed using an appropriate method to form a viscous paste. The resulting paste is a polysalt, whereby the liquid is traditionally a mixture of acids, such as polyacrylic acid, itaconic acid, and malic acid, and the powder (glass) is the base [1]. However, alternative formulations exist, ranging from formulations were the acid is added to the glass and water is used to cause setting,

to formulations in which some of the acid is blended with the glass powder and the rest is present in a dilute solution in water [2,3].

GICs are clinically attractive restorative and luting materials in different therapeutical applications in dentistry, despite the name could simply classify them as dental materials for cementation [4] Owing to their unique properties such as chemical adhesion to tooth structures and base metals, thermal compatibility with enamel, bio-compatibility and low toxicity they are largely used in dentistry, as filling materials or base materials, or alternatively to other glass materials [5,6]. However, the relatively low fracture and wear resistances are two of the major drawbacks of GICs when being compared to modern resin composite materials [7].

The durability of restorative materials is influenced by many variables, such as the hardness, water sorption, and solubility. More specifically, water sorption can change the volume of a material and can cause deterioration of the matrix structure by acting as a plasticizer [8]. The solubility of a cement has an impact on its longevity, stability, and biocompatibility. The rate of dissolution of a cement is not only affected by the testing conditions, but also by the specimen shape and thickness, powder/liquid ratio of the cement, pH, dissolution time, and concentration of the solute [9].

Moreover, flexural strength is a commonly evaluated mechanical property in GICs, which can be defined as "the ability of the material to resist deformation under load" [10]. The three-point flexural test is regarded as a simulation of a clinical situation involving the forces applied by the opposing cusp [11].

There is no perfect antibacterial filling material at the moment, and although glass-ionomers are smart materials because of their fluoride release and cariostatic properties, they are still far from optimal with regard to their antibacterial activity [12,13]. In several studies, the incorporation of antimicrobials (antibiotics, chlorhexidine, zinc) into GICs was investigated, however conflicting results were obtained relating to the influence on the physical–mechanical properties of the GICs [14–16].

Natural products have been used since ancient times in naturopathic medicine. Furthermore, some plant parts and extracts have been broadly used for prevention or treatment of oral diseases in dentistry [17]. Phytomedicines offer effective and promising alternatives to antibiotics for various dental diseases. They have an edge over conventional antibiotic treatments by having a great benefit-to-risk ratio [18]. Many studies have investigated these plants thoroughly, although there are approximately 500,000 plant species worldwide, of which only 1% have been phytochemically investigated [19,20].

Among the plants that show beneficial activity are *Olea europaea, Salvadora persica*, and *Ficus carcia*. *Olea europaea (O. europaea)* (the botanical name of the olive tree, which is native to Mediterranean Europe, Asia, and Africa) [21]. Different percentages of oleuropein (OL), hydroxytyrosol (HT), verbascoside, apigenin-7-glucoside, and luteolin-7-glucoside have been detected in olive leaf extracts. Both OL and HT have been shown to have anti-oxidant and antimicrobial properties [22].

*Salvadora persica (S. persica)* (also called the "toothbrush tree") grows in the Middle East, Asia, and Africa, and is commonly used to make miswaks [23]. The tradition of using a miswak to clean the oral cavity is a part of the Greek–Arab system of medicine and is a centuries old practice. The use of miswaks for oral hygiene serves dual functions: it acts mechanically through friction between plant fibers and the tooth surface and chemically through its unique chemical composition [24].

*Ficus carcia (F. carcia)* is commonly known as the Anjir (India), fig tree (UK), or teen (Arabic). It supposedly originated from Western Asia and was spread to the Mediterranean by humans [25]. *F. carcia* leaves contain numerous active compounds, such as flavonoids, alkaloids sesquiterpenes, and saponins. These active constituents possess different antioxidant, anticancer, anti-inflammation, antiviral, and antibacterial activities [26].

When adding materials such as CHX salts or plant extracts to GICs, it is important to consider the effects that these additives may have on the mechanical and physical properties; the higher the concentration of additives, the greater the likelihood of an adverse effect [27]. Accordingly, in the present study, a conventional GIC was modified with an extract mixture of *Ficus carcia*, *Salvadora persica*, and *Olea europaea* at three different mass ratios with the aim of increasing the antimicrobial activity.

The effects of the modification on the water sorption, solubility, and flexural strength was evaluated by comparing the three plant-modified groups with an unmodified negative control group and a positive control group containing 0.5% CHX.

## 2. Materials and Methods

### *2.1. Preparation of the Plant Extract Mixture*

*O. europaea*, *F. carcia*, and *S. persica* plants were used to prepare the extracts. Each of these plant parts was washed, dried, ground into a powder, and added to the thimble of a Soxhlet extractor (Carl Roth GmbH + Co. KG, Karlsruhe, Germany) (Figure 1). The extractions were performed using ethyl alcohol for several hours. The extraction products were then filtered and proportioned to prepare a mixture containing them all. The plant mixture was then placed at 37 °C in a rotary evaporator (Buchi Rotavapor R-300, Buchi Labor Technik GmbH, Essen, Germany; Figure 2) to remove the ethanol, leaving a crude mixture that was stored in a fridge in a closed flask at 4 °C until use [28].

**Figure 1.** Plant extraction using alcohol in a Soxhlet extractor.

**Figure 2.** Evaporation of alcohol using a rotary evaporator.

### *2.2. Preparation of GIC, Extract and CHX Combinations and Specimen Grouping*

The prepared plant extract mixture (PE) was added to the distilled water used for preparation of conventional GICs (Medicem aqua, Promedica GmbH, Neumuenster, Germany, Lot 1849261) at three different extract-to-water ratios (1:1, 2:1, 1:2), then mixed using a vortex mixer (CATVM4,

Ingenieurbüro CAT, M. Zipperer GmbH, Ballrechte-Dottingen, Germany) to obtain a homogeneous mixture. Each concentration was stored in a sterile bottle using the exact nozzle size supplied by the manufacturer so as not to alter the original powder/liquid ratio. Each prepared liquid (plant extract + distilled water) was mixed with the glass ionomer powder component at the ratio prescribed by the manufacturer (1:2). The modified materials were grouped according to the ratio of extract added to the water, yielding three groups, then compared with a non-modified glass ionomer cement (GIC) as a negative control group and to an 0.5% CHX diacetate (*w*/*w*) (Merck KGaA, Darmstadt, Germany)-modified GIC as a positive control.

Specimen Grouping

The groups included the control (un-modified conventional GIC), 0.5% CHX-modified glass ionomer cement (CHX-GIC) group, and plant-modified groups (extract: water): 1:2, 1:1, 2:1.

### *2.3. Water Sorption and Solubility*

In total, 50 samples of 7 mm diameter and 2 mm thickness were prepared using a Teflon mold ($n$ = 10). For each group, the material was mixed according to the manufacturer's instructions and placed inside the mold on a glass slab. A polyester matrix strip was placed over the cement surface and gently pressed with a glass slide until the mixture had set. Malformed specimens or those with voids were discarded. After one hour, specimens from each group were stored in a desiccator with silica gel (Merck KGaA, Darmstadt, Germany) for 2 h and then incubated in an oven at 37 °C for 22 h, aiming to reach constant mass, with a maximum weight difference of ±0.0005 g. Specimens were weighed on a precision analytical balance instrument (JP105DUG, Mettler-Toledo GmbH, Giessen, Germany) to obtain the initial mass (m1) values.

Each specimen was then immersed in a glass bottle containing 25 mL deionized water and was labelled for identification (Figure 3). The bottles containing the specimens were placed at 37 ± 1 °C for 7 days in an incubator. Afterwards, each specimen was taken out of the water, dried gently with a cotton pellet, and weighed again to obtain the mass values of the specimens after immersion (m2) (Figure 4). The samples were then dehydrated in an incubator at 37 ± 1 °C for 24 h and weighed for the last time to record the final mass after dehydration (m3).

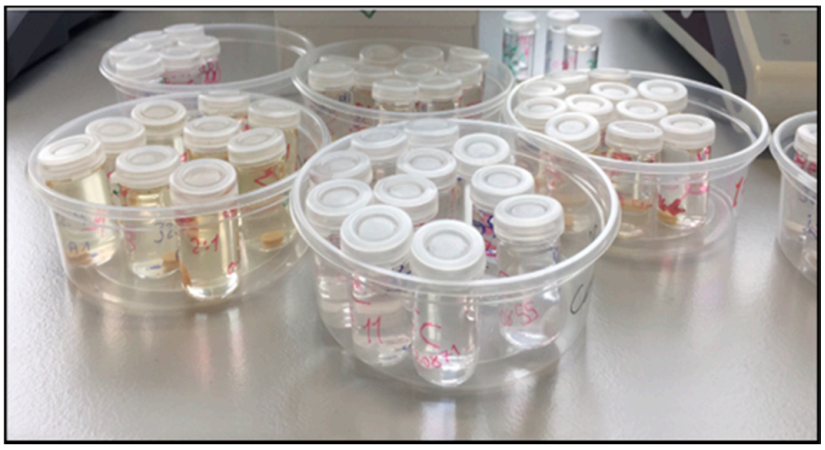

**Figure 3.** Specimens immersed in distilled water for 7 days.

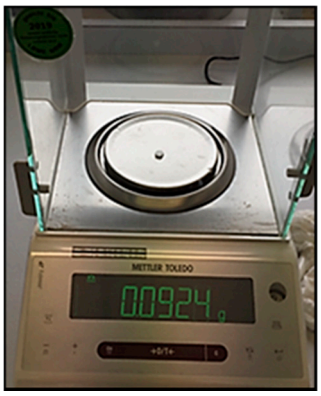


**Figure 4.** Mass determination using sensitive balance.

The amount of water sorption was calculated from the difference between the initial mass and the wet mass ($m_2 - m_1$). The loss of material (solubility) was obtained from the difference between the initial and final drying mass values of each specimen ($m_1 - m_3$). The percentages of water sorption (Wsp) and solubility (Wsol) for each sample were calculated using the following equations [29]:

$$W_{sp} = 100 \cdot \frac{m_2 - m_1}{m_1},$$
$$W_{sol} = 100 \cdot \frac{m_1 - m_3}{m_1}$$

*2.4. Flexural Strength*

The flexural strength (Fs in MPa) was measured according to ISO 9917-2 using $25 \times 2 \times 2$ mm$^3$ rectangular molds ($n$ = 10). Specimens were mixed according to the manufacturer's instructions, placed inside the molds, covered with a polyethylene strip, then allowed to set. Ten minutes after setting, the specimens were removed from the molds and stored at 37 °C in a highly humid environment for 24 h. Specimens containing any voids or imperfections were discarded. The height and width of the specimens were checked using a digital micrometer to an accuracy level of 0.001 mm. The specimens were then subjected to a three-point bending test, with the distance between the two supports set at 20.0 mm [30]. The setup was integrated into a material testing machine (Zwick Zmart Pro, Zwick Roell GmbH & Co. KG, Ulm, Germany) at a crosshead speed of 1.0 mm/min (Figure 5). The **Fs** was calculated according to the following equation:

$$\mathbf{Fs} = \frac{\mathbf{3 \cdot F \cdot l}}{\mathbf{2 \cdot w \cdot h^2}}$$

where **F** is the load at fracture, **l** is the distance between the supports (20.0 mm), **w** is the specimen width, and **h** is the specimen height [31].

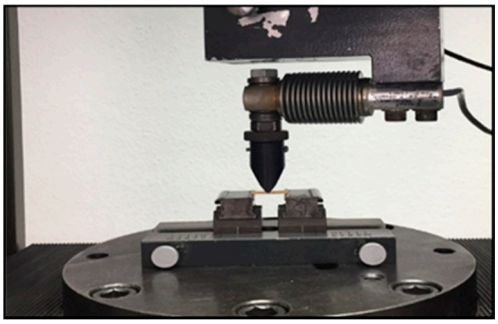

**Figure 5.** Zwick testing machine measuring flexural strength.

### *2.5. Statistical Analysis*

All variables are numerical data presented as the mean (or as the median in cases where non-parametric distribution was observed) and standard deviation (SD). The Ryan–Joiner normality test (similar to Shapiro–Wilk test) was used to examine whether or not the variables followed a normal distribution. Water sorption and solubility showed a parametric distribution. Therefore, one-way analysis of variance (ANOVA) was used for comparison between the three groups. Tukey's post hoc test was used for pairwise comparison between the groups when an ANOVA test was significant. The significance level was set at $p \leq 0.05$.

Observations of the flexure strength showed a non-parametric distribution, thus the Kruskal–Wallis test was used for comparison between the groups, followed by Dunn's post hoc test for pairwise comparison. Statistical analysis was performed using Minitab 17.3.1 for Microsoft Windows (Minitab, Inc., State College, PA, USA).

## 3. Results

### *3.1. Water Sorption and Solubility*

#### 3.1.1. Water Sorption

The analysis of variance (ANOVA) indicated that there was a statistically insignificant difference in the water solubility between the groups ($p$-value = 0.908). The results are illustrated graphically in Figure 6 and the statistics are shown in Table 1. The control group had a mean value of 20.5%, while the CHX-GIC group had a mean value of 19.6%. The mean values for the plant-modified groups at ratios of 1:2, 1:1, and 2:1 were 19.5%, 20.0%, and 19.7% respectively.

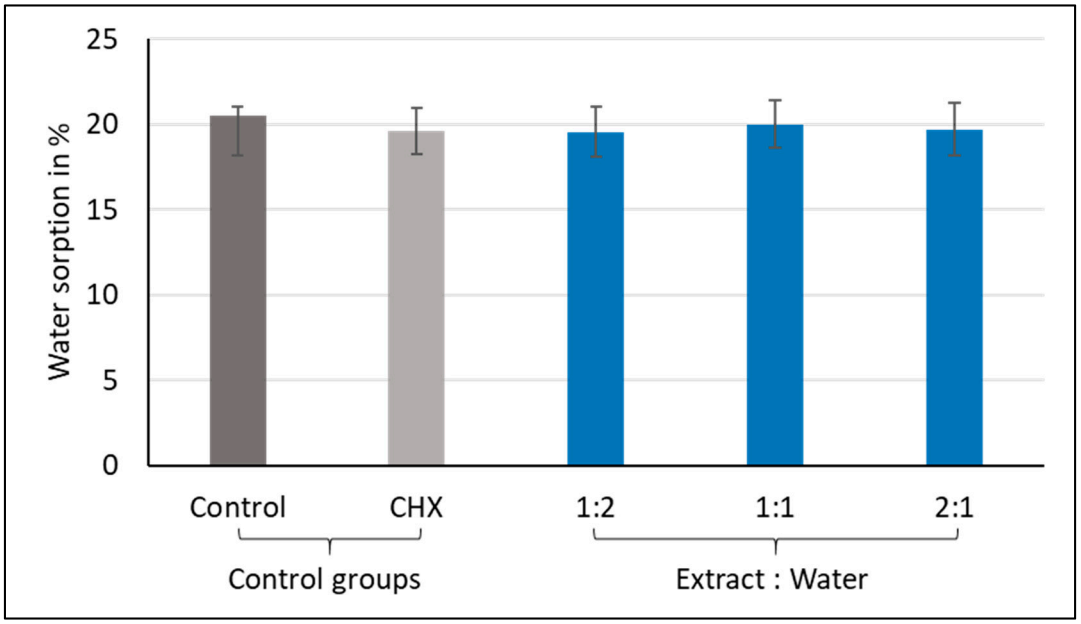


**Figure 6.** Mean water sorption values (%) showing intergroup comparison of the control, CHX, and three plant-modified groups (1:2, 1:1, 2:1).

**Table 1.** Results of ANOVA test for water sorption of control, CHX, and three plant-modified groups (1:2, 1:1, 2:1).

| Group | N | Mean % | St Dev | *p*-Value * |
|---|---|---|---|---|
| Control | 10 | 20.5 | 2.7 | 0.908 |
| CHX-GIC | 10 | 19.6 | 1.2 | |
| 1:2 | 10 | 19.5 | 2.6 | |
| 1:1 | 10 | 20.0 | 1.3 | |
| 2:1 | 10 | 19.7 | 4.3 | |

* Significant at $p \leq 0.05$.

### 3.1.2. Water Solubility

The analysis of variance (ANOVA) indicated that there were statistically significant differences in the water solubility between the groups, with *p*-values < 0.001. The results are illustrated graphically in Figure 7 and the statistics are shown in Table 2. The 2:1 group had the lowest negative mean value (M = −0.3%), which was significantly different from all the other tested groups, followed by the 1:1 group (M = −2.4%), then the CHX-GIC group (M = −3.5%). On the other hand, group 1:2 (M = −5.1%) and the control group (M = −4.9%) showed comparable results, with group 1:2 recording the highest negative mean value.

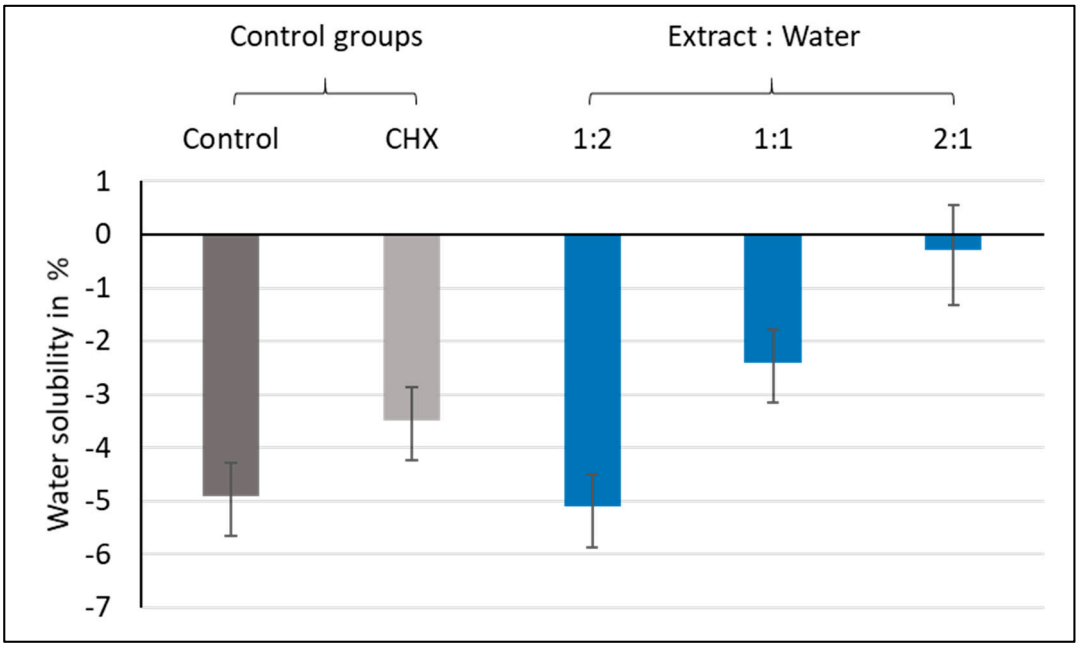


**Figure 7.** Mean water solubility values (%) showing intergroup comparisons of the control, CHX, and three plant-modified groups (1:2, 1:1, 2:1).

**Table 2.** Results for the ANOVA and Tukey post hoc test for water solubility values for the control, CHX, and three plant-modified groups (1:2, 1:1, 2:1).

| Group | N | Mean % | St Dev | *p*-Value * | Pairwise Comparison ** | | |
|---|---|---|---|---|---|---|---|
| Control | 10 | −4.9 | 1.5 | <0.001 | A | | |
| CHX-GIC | 10 | −3.5 | 1.0 | | | B | |
| 1:2 | 10 | −5.1 | 0.6 | | A | | |
| 1:1 | 10 | −2.4 | 0.9 | | | BC | |
| 2:1 | 10 | −0.3 | 1.4 | | | | D |

* Significant at $p \leq 0.05$. ** Groups that do not share a letter are significantly different.

### 3.2. Flexural Strength

The variables showed non-parametric distribution, and thus the Kruskal–Wallis H test was used to test the effects of the plant extract mixture on the flexural strength. The results are illustrated graphically in Figure 8 and the statistics are shown in Table 3. The Kruskal–Wallis H test indicated that there was a significant effect of the plant extract mixture on the flexural strength (H (4) = 28.48, *p*-value < 0.001). Post hoc comparisons using Dunn's test indicated that group 2:1 had the highest median flexural strength (M = 26.1 MPa), followed by group 1:1 (M = 19.6 MPa), while group 1:2 showed the lowest median flexural strength (M = 11.5 MPa). Moreover, group 2:1 was statistically different from the control (M = 11.8 MPa), CHX-GIC (M = 15.3 MPa), and 1:2 (M = 11.5 MPa) groups.

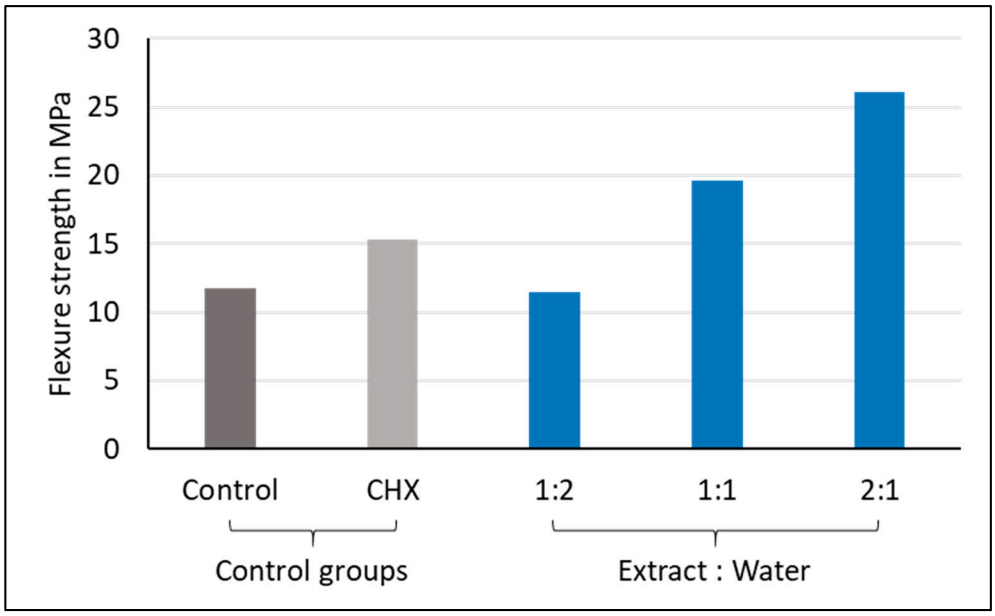


**Figure 8.** Median flexural strength values (MPa) showing intergroup comparisons of the control, CHX, and three plant-modified groups (1:2, 1:1, 2:1).

**Table 3.** Results of Kruskal–Wallis H test and pairwise comparison for the flexural strength values of the control, CHX, and three plant-modified groups (1:2, 1:1, 2:1).

| Variable | N | Median (MPa) | IQR (MPa) | *p*-Value * | Pairwise Comparison ** | | |
|---|---|---|---|---|---|---|---|
| Control | 10 | 11.8 | 5.1 | <0.001 | A | | |
| CHX-GIC | 10 | 15.3 | 10.3 | | A | B | |
| 1:2 | 10 | 11.5 | 5.9 | | A | B | |
| 1:1 | 10 | 19.6 | 4.6 | | | B | C |
| 2:1 | 10 | 26.1 | 9.0 | | | | C |

* Significant at $p \leq 0.05$. ** Medians that do not share a letter are significantly different.

## 4. Discussion

The great potential for exploring natural anti-microbial compounds came from the increasing resistance of many pathogens to currently used agents, such as antibiotics and antiviral agents [32]. Although many natural antibacterial agents have shown effective results against cariogenic salivary flora when used in mouthwashes or toothpastes, there is still a lack of data regarding the effects of their addition on the properties of glass ionomer cements [33,34].

In the present study, an alcoholic extract mixture of *Salvadora persica*, *Ficus carcia*, and *Olea europaea* was prepared using a Soxhlet extractor. The extract mixture was then used to modify a conventional freeze-dried GIC by adding it to the water used for cement preparation at three different extract-to-water mass ratios (1:1, 1:2, 2:1). The extract-modified materials were evaluated and compared with an unmodified GIC (Control) and 0.5% CHX-modified GIC (CHX-GIC) with regard to the water sorption, solubility, and flexural strength.

Although CHX is considered the gold standard for antibacterial applications, its incorporation in GICs frequently results in changes in the physical and mechanical properties [14,35]. This is probably the reason why the combination of chlorhexidine and other antibacterial substances with GICs has still not been incorporated into their production. Here, 0.5% of CHX (*w*/*w*) was chosen in the current study to be added to the GIC powder, based on reports that stated that this percentage might be the best option, since the antibacterial activity increased and the physical–mechanical properties were not compromised at this percentage [36–38].

### *4.1. Water Sorption and Solubility*

Water sorption and solubility are critical parameters in the evaluation of bonding materials and are directly related to the longevity of a cement. Water sorption tests actually measure the net gain in weight of a specimen resulting from diffusion of water molecules and elution of monomers and other small molecules [39].

Two well-known theories could explain the diffusion of water through polymeric materials: one is the free volumetric theory, whereby the water diffuses through microvoids without any mutual relationship with the polar molecules in the material. The other theory is called the interaction theory, whereby water diffuses through material to bind successively to the hydrophilic groups. If there is a negative correlation between the diffusion and equilibrium water uptake, the latter pattern of diffusion supposedly occurs [39].

Initially within GICs, the water sorption process transports calcium and aluminum ions to react with polyacrylic acid. However, over time, excessive water uptake can cause deterioration and degradation of the cement, resulting in impaired structural and mechanical properties [40]. In the present study, the water sorption and solubility were measured after immersion of the specimens for 7 days, because in several previous studies it was stated that the maximum amount of water gain occurs within the first week in most hydrophilic materials [41,42].

For water sorption, all of the tested groups showed water gain at the end of the immersion period. The hydrophilicity of a polymer is determined by its chemistry, polymerization linkages, and the presence of hydroxyl, carboxyl, or phosphate groups, which make them more hydrophilic and more prone to water sorption. Moreover, crack lines were also observed in many specimens of the different groups, which might be partly responsible for the high water sorption values. Kucukyilmaz et al. in 2016 investigated the microleakage scores of GICs and concluded that the observed crack areas and lines in many of the samples resulted in higher rates of dye penetration, which might be the reason for the variations in values from earlier studies [43].

Moreover, the results showed no statistically significant differences between mean values for the control (M = 20.5%), CHX-GIC (M = 19.6%), and the three extract modified groups (1:1, M = 20.0%; 1:2, M = 19.5%; 2:1, M = 19.7%) (see Figure 6). A possible explanation is that there is no variation in their chemical composition, as the tested groups are all basically conventional GICs, and consequently there were no differences in their water sorption capacity values either [44].

Solubility is the ability of a substance to dissolve in another substance, expressed as the concentration of saturated solution of a solvent in a dissolvent [45]. For water solubility, all of the tested groups showed negative mean values (control, M = −4.9%; CHX-GIC, M = −3.5%; 1:1, M = −2.4%; 1:2, M = −5.1%; 2:1, M = −0.3%) (see Figure 7). Negative solubility values may be attributed to incomplete dehydration of these materials, which does not mean that no solubility occurred in these materials, but may hint to their solubility. Negative values were also reported by Toledano et al. in 2006, Keyf et al. in 2007, and Sinthawornkul at el. in 2017 [46–48]. An explanation could be that the acid–base reaction was prolonged and water molecules were continuously bonded into their structures. Therefore, the materials gained weight and expanded [48]. On progression of the acid–base reaction, the GIC takes up water as an integral part of its structure; therefore, the longer the rate of the reaction, the greater the water uptake into the structure of the cement, and vice versa [46].

In addition, there were statistically significant differences among the tested groups—the control and 1:2 groups showed the highest negative mean values, followed by the CHX-GIC and 1:1 groups, while the 2:1 group showed the lowest negative mean values. Such differences could mean that the water molecules did not bond to the structure equally in all groups after the acid–base reaction ended. Therefore, some of the absorbed water molecules were either only trapped in the space of the matrix, filler, or matrix–filler interface. Then, this loosely bonded water was vaporized out of the sample after drying in the desiccator [40].

### *4.2. Flexural Strength*

Flexural strength was chosen for evaluation because it is more sensitive to small changes in a material's structure than the compressive strength and allows the clinical loading situation to be mimicked by giving an appropriate estimate of the tensile strength of a material [49]. However, it is difficult to prepare the beam specimens required for the test without flaws or cracks [50].

Flexural strength was measured according to ISO 9917-2. The test was performed after 24 h of storage, as a GIC's final setting and strength are achieved after 24 h, and they usually present lower strength values during the first hours [50,51]. The values demonstrated in the present study were comparable to the results presented by Kutuz et al. in 2019 [52] and Sajjad et al. in 2019 [53]. The results showed that the plant extract enhanced the flexural strength of the GIC, with the 2:1 group (M = 26.1 MPa) having the highest median flexural strength, which was statistically different from the control (M = 11.8 MPa), CHX-GIC (15.3 MPa), and 1:2 groups (11.5 MPa) (see Figure 8). Moreover, his effect was found to be concentration-dependent, whereby the 2:1 group yielded the highest flexural strength value, followed by the 1:1 group (M = 19.6 MPa); both were significantly different from the control, CHX-GIC, and 1:2 (lowest extract concentration) groups.

This was explained through the chemical analysis of the plant extract, which revealed the presence of cinnamic and bornyl acetic carboxylic acids. It is expected that by adding these acids to glass ionomer liquids, the degree of cross-linking increases, together with polysalt bridge formation,

which subsequently strengthen the mechanical properties of the cement. This is in accordance with Prentice et al., who showed in 2006 that lowering the pH by increasing the concentration of polyacrylic acid with another carboxylic acid improves the release of ions from the surface of the glass ionomer powder and increases the rate of cross-linking [54]. Another explanation that was suggested by Yup et al. in 2001, Behr et al. in 2006, and Moshaverinia et al. in 2008 is that the extract could affect the amount of unreacted powder particles within the matrix, which may act as reinforcing fillers, impeding crack propagation within the cement [55–57].

The test and investigation were done in vitro, but the results greatly contribute to the understanding of the concept of GICs as smart materials rather than as polymeric resin composite materials in dentistry. It remains to be seen if the addition of alcoholic molecules from plants can improve or decrease the wettability of dental tissues in vivo. The adhesion of these materials clinically depends on the linking of COO- -to CA++ in the tooth. However, the drying conditions following alcohol application can critically modify this adhesion and can lead to failure of the restoration, which depends not only on the antibacterial properties of the material itself (GIC), but also on the strength of the bond (gap-free) achieved between the dentine or enamel and the GIC [58].

The limitation of this study is that the testing conditions did not simulate the exact clinical situations. Factors such as the clinical conditions, degree of moisture contamination, powder–liquid ratio, mixing technique, manufacturer, and the batch of the luting cement usually affect the physical and mechanical behaviours of dental cements [59]. In addition, in order to evaluate the flexural strength, only static forces are considered, but not the complex dynamic forces that result in restoration in the oral cavity. However, our in vitro results are important for screening of the sorption, solubility, and flexural strength after the modification of GICs with plant extracts. Other variables could alter the results of the present report. In fact, acidic environments [60] and wear [61] can alter the surface characteristics of dental materials. Therefore, in the future, further studies are needed on the topic.

## 5. Conclusions

Within the context of this study, it can be concluded that the addition of a plant extract mixture in an attempt to improve the antimicrobial properties of the GIC enhanced the flexural strength, without compromising water sorption and solubility behavior of the GIC.

**Author Contributions:** Conceptualization, L.S. and C.B.; methodology, L.S. and C.B.; investigation, L.S. and K.K.; resources, C.B.; writing—original draft preparation, L.S.; writing—review and editing, visualization, L.S., C.B., G.B., and K.K.; supervision, C.B. and G.B. All authors have read and agreed to the published version of the manuscript.

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**Conflicts of Interest:** All authors declare that there is no conflict of interests in this study.

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## 3.3 Study 3

Shear bond strength and film thickness of a
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# Shear Bond Strength and Film Thickness of a Naturally Antimicrobial Modified Dental Luting Cement

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**Abstract:** Although several natural plants and mixtures have been known and used over the centuries for their antibacterial activity, few have been thoroughly explored in the field of dentistry. Thus, the aim of this study was to enhance the antimicrobial activity of a conventional glass ionomer cement (GIC) with natural plant extracts. The effect of this alteration on the bond strength and film thickness of glass ionomer cement was evaluated and related to an 0.5% chlorohexidine modified GIC. Olive leaves *(Olea europaea)*, Fig tree (*Ficus carica)*, and the leaves and roots of Miswak (*Salvadora persica)* were used to prepare an alcoholic extract mixture. The prepared extract mixture after the evaporation of the solvent was used to modify a freeze-dried glass ionomer cement at three different extracts: water mass ratios 1:2, 1:1, and 2:1. An 0.5% chlorhexidine diacetate powder was added to a conventional GIC for the preparation of a positive control group (CHX-GIC) for comparison. The bond strength to dentine was assessed using a material-testing machine at a cross head speed of 0.5 mm/min. Failure mode was analyzed using a stereomicroscope at 12× magnification. The cement film thickness was evaluated in accordance with ISO standard 9917-1. The minimum number of samples in each group was $n$ = 10. Statistical analysis was performed using a Kruskal–Wallis test followed by Dunn's post hoc test for pairwise comparison. There was a statistically insignificant difference between the median shear bond strength ($p$ = 0.046) of the control group (M = 3.4 MPa), and each of the CHX-GIC (M = 1.7 MPa), and the three plant modified groups of 1:2, 1:1, 2:1 (M = 5.1, 3.2, and 4.3 MPa, respectively). The CHX-GIC group showed statistically significant lower median values compared to the three plant-modified groups. Mixed and cohesive failure modes were predominant among all the tested groups. All the tested groups ($p$ < 0.001) met the ISO standard of having less than 25 µm film thickness, with the 2:1 group (M = 24 µm) being statistically the highest among all the other groups. The plant extracts did not alter either the shear bond strength or the film thickness of the GIC and thus might represent a promising additive to GICs.

**Keywords:** medicinal plants; dental luting cement; shear bond strength; film thickness



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## 1. Introduction

During the 19th century, amalgam and gold were commonly used as restorative materials in dental treatments. Nevertheless, their unsatisfactory color created the need for more aesthetically acceptable dental cements and restorative materials [1]. Glass ionomer cements (GIC) were one of the crucial steps in this direction and have become one of the most commonly used restorative materials in dentistry [2,3] GICs are byproducts of an acid–base reaction between weak polyacrylic acids and aluminosilicate glass powder. The set cement contains unreacted glass particles which play a role in in reinforcing the final cement structure [4,5]. Glass ionomer-based cements are the material of choice for cementation, liners, bases, atraumatic preventive treatments, and restoring cervical dental lesions [6,7]. They have the advantage of forming a chemical adhesion with the tooth structure, thus requiring minimal preparation, fluoride release, biocompatibility, antimicrobial activity, recharge ability, and reverse potential to reduce the acidic environment [8–10].

The success of dental materials clinically depends on many factors, among which is the good adhesion to the surface of the tooth to resist various dislodging forces [11]. Shear

bond strength is known as the resistance to dislodging forces, which causes the sliding of the restorative material against the tooth structure. It adopts much importance to the dentist clinically because it has been proven that the major dislodging forces at the tooth restoration interface have a shearing effect [12,13].

Along with the mechanical properties for the selection of a suitable and durable luting agent, there are other clinically related properties that need to be taken into consideration, such as the film thickness [14]. During cementation, achieving a minimum film thickness is very important for the complete seating and adaptation of the prosthetic restorations. Moreover, a thin film thickness decreases the marginal discrepancies, cement dissolution, plaque accumulation, and periodontal disease [14,15].

The use of herbal products is increasing at an exponential rate in both developing and developed countries owing to the free availability, religious beliefs, as well as unique chemical composition [16]. This novel branch has its roots in ancient medicine and the pre-antibiotic era. Herbal extracts were claimed to have the advantage of showing their beneficial effects without the risk of developing microbial resistance. Nowadays, several herbal products are available in the market in different forms, such as toothpastes, oral gels, and mouth rinses [17–19].

*Salvadora persica (S. persica)* is a small tree that belongs to the family Salvadoracea and is commonly known as miswak (toothbrush) tree. Studies of miswak against oral bacteria such as *Streptococcus mutans, salivaris, Staphylococcus aureus*, and *mitis* have proven that the crude extract was significantly effective, with an inhibition zone production of 67 and up to 96% [20,21].

*Ficus carica (F. carcis)* belongs to the family *Moraceae and* is commonly referred to as "Fig". Several authors have claimed that *F. carica* has antioxidant, antiviral, antibacterial, hypocholesterolemia, hypoglycemic, cancer-suppressive, and hypotriglyceridemic effects [22,23].

*Olea europaea (O. europaea)* leaves and olive fruits have an ancient history of therapeutic and traditional practices. The olive tree, leaves, and extracts are an essential part of the Mediterranean culture due to olive polyphenols. Olive leaf polyphenols have been thoroughly investigated because of their anti-inflammatory and antimicrobial activities and anti-hypertensive, anti-diabetic, anti-carcinogenic, and anti-atherosclerotic potentials [24,25].

While many studies support the notion of the protective effect of fluoride in public water and oral health products, the available data still do not endorse the anti-caries ability of fluoride-releasing restorative materials such as GIC [26,27]. Based on the ability of GIC to participate in ion-exchange reactions with the oral environment, many modifications have been carried out to improve its antimicrobial properties [26,27].

In earlier study, an extract mixture of *S. persica, F. carcia,* and *O. europaea* incorporated in a conventional GIC showed a significant antimicrobial activity against *Streptococcus mutans* and *Micrococcus luteus*. Moreover, the chemical characterization of the extract mixture using GC/MS has shown many chemically active compounds, including phenols, flavonoids, alkaloids, carboxylic acids, terpenes, and more [28].Despite the recommendations for the use of these herbal plant extracts, there are only a few available studies that involve the addition of natural herbal extracts to GIC. Additionally, the antimicrobial effects were the focal point of these studies, while the physical-mechanical properties have been overlooked. Thus, the aim of this study was to evaluate the shear bond strength and film thickness of a GIC modified with a natural plant extract, while a 0.5% CHX-modified GIC (positive control) and an unmodified GIC were used for comparison. The null hypotheses were there will be no significant difference between the extract-modified groups, the CHX-modified group, and the control with regard to shear bond strength, failure mode analysis, and film thickness.

## 2. Results and Discussion

### *2.1. Results*

#### 2.1.1. Shear Bond Strength

The variables showed a non-parametric distribution and thus the Kruskal Wallis H test was used to test the effect of the plant extract on the shear bond strength. The results are shown in Table 1 and illustrated graphically in Figure 1. The Kruskal Wallis H test indicated that there was significant difference between the groups, $p = 0.046$. Post hoc comparisons using Dunn's test showed significant differences between the CHX-GIC shear bond strength (M = 1.7 MPa) and the modified groups 1:2, 1:1 and 2:1. However, there were insignificant differences between the control groups and all other groups.

**Table 1.** Results of the Kruskal Wallis H test for shear bond strength.

| Groups | *n* | Median (MPa) | Interquartile Range | *p* * | Pairwise Comparison ** | |
|---|---|---|---|---|---|---|
| Control | 20 | 3.4 | 3.0 | | A | B |
| CHX | 18 | 1.7 | 1.4 | | A | |
| 1:2 | 21 | 5.1 | 7.5 | 0.046 | | B |
| 1:1 | 20 | 3.2 | 5.6 | | | B |
| 2:1 | 18 | 4.3 | 8.7 | | | B |

* Significant at $p \leq 0.05$. ** Groups that do not share a letter are significantly different.

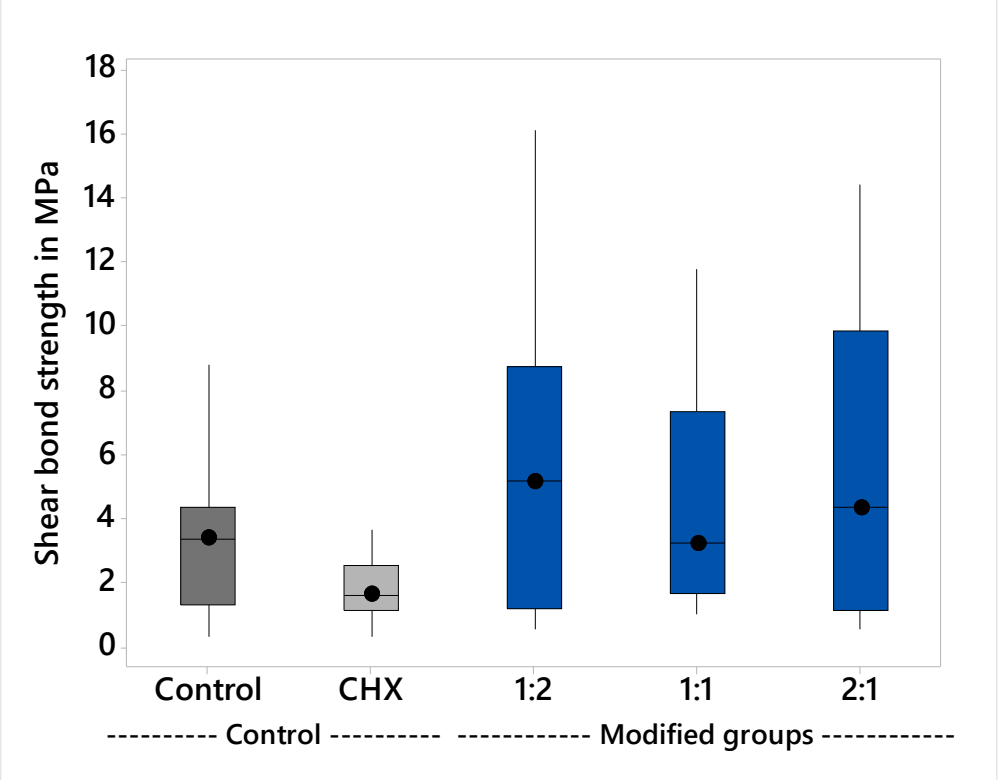


**Figure 1.** Median shear bond strength and interquartile range.

#### Failure Mode

The stereomicroscope examination of the deboned dentin surface after shear bond strength testing revealed that the majority of the fracture modes were cohesive and mixed failure, as presented in Table 2 and illustrated in Figure 2.

**Table 2.** Percentages of the different failure modes for each tested group ($n$ = 10).

| Failure Mode | Control | CHX | 1:2 | 1:1 | 2:1 |
|---|---|---|---|---|---|
| Adhesive % | 10 | 15 | 14 | 21 | 0 |
| Cohesive % | 50 | 38 | 57 | 36 | 40 |
| Mixed % | 40 | 46 | 29 | 43 | 60 |

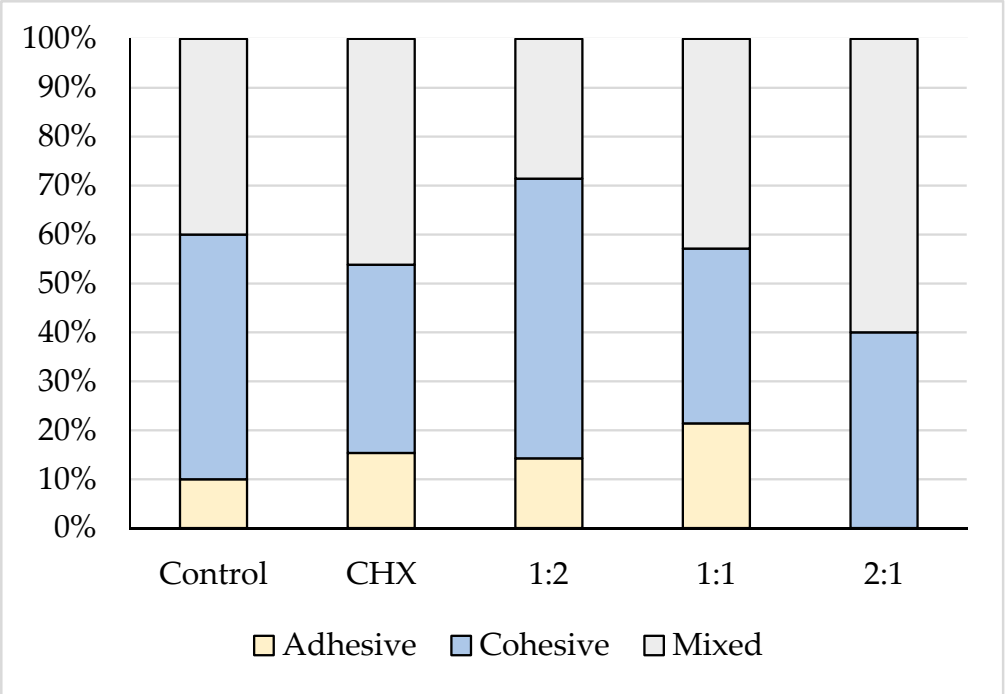


**Figure 2.** A stacked column chart showing the different failure modes of the five tested groups.

2.1.2. Film Thickness

The variables showed a non-parametric distribution and thus the Kruskal Wallis H test was used to test the effect of the plant extract on film thickness; the results are shown in Table 3 and illustrated graphically in Figure 3. The Kruskal Wallis H test indicated that there was significant effect of the plant extract on the film thickness, H (4) = 27.3, $p < 0.001$. The group 1:2 (M = 24 µm) had the thickest film and the post hoc comparisons using Dunn's test showed that it was significantly different from all groups, except group 1:1. All the other groups had insignificant differences compared to each other.

**Table 3.** Results of the Kruskal Wallis H test for film thickness.

| Groups | $n$ | Median (µm) | Interquartile Range | $p$ * | Pairwise Comparison ** | |
|---|---|---|---|---|---|---|
| Control | 10 | 20 | 2.8 | <0.001 | A | |
| CHX | 10 | 20 | 4.0 | | A | |
| 1:2 | 10 | 22 | 3.0 | | A | |
| 1:1 | 10 | 22 | 1.8 | | A | B |
| 2:1 | 10 | 24 | 1.3 | | | B |

* Significant at $p \leq 0.05$. ** Groups that do not share a letter are significantly different.

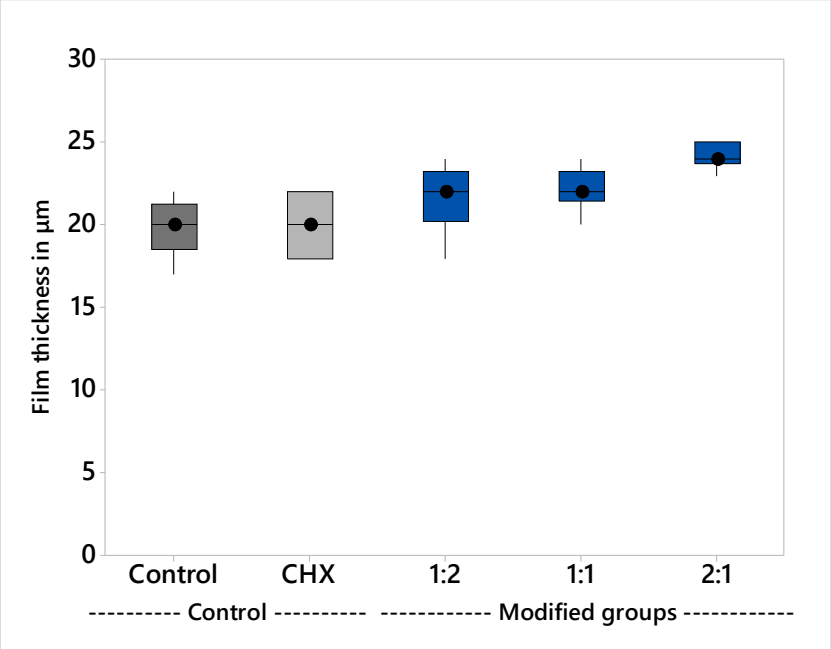


**Figure 3.** Median film thickness and interquartile range.

*2.2. Discussion*

Despite the common usage of glass ionomer cement (GIC) in dentistry because of the anticariogenic property, fluoride release, and rechargeability, the reduction in the bacterial counts and the ability of the conventional glass ionomer cements to completely arrest the caries process is still not reliable for many clinical situations. Therefore, many investigations are concerned with improving the antibacterial activity of GIC to overcome this problem [29,30].

GIC modified with *S. persica*, *F. carcia,* and *O. eoropaea* extract mixtures has shown significant antimicrobial activity against *S. mutans* before, which is the main causative organism of dental caries and *M. luteus*, which is a sensitive marker to the release of antimicrobial agents [28]; thus, this study aimed to assess two important clinical properties of GIC, which are shear bond strength and film thickness.

2.2.1. Shear Bond Strength

Clinical success and the retention of a dental cement are directly affected by its adhesion and bonding to the tooth structure. The mechanism of adhesion of glass ionomer cement to the tooth structure was attributed to the interaction of hydroxyapatite found in the tooth structure with the polyacrylic acid forming strong ionic bonds [31–33].

The bond strength assessment of GIC may be influenced by several factors: testing device, size of the specimen, composition of the tooth structure, storage time, temperature, and the substrate [34]. Enamel is much more susceptible to adhesion than dentin, where values of enamel vary between 2.6 to 9.6 MPa and values of dentin vary from 1.1 to 4.1 MPa [4,35]. Enamel has a surface that is basically homogeneous, and mainly composed of hydroxyapatite, which has high surface energy, whereas dentin has a heterogeneous surface with low surface energy [36]. Moreover, it was found that GIC recorded lower bond strength values to tricalcium silicate-based cements compared to methacrylate- and silorane-based composites [37].

The shear bond strength test in the current study was carried out after 24 h because it was found that bond strengths increase rapidly, with about 80% of the final bond strength being achieved in the first 15 min [4,38]. The results showed that there were no significant differences among the median values of control (M = 3.7 MPa) and CHX-GIC (M = 1.7 MPa). Likewise, there was insignificant difference between the control and the three extract-

modified groups: 1:2 (M = 5.1 MPa), 1:1 (M = 3.2 MPa), and 2:1 (M = 4.3 MPa) groups. This could be due to the amount of CHX (0.5%) added to the CHX-GIC group, and the amount of plant extract in the 1:2, 1:1, and 2:1 groups did not negatively alter or affect the ionic exchange and interaction between the cement and the surface of the tooth. This was in accordance with the results of Becci et al. [39] and Jaidka et al. [40].

On the other hand, the CHX-GIC group showed statistically significant lower median values compared to all the plant-modified groups (1:2, 1:1, 2:1). The reason for this could be due to the presence of Cinnamic and bornyl acetic carboxylic acids in the plant extract mixture [28]. According to Prentice et al. [41, those carboxylic acids might have been existed in a considerable amount that improved release of ions from the surface of the glass ionomer powder through lowering the pH. Moreover, the presence of additional COOH groups from acids might have caused more ionic exchange and interaction with calcium of the tooth within first 24 h. This might explain the slight potential enhancement of the bond strength specifically in group 1:2 (M = 5.1 MPa) compared to CHX, but still it is statistically insignificant compared to the control group (M = 3.7) [41].

Failure Mode

Dental restorations and cements should ideally have high adhesive and cohesive bond strengths to counteract the forces of mastication [42]. In the present study, the deboned dentine surface was observed using stereomicroscope at a 12× magnification in which cohesive and mixed patterns predominated. Choi et al. [43] and Becci et al. [38] accounted cohesive failure prevalence for a low tensile strength of the tested GIC material rather than its true adhesive bond strength to dentin. Lucas et al. [44] attributed this to the strong ionic layer that is formed at the interface between the GIC cements and the calcified structures through an ion exchange process.

For the mixed failure, Palma-Dibb et al. [45] and Carvalho et al. [46] explained it on the basis of the insufficient resistance to early wear and the formation of a glass ionomer matrix. Therefore, part of the glass ionomer remained bonded to the tooth structures, while part was dislodged at the GIC–tooth interface. No correlation was found in the present study between the shear bond strength values and failure modes, because this correlation has been discussed controversially in the literature [47,48]. El Wakeel et al. [49] indicated that there is no relationship between the shear bond strength and the mode of failure.

2.2.2. Film Thickness

Glass ionomer cements have been used widely for the cementation of cast metal and porcelain restorations in dentistry [50]. Film thickness is a significant rheological property that should be taken into consideration during the selection of a suitable and durable luting agent. Film thickness is highly influenced by manipulation variables, such as mixing temperature and powder–liquid ratio. The consistency of the luting cement directly affects the film thickness and the correct adaptation of the restoration. A luting material with a high viscosity requires more time for the optimal seating of the restoration as well as the application of higher seating forces to prevent marginal gaps [51,52].

Film thickness was evaluated consistent with ISO 9917-1. The results showed that all the groups meet the standard, with less than 25 µm film thickness [53]. There was a statistically insignificant difference in the mean values between the control group (M = 20 µm), CHX-GIC (M = 20 µm), and the plant modified groups; 1:1 (M = 22 µm), 1:2 (M = 22 µm). The 2:1 (M = 24 µm) group showed statistically significantly higher mean values compared to all the other tested groups. The results were in agreement with those of Sulaiman et al. [54] and Kious et al. [55]. This could be explained on the basis that the plant extract mixtures did not alter the viscosity of GIC, which directly affects the cement film thickness, where cements of high viscosity showed rapid setting before they can flow properly to achieve a minimum film thickness [56].

The null hypotheses of both shear bond strength and film thickness were rejected based on the results. A limitation of the current study is that it was designed as an in vitro

study and thus the testing conditions did not exactly simulate the oral environment and the clinical situations. Different factors affect the physical and mechanical properties of GIC, such as moisture contamination, the application of a protective coat, mixing time and temperature, batch of cement, and storage medium [57]. Further studies with respect to other bacterial strains and more mechanical and physical properties will be performed.

## 3. Materials and Methods

### *3.1. Plants Extraction and GIC Modification*

#### 3.1.1. Plant Extraction

Three different plants, *Olea europaea* leaves, *Ficus carcia* leaves, and *Salvadora persica* roots, were washed thoroughly with water, dried in air for 6 days at room temperature, and ground using a blender into a fine powder. A standardized amount (80 g) from each plant powder was placed into a Soxhlet extractor (Carl Roth GmbH + Co. KG, Karlsruhe, Germany) separately and an extraction process was carried out using 250 mL of ethyl alcohol (70%) at 75 °C. The resultant product of each process was then filtered using Whatman filter paper no. 1 and mixed together to prepare an extract mixture. A rotary evaporator (Buchi Rotavapor R-300, Buchi Labor Technik GmbH, Essen, Germany) was used to evaporate the solvent at 37 °C, leaving a concentrated crude mixture that was stored at 4 °C in a glass bottle until usage [58].

#### 3.1.2. Modification, Preparation and Specimens Grouping of GIC

Conventional freeze-dried glass ionomer cement (Medicem aqua, Promedica GmbH, Neumuenster, Germany, Lot 1849261) that was supplied in the form of powder/water version was used. The distilled water used for the preparation of GIC was modified with the extract mixture at three different extracts of water mass ratios, giving three plant-modified groups (1:2, 1:1, 2:1). Plastic bottles with the exact nozzle size as those supplied by the manufacturer were used to store the different groups in order not to alter the recommended powder/liquid ratio (1:2) upon cement preparation. Fresh specimens of each of the modified groups were prepared according to the recommended powder/liquid ratio (1:2) for each testing procedure and then compared with two control groups:

- Negative control: prepared by mixing the powder of GIC with the exact amount of distilled water as per the manufacturer's instructions (1:2), without any modification.
- Positive control: prepared by adding 0.5% CHX diacetate powder (*w*/*w*) (Merck KGaA, Darmstadt, Germany) to GIC powder (CHX-GIC) to be mixed with distilled water (1:2).

Group names:

1. Control: (unmodified GIC).
2. CHX-GIC: (0.5% CHX modified GIC).
3. Extract- mixture modified groups:
    a. 1:2 (extract: water).
    b. 1:1 (extract: water).
    c. 2:1 (extract: water).

### *3.2. Shear Bond Strength*

Ninety-seven carious and crack free bovine teeth were selected and stored in NaCl until usage. The teeth were embedded in acrylic blocks. The enamel surface of the teeth was removed using silicon carbide abrasive paper on a polishing machine in order to obtain flat smooth dentin surfaces. Polycarboxylic acid (25%) was used as a dentine conditioner for 25 s then rinsed and air-dried [26,27]. A split teflon mold of a 4 mm diameter and 3 mm height was clamped onto the exposed dentin surface of the tooth using a metallic device with springs and screws for opening and closing (Figure 4). The cement was mixed as per the manufacturer instructions (1:2), packed and condensed inside the mold, and allowed to set. One hour later, the metallic device was opened and the mold was removed, leaving the

specimen attached to the dentine. Teeth with the bonded specimens were then stored at 37 °C in deionized water for 24 h. Each specimen was placed in a universal testing machine (Zwick Zmart. Pro, Zwick/Roell, Ulm, Germany) and subjected to dislodging forces at a crosshead speed of 0.5 mm/min using a sharp knife-like mandrel that was attached to the upper assembly (Figure 5a,b). The dislodging force was recorded and then the bond strength of GIC to dentine was calculated according to the following equation [59,60]: shear bond strength [MPa] = force / area.

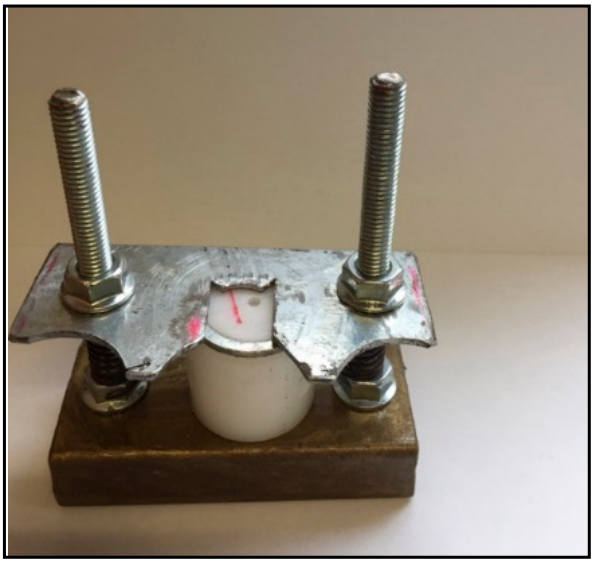

**Figure 4.** Teflon mold fixed by a metallic device with screws on the tooth surface for bond strength specimen preparation.

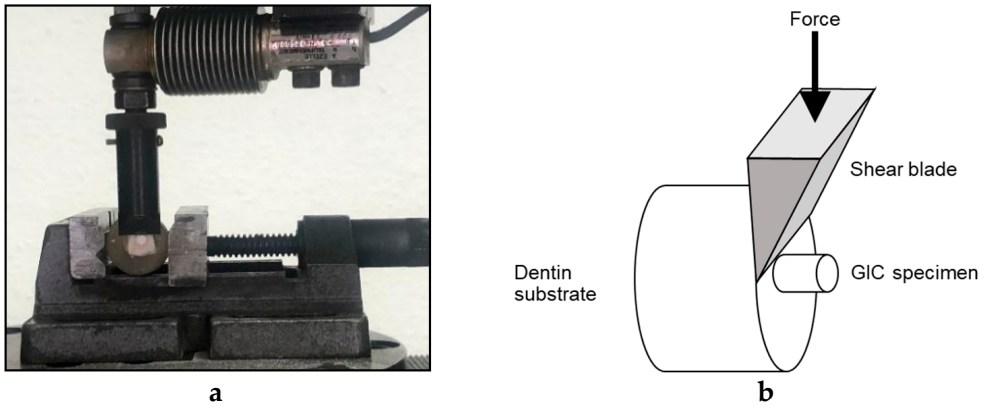


**Figure 5.** (**a**) Zwick testing machine dislodging GIC specimen; (**b**) Schematic diagram showing shear bond strength testing.

Failure Mode Analysis

The different failure modes for all the tested groups ($n$ = 10) were evaluated by one observer under an optical microscope (Stereomicroscope SR, Carl Zeiss AG, Oberkochen, Germany) at a 12× magnification. Failure modes were categorized into three groups: adhesive failure when the GIC was removed from the dentin surface without residual debris, cohesive failure when a fracture occurred inside the cement or the tooth, and mixed failure when a combination of both cohesive and adhesive failures was observed (Figure 6a–c) [61].

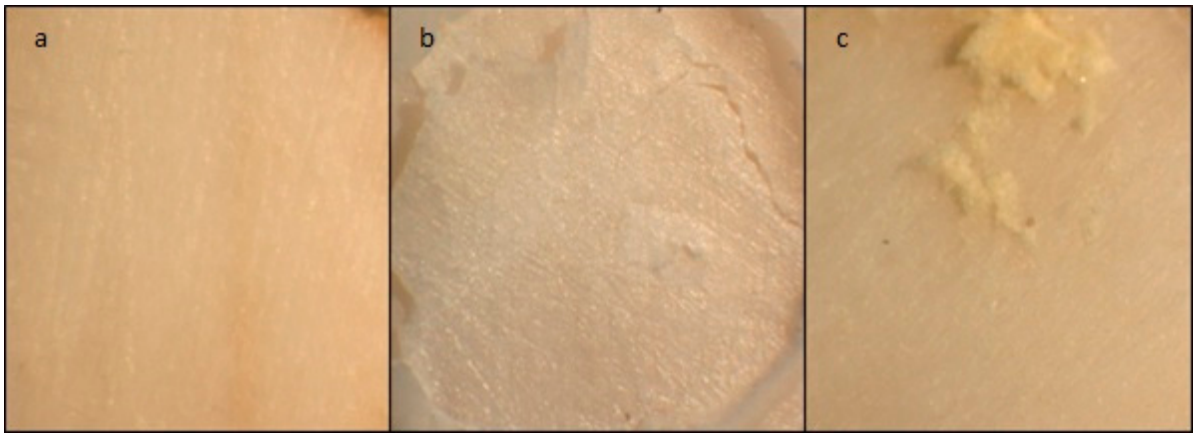


**Figure 6.** Stereomicroscope images of dentine showing the three failure modes at a magnification of 12×. (**a**) adhesive failure; (**b**) cohesive failure; (**c**) mixed failure.

### *3.3. Film Thickness*

The test was conducted according to ISO 9917-1:2007 for glass ionomer cement. The thickness of two flat, uniform, rectangular glass plates stacked in contact was measured four times to the nearest 0.1 µm with a digital micrometer (Digimatic, Mitutoyo Europe GmbH, Neuss, Germany). This reading was recorded as Reading A. The cement for each group ($n$ = 10) was prepared according to the manufacturer's instructions and then a standardized amount of each cement mixture was placed between the two glass plates. A 147 N load was applied on the upper glass plate using a universal testing machine; see Figure 7a,b. Seven minutes later, the overall thickness of the plates with the cement between was recorded as Reading B. The difference between the thickness of the plates with and without the material between (B−A) was considered as the final combined film thickness for the specimen being tested [53].

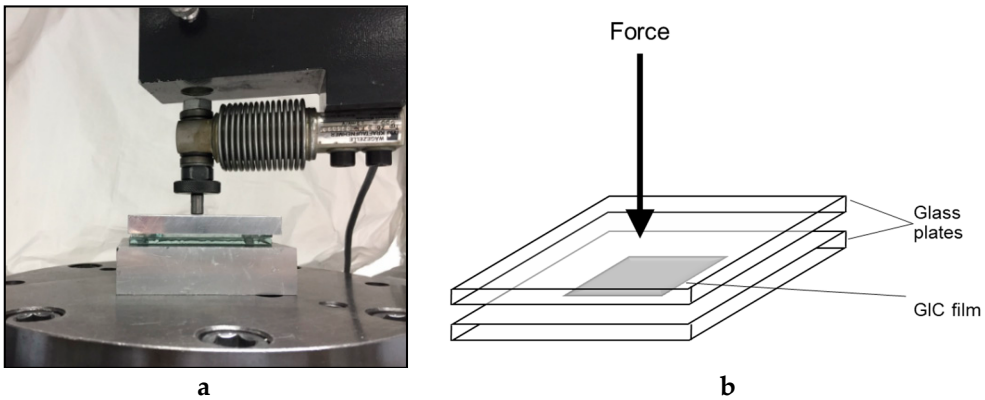


**Figure 7.** (**a**) Loading of two glass plates with film thickness specimen in between; (**b**) Schematic diagram showing film thickness testing.

### *3.4. Statistical Analysis*

The Ryan–Joiner normality test (similar to Shapiro–Wilk test) was used to test whether or not the variables followed a normal distribution. The numerical data showed a non-parametric distribution, and thus were presented as a median and interquartile range, $p \leq 0.05$. Furthermore, the Kruskal–Wallis test was used for comparison between the groups, followed by Dunn's post hoc test for pairwise comparison. Statistical analysis was performed via Minitab 17.3.1 for Microsoft Windows (Minitab, Inc., State College, PA, USA).

## 4. Conclusions

Within the limitations of the current study, it can be concluded that the addition of a plant extract mixture in an attempt to enhance the antimicrobial activity did not negatively alter the shear bond strength and film thickness properties of GIC, and thus this might have potential for GIC modifications.

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**Data Availability Statement:** Ethical guidelines were followed strictly.

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**Conflicts of Interest:** All the authors declare that there is no conflict of interest in this study.

**Sample Availability:** Samples of the compounds are not available from the authors.

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# 4. Discussion with References

## 4.1 Discussion

Numerous studies revealed that incorporation of antibacterial agents in restorative materials have many therapeutic benefits, but frequently results in impaired physical and mechanical properties. These limitations provoke the need to develop some innovative strategies that can act against dental caries without altering the material properties. One of such strategies is to explore the abundantly available medicinal plants in nature that have proven a profound antimicrobial activity [1, 2].

In the current study, roots of *Salvadora persica* and *leaves* of *Ficus carcia*, and *Olea europaea* were extracted using Ethyl alcohol to prepare an extract mixture. The prepared mixture was used to modify a conventional GIC yielding three modified groups that were named according to the mass ratio of extract added to water used for preparation of GIC (1:1, 1:2, 2:1). The modified materials were evaluated and compared with a conventional GIC (Control) and 0.5 % CHX modified GIC (CHX-GIC) with regard to the antimicrobial activity, compressive strength, water sorption, solubility, flexural strength, shear bond strength, and film thickness.

Results of diffusion assays against *S. mutans* showed that plant extracts with the different concentrations were effective in inhibiting the bacterial growth compared to the control group. The extract mixture inhibitory effect was more pronounced in group 2:1 that showed the statistically highest inhibition zones compared to groups 1:1 and 1:2. Furthermore, the 2:1 plant modified group showed mean inhibition zones that were significantly higher than the control group against *M. luteus*. However, CHX-GIC group was significantly of the highest mean values followed by 2:1 group compared to the other tested groups. This can be explained on the basis that *M. luteus* is very sensitive to chlorhexidine that can be efficiently taken up by the bacteria according to Wendel *et al.* [3].

The antimicrobial activity might be attributed to the different phytochemical constituents detected in the plant extract. GC/MS analysis in the present study revealed the presence of terpenoids, coumarins trimethoxy cinnamic acid phenols, flavonoids and saponins. Terpenoids cause bacterial membrane disruption [4], while coumarins and phenols

damage the bacterial cell membrane and membrane permeability [5,6]. Moreover, flavonoids and saponins cause alteration of cytoplasmic membrane function [7, 8].

Compressive and flexural strength test results were comparable. There was insignificant difference between all of the control, 1:1, 1:2 and CHX-GIC groups. Such findings are in accordance with several studies [9, 10], that proved that the presence of antimicrobials at certain percentages did not alter the mechanical properties of GIC. However, others contradict the results of current study based on its conditions and findings [11-13].

The 2:1 group showed significant enhancement of both, compressive strength and flexural strength values compared to the other tested groups. This could be explained on the basis of the chemical analysis that identified silica in *Salvadora persica* [14]. Silica was claimed to improve the strength through its ability to adhere to the GIC matrix by chemical bonding and therefore, reinforcing the cement [15, 16].

Moreover, GC/MS analysis revealed the presence of Cinnamic and bornyl acetic carboxylic acids in the extract mixture. It was assumed that by adding these acids to GIC, the degree of cross-linking and polysalt bridge formation increases and subsequently the mechanical properties of the set cement [17]. Another explanation could be that the extract affected the amount of unreacted powder particles within the matrix, which may act as strengthening fillers, hindering crack propagation within the cement [18-20].

With regard to water sorption, all of the tested groups experienced water gain at the end of the immersion period with no statistically significant differences between mean values of all the tested groups. A possible explanation is that there was no alteration in their chemical composition and thus there were no variations in their sorption capacity values as well [21].

For water solubility, negative mean values were recorded for the control and modified groups which might be attributed to incomplete dehydration of these materials. Several authors accounted the negative values to the prolongation of the acid–base reaction that allows the water molecules to continuously bond into the cement structure. Therefore, the cement gained weight and expanded [22-24]. Moreover, there were statistically significant differences among the groups. Such observation could mean that the bonding of the water

molecules to the structure was not equal for all the groups. Therefore, some of the unbounded water molecules were only trapped in the matrix or the filler and vaporised out of the cement in the desiccator [25].

Shear bond strength test showed comparable results between the control and CHX-GIC as well as the control and the three extract modified groups. This could indicate that the ionic exchange and interaction between the cement and the surface of the tooth were not altered by the addition of the extract mixture or the CHX (0.5 %) [26, 27]. The plant modified groups (1:2, 1:1, 2:1) showed statistically significant higher median values compared to the CHX-GIC group. The reason could be due to the presence of carboxylic acids in the plant extract mixture that might explain the slight probable improvement of the bond strength specifically in group 1:2 [17, 28].

Failure mode analysis revealed the predominance of cohesive and mixed patterns. Cohesive failure was attributed to the low tensile strength of the material instead of its true bond strength to dentin and to the ionic exchange process at the interface between the cements and the tooth [29-31]. Mixed failure pattern was justified with the inadequate resistance to early wear, and the formation of cement matrix [32, 33]. No correlation was made between bond strength values and failure modes because some authors claimed that it is controversial [34, 35]. whereas, others indicated that there is no relationship between them at all [36].

Film thickness test results showed that all the groups meet the ISO 9917-1 standard with having less than 25µm film thickness [37]. There was no statistically significant difference in the mean values between the groups except for the 2:1 group that showed statistically highest mean values. This could indicate that the extract mixture did not alter the viscosity of GIC which directly influences the film thickness [38].

## 4.2 Conclusions

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

1.The attempt to enhance the antimicrobial activity of GIC with natural plant extracts might be a very good potential for the prevention of recurrent caries.

2. Compressive and flexural strength of GIC were enhanced at the plant extract high concentration.

3. Water sorption, solubility, film thickness of GIC were not altered by the plant extracts.

## 4.3 References

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