EGYPTIAN
DENTAL JOURNAL
www.eda-egypt.org

Vol. 68, 3857:3868, October, 2022

Print ISSN 0070-9484 • Online ISSN 2090-2360

EDJ
EGYPTIAN DENTAL JOURNAL

Conservative Dentistry and Endodontics

Submit Date : 22-05-2022 • Accept Date : 16-06-2022 • Available online: 01-10-2022 • DOI : 10.21608/edj.2022.140174.2118

ESSENTIAL OIL MODIFICATION OF GLASS IONOMER CEMENT: ANTIBACTERIAL ACTIVITY AND COMPRESSIVE STRENGTH

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ABSTRACT

Objective: The current study was conducted to investigate the effect of essential oil (EO) modification of glass ionomer cement (GIC) on antibacterial activity and compressive strength.

Methods: A total of 100 specimens were prepared; 50 specimens for the antibacterial activity test (3 mm x 2 mm) and 50 specimens for the compressive strength test (4 mm x 6 mm). Specimens were divided into five groups (n=10) according to the type of the essential oil used in modification of GIC; Group 1: unmodified glass ionomer cement (control group); Group 2: 2.5% bergamot modified GIC; Group 3: 2.5% peppermint modified GIC Group 4: 2.5% lemongrass modified GIC and Group 5: 2.5 % fennel modified GIC. Antibacterial activity was assessed using agar diffusion test. Compressive strength test was performed using universal testing machine. Data were tabulated and statistically analyzed.

Results: Bergamot modified GIC recorded the highest mean inhibition zone, followed by fennel, then lemongrass and peppermint. The lowest mean value was revealed by the control group. Regarding compressive strength results, control group showed the highest mean value, followed by lemongrass, then peppermint and bergamot. The lowest mean value was recorded by fennel modified GIC.

Conclusion: Essential oil modification of GIC was effective in enhancing the antibacterial activity although it adversely affects compressive strength in comparison to the control group. Lemongrass and peppermint essential oil modification of GIC were effective in improving antibacterial property while preserving an acceptable compressive strength according to the ISO standards.

KEY WARDS: Essential oil, Bergamot, Peppermint, Lemongrass, Fennel

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INTRODUCTION

Dental caries is a multi-factorial disease that occurs as a result of ecological imbalance between protective mechanisms and oral microbial biofilms. It causes destruction of dental hard tissues by bacterial acids due to fermentation of dietary carbohydrates [1]. Streptococcus mutans plays the main role in development of dental caries as it is predominantly isolated from initial and advanced carious lesions [2].

Microbial biofilms are controlled using different mechanical and chemical methods for plaque control. Mechanical plaque control could be held either by mastication as natural mechanism or by using tooth brushes, dental floss and fissure sealants [3]. One of the most frequent approaches used for chemical plaque control is the use of antibacterial agents [4].

Restorative materials with antibacterial properties are highly recommended. Fluoride release capacity of glass ionomer cements supports its anticariogenic potential [5]. However, the bacterial spectrum that could be inhibited due to fluoride release is still limited [6]. Addition of various synthetic constituents to glass ionomer cements was proposed in an attempt to enhance its antibacterial activity as chloroxylenol [6], chlorhexidine gluconate [7], doxycycline hyclate [8], tetracycline [9], ciprofloxacin, metronidazole and minocycline [10].

Increasing microbial resistance to antibiotics has promoted developing natural antibiotics of plant origin as a next-generation for synthetic antibiotics [9]. Essential oils (EOs) are naturally derived components found in flowers, fruits, herbs, seeds, leaves and roots of different plants [11]. They consist mainly of mixture of volatile compounds which are classified in two main groups; terpenes and terpenoids as well as aromatic and aliphatic constituents, in addition to other components in lower concentrations[12]. Essential oils have antibacterial potential via destroying bacterial cell walls and disrupting their enzymatic activity [13].

Bergamot is a hybrid fruit between bitter orange and lemon [14]. Its essential oil is extracted from the peel of the Citrus bergamia Risso et Poiteau plant. It is reported to have valuable biological activities including antifungal, anti-inflammatory [15] as well as antibacterial activity against mutans streptococci [14]. Peppermint is a medicinal plant that also known as Mentha piperita from the family of Lamiaceae. Menthol is the main component of peppermint. It possesses an antibacterial activity against streptococcus mutans [16]. Lemongrass (Cymbogon citratus) is a plant that belongs to the Poaceae family. It possesses antimicrobial, anti-rheumatic, analgesic, antispasmodic and antipyretic properties [17]. It was previously reported to have an antibacterial effect against streptococcus mutans [9]. Fennel or Foeniculum vulgare is a medicinal plant from the family of Apiaceae. It has anti-inflammatory, antioxidant [16] and antibacterial potential [18].

Although, several researchers reported an antibacterial activity of the previously mentioned essential oils against streptococcus mutans [9,13,14,16], no researches assessed the effect of modification of glass ionomer cement using bergamot, peppermint, lemongrass and fennel on antibacterial activity.

Glass-ionomer cement (GIC) is a water-based material that sets depending on acid-base reaction. This setting reaction is a slowly progressing process associated with a gradual increase in material mechanical properties [19]. Dental restorations should have acceptable mechanical properties as they are subjected to various stresses inside the oral cavity during functional loading as compressive, tensile and shear stresses [20]. Compressive strength test is advocated by the ISO standards for evaluating the mechanical properties of dental restorations because most of the applied masticatory forces are compressive in nature [21].

Hence, the current study was conducted to assess the effect of essential oil modification of glass ionomer cement on the antibacterial activity and compressive strength.

MATERIALS AND METHODS

Materials used in the study

In the current study, four commercially available essential oils were used; 2.5% bergamot, 2.5% peppermint, 2.5% lemongrass and 2.5 % fennel (Harraz for Food Industry and Natural Products, www.harraz.eg, Egypt). A conventional glass ionomer filling material was used in the current study (FILLBEST, BMS DENTAL S.r.I., Via M. Buonarroti, Zona Industriale 56033 Capannoli (PISA), WWW.bmsdental.it, Italy).

Analysis of the chemical components of the essential oils

Analysis of the chemical components of the essential oils used in the study was performed using gas chromatography–mass spectrometry analysis (GC-MS) at Central Laboratories Network, National Research Centre, Cairo, Egypt. The GC-MS system (Agilent Technologies) was equipped with gas chromatograph (7890B) and mass spectrometer detector (5977A). Essential oil samples were diluted using hexane (1:19, v/v). The GC was equipped with DB-WAX column (30 m x 250 µm internal diameter and 0.25 µm film thickness). Analysis was carried out using hydrogen as the carrier gas at a flow rate of 2.0 ml/min, injection volume of 1 μl and the following temperature program: 40 °C for 1 min; rising at 4 °C /min to 150 °C and held for 6 min; rising at 4 °C/min to 210 °C and held for 1 min. The injector and detector were held at 280 °C and 220 °C, respectively. Mass spectra were obtained by electron ionization (EI) at 70 eV; using a spectral range of m/z 50-550 and solvent delay 4 min. Identification of different constituents was determined by comparing the spectrum fragmentation pattern with those stored in Wiley and NIST Mass Spectral Library data [22].

Specimens grouping and preparation

A total of 100 specimens were prepared for the present study. Fifty specimens were prepared for the antibacterial activity test, 3 mm in diameter x 2 mm in height, and 50 specimens for the compressive strength test, 4 mm in diameter x 6 mm in height. Specimens for each test were prepared using a custom made split teflon mold with the intended dimensions. For each test, specimens were divided into five groups (n=10) according to the type of the essential oil used in modification of the glass ionomer cement; Group 1: unmodified glass ionomer cement (control group); Group 2: 2.5% bergamot modified GIC; Group 3: 2.5% peppermint modified GIC Group 4: 2.5% lemongrass modified GIC and Group 5: 2.5 % fennel modified GIC.

All specimens were freshly mixed with a powder / liquid ratio as recommended by the manufacturer. For unmodified glass ionomer specimens (control group), one scoop of powder and one drop of liquid were mixed on a clean glass slab until a homogenous mix was obtained. The mix was applied inside the split teflon mold which was placed on a glass slide covered with celluloid strip (Stripmat, POLYDENTIA, CH-6805 Mezzovico, Switzerland). Another celluloid strip was applied on the top surface of the split teflon mold and gently pressed under another glass slide to achieve well-packed smooth surfaced specimens. For essential oil modified glass ionomer cement specimens, one drop of the essential oil was applied to the drop of the glass ionomer liquid directly before manipulation, mixed with one scoop of powder and applied into the mold in the same manner as mentioned before.

Antibacterial activity test

Antibacterial activity was assessed using agar diffusion test. ATCC 25175 Type strain Streptococcus mutans (16S rRNA gene, Serotype c. carious dentin) were obtained from MIRCEN (Microbiological Resources Centre, Cairo, Egypt) to be used in this study. Bacteria were cultured at 37°C overnight in Trypticase Soy agar (BD 236950, Difco TM, USA) and used as inoculums. The McFarland 0.5 turbidity standard (Densimat, BioMerieux, France) was used to determine the turbidity of the suspension. The concentration of bacteria is standardized to about 1xCFU/ml and used as a working microbial solution.

A total of 100 µl of the previously prepared working microbial solutions was spread evenly over Trypticase Soy agar. The plates were incubated at 37°C in a bacteriological incubator. The antibacterial effect for all experimental groups were assessed by measuring the diameter of bacterial growth inhibition zones after 24 and 48 h. The diameter of the bacterial growth inhibition zones was measured in millimeters using an electronic digital caliper at two different points to obtain two measurements for each specimen and then a mean value was determined for each one.

Compressive strength test

Specimens prepared for compressive strength testing were subjected to compressive loading using a universal testing machine (Lloyd LR 5K, Lloyd Instruments Ltd, Hampshire, UK) operating using Nexygen software version 4.6. The load was applied along the long axis of the specimens with a load cell 5KN at a cross head speed 0.5 mm/min. The maximum load at fracture was recorded and the compressive strength was calculated in MPa by dividing the load with the cross-sectional area of the specimen.

Statistical analysis

Numerical data were represented as mean and standard deviation (SD) values. Shapiro-Wilk's test was used to test for normality. Homogeneity of variances was tested using Levene's test. Data showed parametric distribution and variance homogeneity. Bacterial inhibition zone data were analyzed using two-way mixed model ANOVA. Comparison of simple main effects was done utilizing one-way ANOVA followed by Tukey's post hoc test for independent variable and paired t-test for repeated measurements with p-values adjustment for multiple comparisons using Bonferroni correction. Compressive strength data were analyzed using one-way ANOVA followed by Tukey's post hoc test. The significance level was set at $p<0.05$ within all tests. Statistical analysis was performed with R statistical analysis software version 4.1.3 for Windows*.

RESULTS

Results of chemical analysis of essential oils

Results of chemical analysis of different constituents of the essential oils used in the study are presented in tables 1-4.

Results of antibacterial activity (bacterial inhibition zone)

Bacterial inhibition zones as shown in table 5 revealed statistically significant differences between different essential oil modified GIC groups at both time intervals ($p<0.001$). Bergamot recorded the highest mean value at both 24 h and 48 h, followed by fennel, then lemongrass and peppermint. The lowest mean value was revealed by the control group. Regarding time intervals, bergamot showed no significant difference between both time intervals ($p=0.057$). However, other essential oils modified GIC groups reported significant increase of bacterial inhibition zones after 48 h ($p<0.05$).

Results of compressive strength test

Results of compressive strength (table 6) presented a significant difference between different experimental groups ($p<0.001$) with the control group showing the highest mean value, followed by lemongrass, then peppermint and bergamot. The lowest mean value was recorded by fennel modified GIC.

* R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

TABLE (1): Chemical components of bergamot essential oil

Peak	RT	Name	Formula	Area	Area Sum %
1	4.918	beta-Pinene	C10H16	1436508.16	5.77
2	6.159	beta-Myrcene	C10H16	522061.97	2.1
3	7.18	Limonene	C10H16	10672395.6	42.87
4	8.171	gamma-Terpinene	C10H16	1421897.98	5.71
5	8.337	Santolina triene	C10H16	145491.92	0.58
6	8.628	Prehnitol	C10H14	141376.17	0.57
7	16.955	trans-Geraniol	C10H18O	3711105.3	14.91
8	17.258	Linalyl acetate	C12H20O2	6707585.84	26.94
9	22.362	Linalyl propionate	C13H22O2	136275.26	0.55

Table (2): Chemical components of peppermint essential oil

Peak	RT	Name	Formula	Area	Area Sum %
1	4.847	beta-Pinene	C10H16	89321.03	0.56
2	6.871	Limonene	C10H16	275114.49	1.74
3	7.138	Eucalyptol	C10H18O	221432.6	1.4
4	8.568	Prehnitol	C10H14	62432.15	0.39
5	14.248	Cyclohexanone, 5-methyl-2-(1-methylethyl)-, cis-	C10H18O	3937906.12	24.87
6	14.937	l-Menthone	C10H18O	1320313.69	8.34
7	17.192	Linalyl acetate	C12H20O2	853735.12	5.39
8	18.13	neo-Menthol	C10H20O	874380.42	5.52
9	19.086	Pulegone	C10H16O	316184.04	2
10	19.566	Levomenthol	C10H20O	6131402.99	38.73
11	20.813	Camphene	C10H16	73264.36	0.46
12	21.478	Carvone	C10H14O	1675976.56	10.59

Table (3): Chemical components of lemongrass essential oil

Peak	RT	Name	Formula	Area	Area Sum %
1	6.123	beta-Myrcene	C10H16	316384.96	1.71
2	10.485	6-Methyl-5-hepten-2-one	C8H14O	184310.9	0.99
3	16.813	trans-Geraniol	C10H18O	176103.12	0.95
4	17.228	Linalyl acetate	C12H20O2	286783.22	1.55
5	20.445	Carveol	C10H16O	6743170.97	36.39
6	21.923	Z-Citral	C10H16O	9528558.03	51.42
7	22.439	Linalyl propionate	C13H22O2	496473.57	2.68
8	24.784	Geraniol formate	C11H18O2	799011.31	4.31

TABLE (4): Chemical components of fennel essential oil

Peak	RT	Name	Formula	Area	Area Sum %
1	6.865	Limonene	C10H16	477574.94	2.76
2	11.999	Fenchone	C10H16O	142100.55	0.82
3	19.941	Estragole	C10H12O	16004531.9	92.51
4	22.386	Linalyl propionate	C13H22O2	124798.78	0.72
5	23.532	2,4-Decadienal, (E,E)	C10H16O	181885.75	1.05
6	23.727	Anethole	C10H12O	368684.57	2.13

TABLE (5): Bacterial inhibition zone of different essential oil modified GIC groups at different time intervals

Time	***Bacterial inhibition zone (mm) (Mean ± SD)***					***f-value***	***p-value***
	Control	***Bergamot***	***Peppermint***	***Lemongrass***	***Fennel***		
24h	$0.00±0.00^{E}$	$8.51±0.17^{A}$	$6.19±0.03^{D}$	$7.19±0.06^{C}$	$7.58±0.03^{B}$	**8057.57**	**<0.001***
48h	$0.00±0.00^{E}$	$8.73±0.08^{A}$	$6.25±0.06^{D}$	$7.32±0.04^{C}$	$7.64±0.04^{B}$	**22911.31**	**<0.001***
t-value	**NA**	**7.03**	**10.86**	**26.00**	**32.35**		
p-value	**NA**	**0.057**	**0.030***	**0.007***	**0.005***		

NA: Not Applicable, means with different superscript letters within the same row are significantly different*significant

TABLE (6): Compressive strength of different essential oil modified glass ionomer cements

Compressive Strength (MPa) (Mean ± SD)					***f-value***	***p-value***
Control	***Bergamot***	***Peppermint***	***Lemongrass***	***Fennel***		
$131.83±3.01^{A}$	$84.55±4.61^{D}$	$105.28±7.91^{C}$	$116.20±5.61^{B}$	$73.04±3.09^{E}$	**104.79**	**<0.001***

Means with different superscript letters within the same row are significantly different*significant (p<0.05)

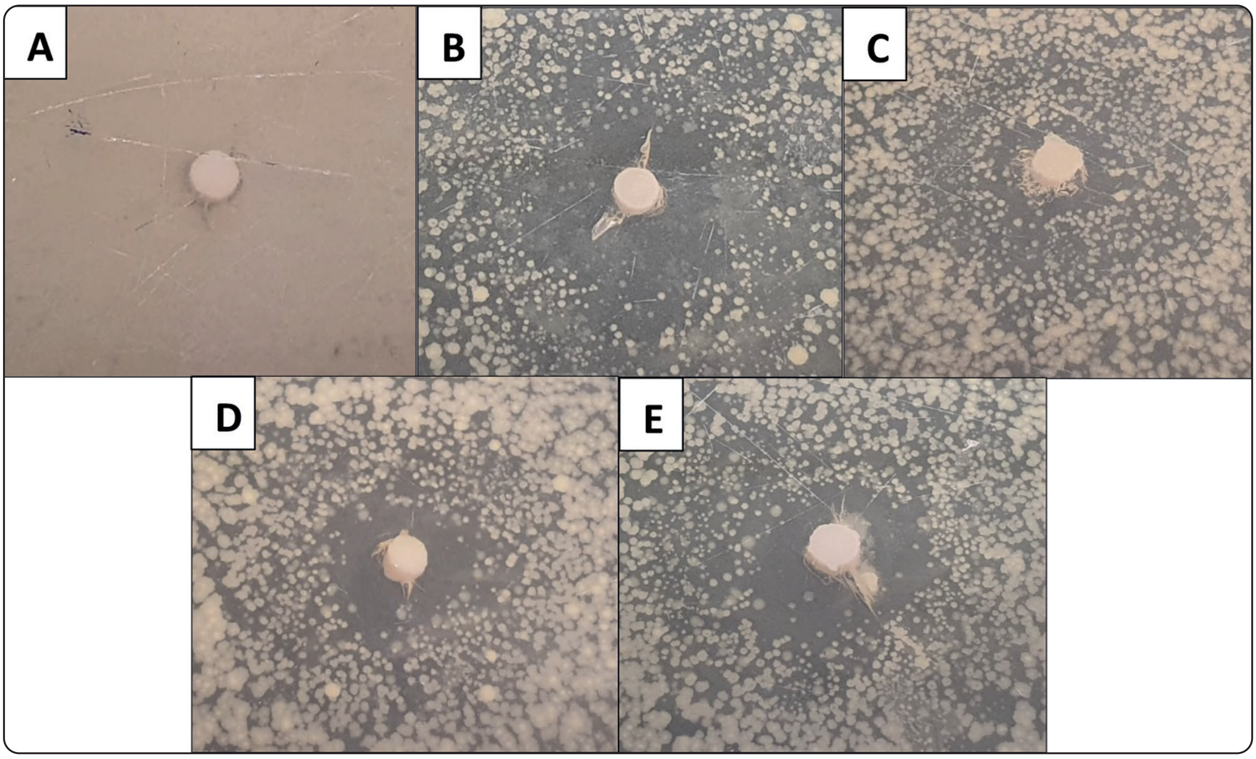

Fig. (1): Bacterial inhibition zone of all experimental groups; (A): Unmodified GIC (Control), (B): Bergamot modified GIC, (C): Peppermint modified GIC, (D): Lemongrass modified GIC and (E): Fennel modified GIC

DISCUSSION

The current study assessed the effect of essential oil modification of glass ionomer cement on antibacterial activity and compressive strength. Medicinal plants are considered major source of a wide variety of chemical compounds that possess multiple biological activities as antifungal and antibacterial activities in addition to treatment of other various diseases [13].

Results of the current study revealed that all tested essential oil modified GIC groups showed significantly higher antibacterial activity in comparison to the control group. Essential oils possess multiple antibacterial mechanisms. They can attack the cytoplasmic membrane of bacterial cell increasing its permeability which subsequently affects its electron transport function, nutrient absorption, nucleic acid synthesis and ATPase enzyme activity [23,24]. Hydrophobicity of essential oils allows them to penetrate bacterial membrane phospholipids and mitochondria leading to bacterial cell death [16].

In addition, phenolic compounds found in essential oils enhance their antimicrobial properties specially against gram positive bacteria [25]. Interaction of the hydroxyl group of phenolic compounds with the bacterial cytoplasmic membrane changes its permeability and affects lipid ordering and stability leading to disruption of the cellular components [26].

Bergamot modified GIC showed the highest bacterial inhibition zone than the control group and other essential oil modified GIC groups (table 5). This might be attributed to its chemical composition. The monoterpene limonene is the major component in citrus essential oils ranging from 32 to 98% [27]. According to the results of the gas chromatography–mass spectrometry analysis of the current study (table 1), limonene recorded the highest percentage of the constituents of bergamot representing 42.87 %. *Subramenium et al, 2015*[28], found that limonene has 75-95 % antibiofilm activity against streptococcus mutans. They stated that limonene is able to inhibit bacterial adhesion to tooth surface and inhibit bacterial acid production in addition to its ability to downregulate several genes (covR, mga and vicR). Those genes play a critical role in regulating surface-associated proteins in streptococcus mutans.

Presence of other constituents as linalyl acetate and trans-Geraniol could potentiates its antibacterial activity [29]. Bergamot essential oil used in the current study is composed of linalyl acetate (26.94 %) and trans-Geraniol (14.91 %) as reported in table 1. Linalyl acetate is able to disrupt the lipid fraction of microorganism plasma membrane, resulting in alterations of cell membrane permeability and leakage of intracellular materials [30]. *Guimarães et al, 2019* [31], reported that alcohol compounds like Trans-geraniol is one of the effective and fast acting antimicrobial agents and attributed this effect to the presence of hydroxyl groups. Another antibacterial mechanism of geraniol was described via its ability to adhere to bacterial cell membrane lipids and interacts with its components and hence increase its permeability in addition to its ability to bind to important intracellular sites resulting in cell destruction [32].

The higher percentage of the previously mentioned constituents of bergamot essential oil in addition to their antimicrobial properties via multiple mechanisms might explain its highest antibacterial results in comparison to other experimental groups.

According to the findings of the current study, peppermint recorded antibacterial activity higher than the control group, however it was the least among all tested essential oil groups. *Sreevidhya, 2014* [33], revealed an antibacterial activity of peppermint against streptococcus mutans. This finding was previously explained by the presence of menthol and carvone constituents[16]. Also, *Ambrosio et al, 2021* [34], reported antibacterial potential and antioxidant activity for essential oils containing menthol and carvone.

Peppermint essential oil used in the current study is composed of 38.73 % Levomenthol, 5-52% neo-menthol and 8.34% I-menthone (table 2). Menthol showed an antibacterial mechanism as that described before for linalyl acetate monoterpene based on its lipophilicity which make it able to disrupt the lipids of microorganism plasma membrane with negative impact on cell permeability [30]. Carvone represents 10.59 % of the chemical composition of peppermint (table 2). It is considered a powerful antimicrobial agent able to penetrate bacterial cell membrane increasing cell permeability [35]. In addition to the presence of minor constituents as linalyl acetate (5.39 %) and limonene (1.74 %).

Lemongrass essential oil showed antibacterial activity higher than the control group which might be attributed to its chemical composition (table 3). *Ilango et al, 2019* [9], reported antibacterial activity of lemongrass essential oil against streptococcus mutans at minimum inhibitory concentration of 10 μl. The major component of lemongrass as reported in table 3 is Z-Citral which represents more than 50% of the components. Z-citral is a monoterpene which causes loss of membrane integrity of bacterial cells increasing cell membrane permeability via hydrophobic interactions with the membrane[36,37]. It could also induce changes in the intracellular pH and intracellular ATP concentration in the bacterial cell as reported by *De Silva et al, 2017* [38].

Presence of carveol as one of the major components of lemongrass (36.39%) plays a great role in its antibacterial activity. It has been previously reported that carveol has a strong antibacterial activity against gram positive bacteria [31,34]. *Lopez-Romero et al, 2015* [39], recorded changes in the hydrophobicity, surface charge and bacterial cell membrane integrity after exposure to essential oils containing carveol.

Fennel reported antibacterial activity against streptococcus mutans higher than control group, lemongrass and peppermint essential oil modified groups. Previous research demonstrated an antibacterial activity of fennel against streptococcus mutans [16]. According to the chemical analysis results of the current study (table 4), the major constituent of fennel EO is estragole representing 92.51 %. *Andrade et al, 2015* [40], reported high inhibition zone of estragole against gram positive bacteria.

Several mechanisms could be responsible for the development of microbial resistance to antibacterial agents as poor diffusion of the antibiotics through the biofilm, phenotypic changes of the cells forming the biofilm and the expression of efflux pumps. Efflux pumps allow the microorganisms to regulate their internal environments by removing toxic substances such as antimicrobial agents and metabolites [41]. Hence, efflux pump inhibitors are important to enhance the efficiency of antimicrobial agents. *Da Costa et al, 2021* [42], demonstrated that estragole is a potent efflux pump inhibitor.

It has been reported that the biological activity of the major components of essential oils is modulated by the minor components. The synergism among the major and minor components potentiates the antimicrobial activity of the essential oils [34,43]. *Alexa et al, 2019* [14], highlighted the effect of synergism of different components by testing antibacterial activity of different essential oils in binary and tertiary mixtures where they reported higher results than using essential oil in a single form. The findings of the current study cannot be directly correlated with other studies since, no previous researches investigated the effect of essential oil modification of GIC using bergamot, peppermint, lemongrass and fennel on the antibacterial activity.

Though the tested essential oils improved the antibacterial potential of glass ionomer cement, it is important not to jeopardize its mechanical properties. Compressive strength results demonstrated a significant decrease for all essential oil modified GIC groups in comparison to the control group. *Sherief et al, 2021* [44], reported low compressive strength of glass ionomer cement modified using cinnamon and thyme essential oil. They explained this finding by the disability of essential oils to chemically bond to the glass and the polyalkenoate matrix resulting in high structural micro-porosity and diminished strength properties. Another explanation was attributed to the fact that cross-linking in GIC occurred due to coordination of Al^{3+} and Ca^{2+} with the COOH groups of the polyacrylic acid. Presence of essential oil within GIC deprives many COOH groups from interlinking within the glass ionomer matrix and causes deceleration of the setting reaction which adversely affect the compressive strength [10, 44]. In addition, changing the powder/liquid ratio of GIC by adding essential oil to polyacrylic acid during material manipulation could adversely affect its mechanical properties [10].

Among essential oil modified groups, lemongrass recorded the highest compressive strength value followed by peppermint then bergamot and the least value was demonstrated by fennel. This might be attributed to chemical structure of different components of the tested essential oils. Compounds containing functional OH and COOH groups as those present in the chemical structure of glass ionomer for inducing acid-base reaction between glass particles and polyacrylic acid might improve compressive strength of GIC [45]. Compressive strength results (table 6) demonstrated that lemongrass (116.20±5.61) and peppermint (105.28±7.91) essential oils modified GIC groups recorded mean values above 100 MPa. Although these values were significantly lower than the control group, it satisfies the requirement of the ISO standards 9917-1:2007 which reported that compressive strength of glass ionomer cements should not be less than 100 MPa [46].

Among essential oil modified groups, bergamot and fennel recorded higher antibacterial activity in comparison to lemongrass and peppermint while, for compressive strength results, lemongrass and peppermint showed higher results. It seems like there is an inverse relation between antibacterial activity and compressive strength. In the agar diffusion test, the diameter of inhibition zone is greatly affected by the solubility and diffusion characteristics of the substances being tested [47]. Although solubility of glass ionomer and rate of diffusion of the essential oil might positively affect the antibacterial activity, they also negatively affect the compressive strength.

CONCLUSIONS

Within the limitations of the current study, it could be concluded that essential oil modification of GIC was effective in enhancing antibacterial activity although it adversely affects compressive strength in comparison to the control group. Lemongrass and peppermint essential oil modification of GIC was effective in improving antibacterial property while preserving an acceptable compressive strength according to the ISO standards. Nevertheless, further studies should be conducted to assess the effect of the tested essential oils in different concentrations on other mechanical, physical and optical properties.

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