Advances in Medical, Pharmaceutical and Dental Research Journal (AMPDR)- ISSN 2812-4898
http://dx.doi.org/10.21622/AMPDR.2024.04.2.798

Dec. 2024
Volume 4
Issue 2

Comparative Evaluation of Bond Strength of Essential Oil Modified Composite Resin to Enamel (In-Vitro)

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Received on, 26 January 2024 - Accepted on, 05 July 2024 - Published on, 01 September 2024

ABSTRACT:

Objectives: *The aim is to evaluate the bond strength of essential oil-modified composite resin to enamel.*

Materials and Methods: *84 molars were embedded in acrylic molds with the buccal surfaces facing upwards, then they were divided into three groups according to the type of essential oil used (n=28): Group 1: Cinnamon oil, Group 2: Thyme oil, Group 3: Clove oil. Each group was then divided into four subgroups according to the concentration of essential oil used to modify the Flowable Composite Resin (CR) (n=7): Subgroup 1: negative control, Subgroup 2: concentration 1µL/2g CR, Subgroup 3: concentration 2µL/2g CR, Subgroup 4: concentration 5µL/2g CR. plastic molds were fixed and the flowable composite was applied and cured. Finally, the shear bond strength test was conducted.*

Results: *Mean SBS values of Cinnamon group was significantly higher than the control subgroup (P = 0.001), but this did not apply to Thyme (P = 0.189) and Clove Oil (P = 0.386) groups. The source of the significance of the SBS values in the Cinnamon oil group was between the control subgroup and every experimental one.*

Conclusion: *Mean SBS values of Cinnamon oil modified composite resin were significantly higher than the control group. Mean SBS values of Thyme oil and Clove oil modified composite resins were not significantly higher than the control group.*

KEYWORDS:

Phytodentistry, Essential oils, Composite resin, Secondary caries, Shear bond strength.

1. Introduction

Despite the development in public policies, dental caries is still the most common and costing disease around the world, presenting an international problem that must be treated by the authorities and professionals, [1] especially in developing countries. [2] The amount of dental plaque forming around the restoration margins and its ability to cause dental caries depends on the type of restoration used, which further indicates that resin-based materials cause a greater accumulation of plaque in comparison to amalgam, GIC and silicate. [3,4,5]

Furthermore, composite restorations seem to accumulate greater amounts of biofilms over a long period in comparison to enamel and other restorations, which is strong evidence that these biofilms contribute to the mechanical and chemical degradation of these restorations, due to their low anti-cariogenic properties. [6] One of the main causes of this failure is secondary caries. [7] Since the origin of dental caries is related to bacteria, the antibacterial treatment is the main support for the treatment. [8]

During the past decades, the antibacterial treatment of dental caries was taken into account in order to prevent the formation of caries. [9,10] The literature provides information concerning many modifications of composite resins and bonding agents in order to reinforce their physio-mechanical and antibacterial properties [11,12] by adding antibacterial agents either to the resin matrix or to the filler particles. [13]

Among these antibacterial agents that were incorporated in composite resins and mostly mentioned in the literature are: nanoparticles, titanium dioxide, zinc oxide, calcium phosphate and fillers containing fluoride, and fluoric compounds. [14] These agents must fulfill some criteria like good

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biocompatibility, low water absorption, miscibility, and color matching, without sacrificing the mechanical properties of the restoration. [11]

In this regard, plant products (essential oils, extracts, and isolated compounds) were introduced as novel therapeutic and anti-cariogenic agents in order to replace synthetic compounds with natural compounds which have much fewer side effects providing safer and more effective substitutes in managing dental caries. [15] Essential oils have earned attention among other plant products with promising antibacterial properties, [16] among which the cariogenic bacteria. [17]

Essential oils are used in a wide domain due to their wide antibacterial spectrum, safe usage in the field of general health, and low cost. In addition, their complex chemical structure minimizes the risk of the emergence of bacterial resistance. [18] Since no bacterial resistance was observed when essential oils were used, this would be promising in the treatment of human diseases. [19] Plants have been used in dental domains since old times and PhytoDentistry is now being used increasingly in modern treatment plans. [20]

So, the world is heading towards herbal medicines. [21] Discovering the plants used in traditional medicine may end up in developing preventive and therapeutic strategies in the field of oral health. [22]

There are two strategies to overcome bacterial resistance and eliminate -or at least- reduce the allergic reactions that appear against them, which are either developing new generations of antibiotics or replacing them with natural products, which for decades were known for their antibacterial, antifungal, and antiviral properties in traditional medicine, and scientific evidence about these properties and other properties is available, and this encouraged the incorporation of these materials in modern medicine. [23]

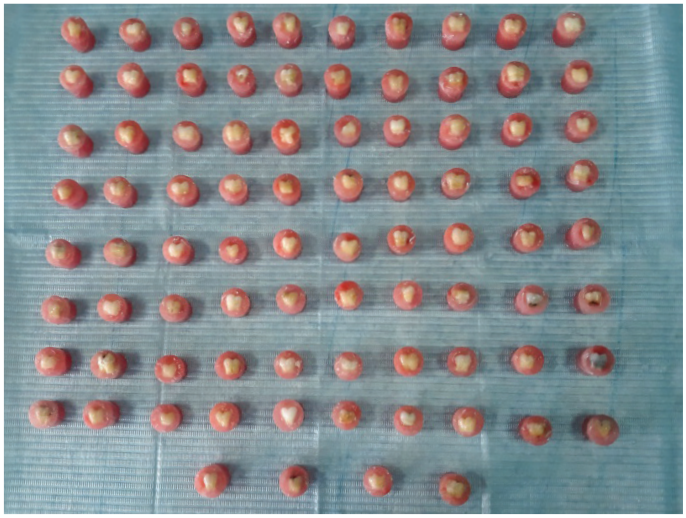
Among these essential oils, which were tested and have the strongest antibacterial activity against oral pathogens are cinnamon, thyme, and clove essential oils. [24] The physio-mechanical properties of the bonding agent modified with essential oil derived from Butia Capitata seeds did not statistically differ from the non-oil-containing bonding agent [25] and the physio-mechanical properties and the bond strength of the bonding agent containing Thymol did not statistically differ from the control group. [26] In addition, a recent study found that incorporating cinnamon essential oil in the composition of the flowable composite did not statistically affect the mechanical properties, whereas it could enhance the antibacterial activity reducing the risk of the formation of secondary caries. [27]

So far, there is a lack of studies aiming to investigate the potential of the application of essential oil in dentistry; hence there is a need for more studies to assess this potential in adjunctive anti-cariogenic chemotherapy. [28] Therefore, this study is conducted to evaluate the bond strength of essential oil-modified composite resin to enamel and to compare it with non-oil-containing composite resin.

2. MATERIALS AND METHODS

TEETH PREPARATION

Eighty-four freshly extracted human molars were washed under running water to remove the organic debris with the help of manual scalers U15 and CK6 then preserved in distilled water, which was replaced weekly. The roots were cut 2 mm below the cementoenamel junction using low-speed diamond disks along with constant water flow [29] then the pulp tissue was removed using a probe. A cotton pellet was inserted into the pulp chamber space then the crowns were embedded into self-curing acrylic resin molds ensuring that the buccal surfaces were facing upwards, subsequently, all molds (Figure 1) were immediately put in a bowl containing water until the complete set of acrylic resin. [29] Standard enamel surfaces (Figure 2) observed by unaided eye were established by grinding the buccal surfaces with silicone carbide papers grit 400. The specimens were examined under a light microscope to eliminate the presence of cracks or the exposure of superficial dentin. [30] The areas adjacent to the standard enamel area were covered with nail polish. The specimens were divided randomly using a closed box into three equal groups (n=28) according to the type of essential oil used; these three groups were subsequently divided into four subgroups (n=7) according to the amount of oil incorporated in the flowable composite (Figure 3).



Fi g.1 Teeth embedded in acrylic resin molds

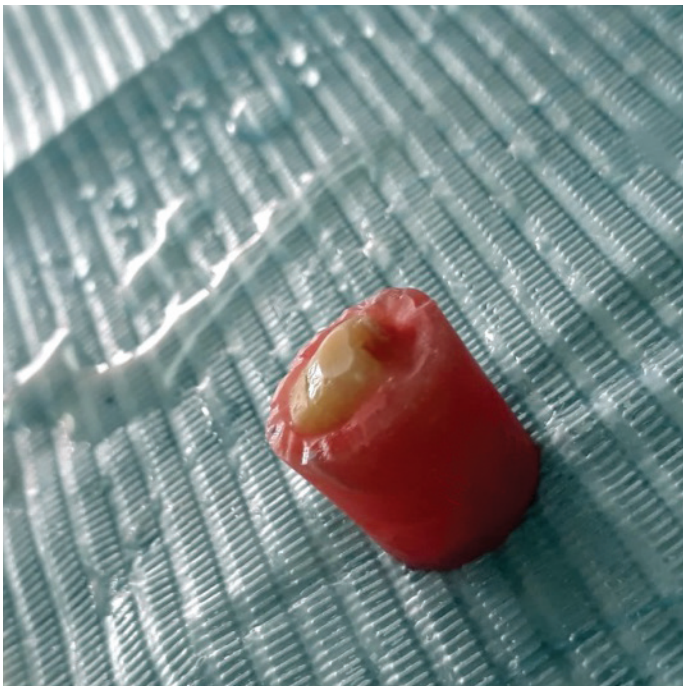


Fig. 2 The standard enamel surface

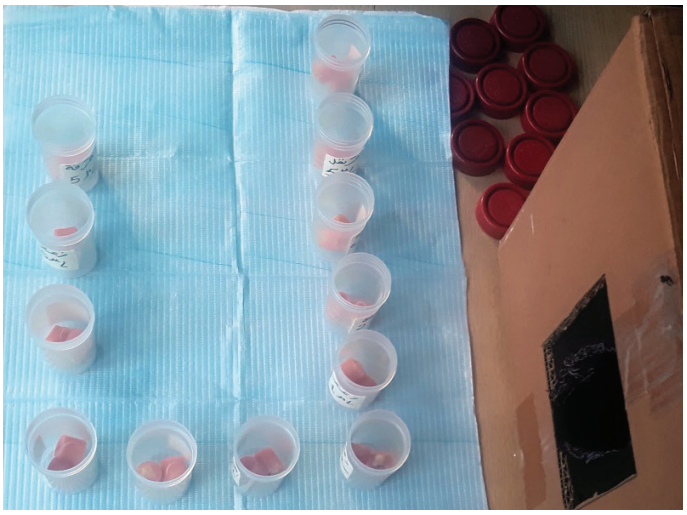


Fig. 3 Dividing of the study sample

PREPARATION OF ESSENTIAL OIL MODIFIED COMPOSITE RESIN

Three Essential oils of the highest antimicrobial properties were chosen to modify the composite resin which are cinnamon, thyme and clove essential oils. The preparation of the modified composite resin is established by adding each of the amounts (1,2,5 µl) of each of the three essential oils chosen (Figure 4), which are kindly provided by (Center Farma Group, Turkey) and delivered by an adjustable micropipette (Toppette pipettor, DRAGONLAB, China) to 2g of flowable composite (Tetric-N-Flow Bulk-Fill, Ivoclar Vivadent, Germany) measured by an electronic pocket scale (Pocket scale, Zhejiang junkaishun, China). The mixing was done using a glass jar and rod in the box to eliminate the effect of light on the mixture as much as possible until a homogenous consistency is obtained. [24,27]

Fig. 4 Delivering of the essential oil

After the mixing procedure, the mixture is aspirated into a 1 ml syringe covered with black tape to protect it from light. The enamel is etched for 15 seconds using phosphoric acid etching gel 37% (N-Etch, Ivoclar Vivadent, Germany) according to the manufacturer's instructions; the etching gel is then washed away by a pressed water-air stream for 10 seconds; subsequently, the enamel surface is dried gently using a pressed air stream for 5 seconds and the bonding agent (Tetric-N-Bond, Ivoclar Vivadent, Germany) is applied on all test specimens using a bonding brush for each single specimen then is dispensed gently with a pressed air stream. A partially transparent plastic mold (2 mm inner diameter, 2 mm height) measured by a vernier caliper is fixed on the bonded area according to ISO specifications that recommend the limitation of the bonding area, which is very important for the experiment. [31] The bonding agent is cured for 10 seconds according to manufacturer's instructions; after that, the plastic mold was filled with the flowable composite and cured for 10 seconds (Figure 5); The mold is carefully removed and the flowable composite is cured for another 10 seconds. All specimens are preserved in distilled water at room temperature for 24 hours before the SBS test.

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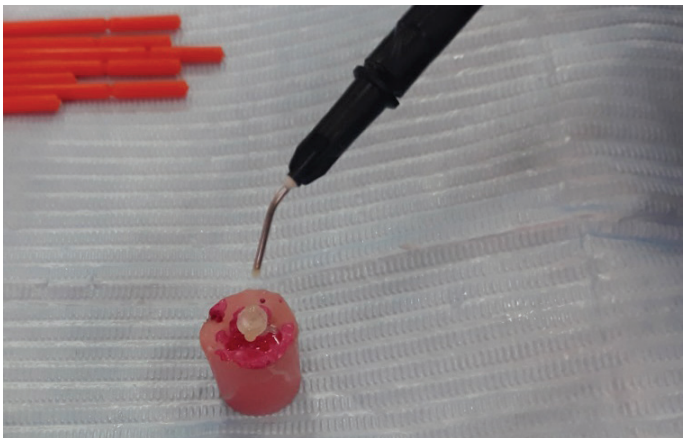

Fig. 5 Application of composite resin on enamel

SHEAR BOND STRENGTH TEST

The specimens are fixed in a metal holder and mounted on the universal testing machine (S.A.E. IBERTEST/Spain), subsequently; the shear force is applied parallel to the acrylic cylinder base and at the tooth-restoration interface using a knife-edged metal tip with a crosshead speed of 0.5 mm/min (Figure 6). [32] The maximum force needed to break the bond is recorded (in Newton) and divided by the bonded area (in mm^2) in order to obtain the Shear bond strength values (in MPa).

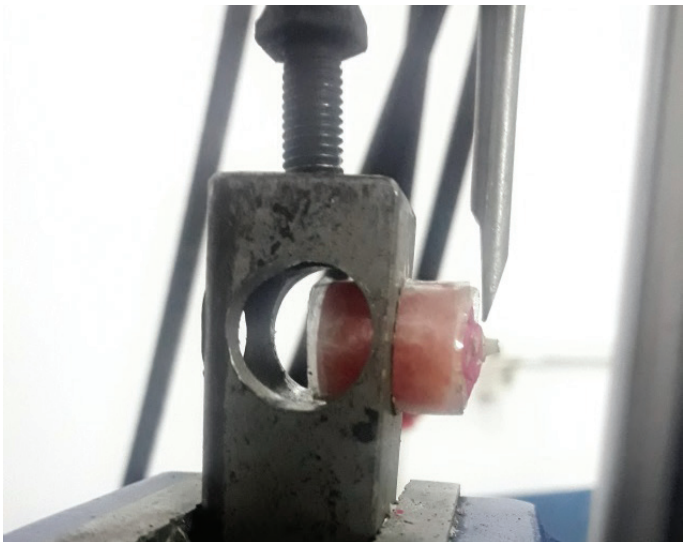

Fig. 6 Application of the shear force

3. STATISTICAL ANALYSIS

Data are collected to report the descriptive statistics of the shear bond strength in (MPa) for all subgroups in addition to the frequency and percentages. First, the test of normality is conducted to check whether the data are normally distributed or not using the Kolmogorov-Smirnov test, then, the homogeneity of variances is checked using the Levene's test for equality. Since there is normality but no homogeneity between the subgroups, the Kruskal-Wallis test as a nonparametric -more truthful- substitute for One-way ANOVA is chosen to compare the SBS means of the four subgroups of each group. Finally, Tamhane's T2 post hoc Test is performed to determine the subgroups responsible for the significance in case it is found. All these tests are set at a significance level of $P < 0.05$. The statistical analysis is achieved by using SPSS Version 22 (SPSS for Windows, Chicago, SPSS Inc., USA).

4. RESULTS

Frequency and percentage of the study specimen are demonstrated in (Table 1). Pie charts illustrating the shares of the subgroups for each oil used are shown in Figure 7. Descriptive statistics of SBS values expressed in MPa for the four subgroups of each group are presented in Table 2 and Figure 8. There is a statistically significant difference in SBS values of Cinnamon Oil group ($P = 0.001$) but this did not apply to thyme and clove oil groups ($P = 0.189$) and ($P = 0.386$), respectively. Comparison of mean values of the study variables in relation to SBS values and the statistical significance is presented in Table 3. finally, using the post hoc test, the statistical analysis of cinnamon oil group showed significant differences of the SBS values between the control subgroup and every experimental one (1µL/2g CR, 2µL/2g CR, 5µL/2g CR) ($P = 0.012$) and ($P = 0.025$) and ($P = 0.032$), respectively; on the contrary, there were no significant differences between the experimental subgroups.

Table 1. Frequency and percentage of the study specimen

Groups	Subgroups	Frequency	Percentage
Cinnamon Oil Group	negative Control	7	8.34 %
	1µL/2g CR	7	8.34 %
	2µL/2g CR	7	8.34 %
	5µL/2g CR	7	8.34 %
Thyme Oil Group	negative Control	7	8.34 %
	1µL/2g CR	7	8.34 %
	2µL/2g CR	7	8.34 %
	5µL/2g CR	7	8.34 %
Clove Oil Group	negative Control	7	8.34 %
	1µL/2g CR	7	8.34 %
	2µL/2g CR	7	8.34 %
	5µL/2g CR	7	8.34 %
	Sum	84	100%

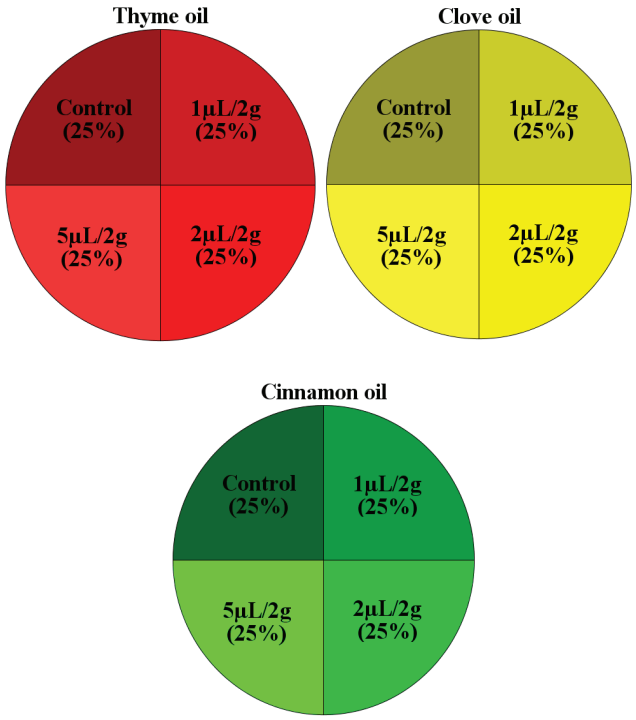

Fig. 7 Percentages of the study specimen

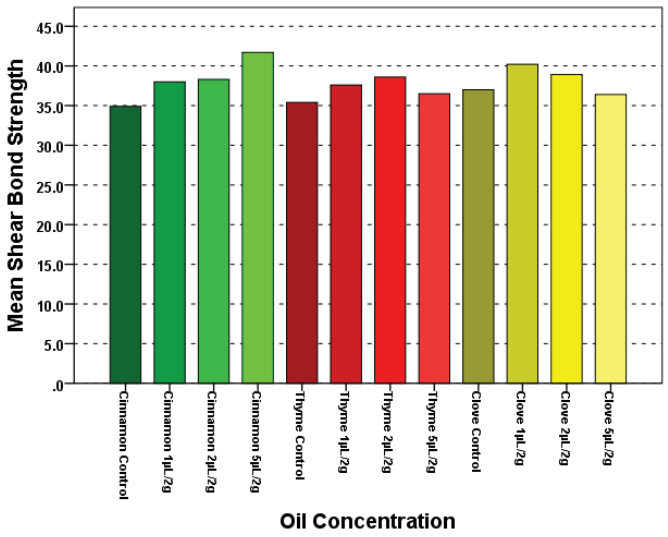

Fig. 8 Mean SBS values of the study subgroups

Table 2. Descriptive statistics of SBS values expressed in Mega Pascal (MPa)

Groups	Sub-groups	Mean	Standard Deviation	Median
Cinnamon Oil Group	negative Control	34.961	1.680	35.3
	1µL/2g CR	38.043	0.840	37.9
	2µL/2g CR	38.314	1.865	37.7
	5µL/2g CR	41.786	4.405	39.9
Thyme Oil Group	negative Control	35.486	4.448	34.6
	1µL/2g CR	37.600	5.540	40.9
	2µL/2g CR	38.671	1.073	38.5
	5µL/2g CR	36.557	1.013	36.2
Clove Oil Group	negative Control	37.057	4.813	35.9
	1µL/2g CR	40.600	4.370	40.6
	2µL/2g CR	38.900	6.197	35.6
	5µL/2g CR	36.443	2.117	35.8

5. DISCUSSION

Modifications of composite resin have been widely investigated recently, as incorporating these agents in its chemical structure affected its clinical performance and physio-mechanical and aesthetic properties as well as polishability and antibacterial properties. [12] Essential oils are a promising group of antibacterial agents which possesses anticariogenic properties. [33] Unfortunately, all composite restorations lose their antibacterial properties after they are completely polymerized. [34] Cinnamon, thyme and clove essential oils are effective antibacterial phenols. [35]

This study evaluates the effect of three antibacterial essential oils on the shear bond strength of Tetric®-N-Flow Bulk-fill composite to dental enamel; the company confirmed that these oils are pure essential oils extracted by steam distillation with no organic solvents or fragrances or any other artifacts that could affect the SBS. Flowable composite resin is chosen to be modified instead of the highly filled one due to its low viscosity, which allows producing a more homogenous mixture and consequently, the essential oil remains at the same concentration in all of its parts; in addition, it is also chosen by other studies. [24,27] A glass jar and rod are chosen instead of metal or plastic to conduct the mixing procedure to avoid any undesirable chemical reaction between them and the mixture.

Factors affecting the shear bond strength values vary from factors concerning the tooth and others concerning the restorative material, for instance, using the conventional etching method and bonding to non-fluorosed enamel positively influence the bond strength. [36] These findings apply to this study where the conventional etching method is used and the bonding procedure is conducted on sound enamel surfaces with no white lesions or fluorosis. The clinically acceptable shear bond strength values are estimated to range between (7,53 - 20,57) MPa [37] and this applies to this study because none of the twelve subgroups had a mean SBS value of less than 30 MPa.

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In this study, data in all subgroups are normally distributed which corresponds with [30,38] who also evaluated SBS to enamel but contradict with ISO standards which report that SBS values are often not normally distributed according to ISO standards. SDR flow Bulk-fill composite is modified with cinnamon oil of three amounts (1,2,5) µl and evaluated three mechanical properties; the results show that cinnamon oil modified composite resin until (2 µl/2g CR) had clinically comparable physio-mechanical properties and can be used in pediatrics and as an antibacterial liner. [27] These findings correspond with this study, as the bond strength is not influenced negatively by the addition of the three oils, but by contrast, increased significantly (slightly above than 40 MPa when 5 µl of Cinnamon oil is added) even though a significant increase in SBS is not expected to be found in all experimental groups of the three oils. Unfortunately, these two studies [24,27] do not mention the reason of choosing the three amounts of oil (1,2,5) µl, but their results showed that these amounts are effective against oral pathogens after the modification and do not affect the physio-mechanical properties negatively; therefore, they are chosen in this study to measure the alterations in SBS values; moreover, these studies are not specific in determining whether the mechanical mixing is manual or automatic.

Lapinska and others demonstrate that the most powerful essential oils against oral pathogens are cinnamon, thyme and clove essential oils [24] and these findings correspond to those found in other studies [17,33]; therefore, these three essential oils are chosen in this study to modify the composite resin. Previous studies seem promising and suggest that incorporating antibacterial agents in dental materials may improve their antibacterial properties without influencing the physio-mechanical performance. [39,40]

Regarding thyme oil, a more recent study has investigated the effect of modifying an orthodontic total-etch bonding system with thyme oil on the shear bond strength of orthodontic brackets; the findings showed that the concentration of 3% caused a significant decrease in the bond strength in contrast with 1% where the decrease is acceptable. [41] More studies must be conducted to evaluate the validity of these natural products in operative dentistry.

Nevertheless, this study has certain limitations. Firstly, this study and other studies [24,27] used a methacrylate-based composite, known for its hydrophobic features, to be modified with essential oils. But a more hydrophobic composite which is the silorane-based composite [42] seems to be a more convenient choice to be modified by the hydrophobic essential oil by obtaining a more homogenous essential oil modified composite. Secondly, the specimen that consists of 84 molars has a subgroup of only (n=7), but it was higher than the minimum statistically accepted level (n=5). Thirdly, this study uses Tetric®-N-Flow Bulk-fill to be modified with essential oils instead of SDR flow Bulk used in the previous studies due to economic sanctions. Fourthly, the mixing procedure is performed manually which risks obtaining a less homogenous mixture and may produce some voids influencing the mechanical performance.

All dissolved agents added to composite tend to be released in large amounts within only few days followed by a decrease in concentration. Moreover, this release leaves restoration having a porous structure which in turn harms the mechanical properties, [43] and essential oils belong to these agents. [24] In this study, the bond strength does not diminish, perhaps due to the storage in distilled water for 24 only or due to the tiny amounts of oils added to the composite resin.

However, even though essential oils do not possess long-term cytotoxicity and genotoxicity, [44] excess volatility and chemical instability of some of their compounds in the presence of air, humidity, light and oxygen may affect the clinical performance negatively, but they can be protected from degradation by encapsulation with polymeric particles like chitosan [45] and starch [46] and to control the release of Essential oil from them. Moreover, Essential oils can also be capsuled by metallic nanoparticles [47] which increase their stability [48]; therefore, they can be protected from the surrounding affecting factors like air and humidity reinforcing their biological activities. [49]

Determining the effective compounds contained in essential oils and comprehending their mechanism of action allow choosing the best conditions for growing and harvesting the aromatic plant as well as the best way to extract the best structure of the oil from it and use it; and once the active compound is known, it is then possible to prepare and develop synthetic analogs. [50] Before conducting clinical studies, overall studies concerning the safety of topical application of these products must be performed. [15] Long-term performance and aesthetic properties of essential oil modified composite resin must be investigated as well as investigating the potential allergic reactions and cytotoxic effects; as a study reported that essential oils have cytotoxic effects and their severity varies depending on the type of oil and its concentration. [44]

Most in-vitro studies investigating the antibacterial properties of essential oils are carried out against pure planktonic cultures with the absence of saliva, whereas the oral cavity is a complex environment

harboring a vast group of microorganisms which interact with one another in the form of a complex-structured biofilm. Therefore, antibacterial properties of essential oils against oral biofilms must be investigated in-vitro before conducting clinical experiments and preparing a commercial product.

Since this study assesses only one type of flowable composite resin, its results cannot be translated to other types due to the variance in structure between each other. So far, there are no investigations about the mechanism and duration of releasing essential oils from composite resins. It seems promising that incorporating these natural products which have plenty of advantages and have proved their anticariogenic properties in dental treatments would provide novel treatment plans for many patients who suffer from oral diseases around the globe.

Table 3. Comparison of mean values of the study variables in relation to shear bond strength values and the statistical significance

Groups	Chi-square	Degree of Freedom	P value
Cinnamon Oil group	16.416	3	0.001
Thyme Oil group	4.779	3	0.189
Clove Oil group	3.036	3	0.386

6. ACKNOWLEDGEMENTS

The authors are grateful for Dr. Yousef Deeb for operating the Universal testing machine. Last but not least, the authors would like to express sincere thanks to Dr. Haytham Kosheh for providing the suitable micropipette.

THE SOURCE OF FUNDING

The authors declare that this study is completely self-financed.

CONFLICTS OF INTERST

The authors declare that there are no potential conflicts of interests concerning the study, authorship or publication of this study.

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Advances in Medical, Pharmaceutical and Dental Research Journal (AMPDR)- ISSN 2812-4898
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