

Influence of Adding Bergamot Essential Oil Against *Staphylococcus Aureus* Activity and Some Properties of Dental Stone

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Abstract

An extremely critical field in dentistry to be investigated is the cross-contamination between dental patients and dental health care personnel regarding which dental cast is considered as a medium. Many researchers suggested the disinfection of dental stone by incorporating a certain disinfectant in the mixing liquid to gain antimicrobial activity while preserving acceptable properties of the material. This study was aimed to evaluate the antibacterial activity of bergamot essential oil incorporated in mixing liquid of type III dental stone against *S. aureus* and to evaluate the compressive strength and reproduction of details properties of the incorporated stone material. **Methods:** A total of 90 specimens, 30 specimens for each test with dimensions of 5 mm in diameter and 3 mm in thickness for antibacterial disk susceptibility test, 20 mm in diameter and 40 mm height for compressive strength test and 30 mm in diameter and 20 mm height for reproduction of details test were prepared by addition of various percentages of BEO (8% and 10% by volume) and 0% as a control group. After isolation and identification of *S. aureus*, antibacterial tests were carried out to determine the minimum inhibitory concentration of the essential oil by broth micro dilution method, followed by both direct colony count and antibacterial disk susceptibility tests. Dental stone specimens were tested for compressive strength after 1 hour from the start of the mix. Regarding reproduction of details quality, specimens were evaluated by optical microscope. **Results:** Antibacterial tests revealed that a concentration of 8% is effective against *S. aureus*. The essential oil increased the compressive strength of dental stone and had no adverse effect on the reproduction of details quality. **Conclusion:** Bergamot essential oil could act as a potent antibacterial agent against *S. aureus* to obtain a disinfected dental cast with improved compressive strength and unaffected reproduction of details quality.

Keywords: Bergamot essential oil, *Staphylococcus aureus*, Type III dental stone, Compressive strength, Reproduction details.

Introduction

Dental stone is one of the widely utilized Gypsum materials in dentistry as a pouring material for reproducing dental casts from dental impressions, because it possesses excellent compatibility with any impression material. In addition to other desirable qualities which most importantly include accuracy, dimensional stability and durability, however, dental stone misses an important property of being antimicrobial (Sakaguchi et al., 2019). When dental impressions are not sterilized or disinfected due to multiple difficulties, it will result in reproducing infectious dental casts that act as a medium for cross-contamination (Leung and Schonfeld, 1983), this is one of the essential issues to be investigated nowadays due to the fact that it occurs mainly between dental patients and dental health care personnel especially dental laboratory personnel (Kohn et al., 2003). For this issue to be solved many researchers suggested the disinfection of dental stone by incorporating a disinfectant chemical into the mixing liquid (Breault et al., 1998; Abdelaziz et al., 2002a; Abdelaziz et al., 2002b).

Essential oils which are represented as naturally occurring, volatile, aromatic mixtures that possess antibacterial, anti-fungal, and antioxidant properties which gave them the access into pharmaceutical industries (Guenther, 2008; Baratta et al., 1998; Mawlood and Naji, 2021). Bergamot essential oil is one of these oils, its antibacterial properties have been studied against a variety of microorganisms such as *S. aureus* showing promising results (Sánchez-González et al., 2010). Such results have made bergamot essential oil a good candidate for the present study which explored its antibacterial effect after incorporation in the mixing liquid of dental stone against *S. aureus*, In addition to determine the effects of adding BEO on the compressive strength and the reproduction of details properties of the stone material.

Materials and methods

Five *S. aureus* specimens were obtained from patients' throat swabs at the Department of Prosthodontics, College of Dentistry, University of Baghdad. Then, the bacteria were isolated and identified to carry out this study.

- Minimum inhibitory concentration was carried out in accordance with the Clinical and Laboratory Standards Institute by broth micro dilution method experimenting ten concentrations in two fold serial dilutions ranging from 64% to 0.125% and 0% as control and repeated thrice for each bacterial specimen.

Bacterial inoculum was prepared by direct colony suspension method from 18-24 h culture with reference to 0.5 McFarland standard to achieve an inoculum of $1-2 \times 10^8$ (CFU)/mL then diluted in muller Hinton broth, so that the final inoculum density is approximately ranging from $2-8 \times 10^5$ CFU/mL (Wikler et al., 2012).

A 100 μ L of mueller Hinton broth was dispensed into columns of wells in micro titer plate utilizing micropipette excluding the second column, 200 μ L of 64% essential oil dilution was added to the second column and then two fold serial dilution was performed from third to eleventh column of wells. Around 10 μ L of bacterial inoculum was dispensed into wells except for twelfth column which act as negative control instead a 100 μ L of nutrient broth was dispensed into it. First column of wells was considered as a positive control with mueller Hinton broth and bacterial inoculum only, while twelfth column as negative control with mueller Hinton

broth and nutrient broth only. Then plates were incubated for 18-24 h at $35\pm 2^{\circ}\text{C}$. After incubation, plates were inspected by the naked unaided eye for the absence of turbidity, the lowest concentration that yielded a clear broth with no turbidity and no visible bacterial growth was determined as the minimum inhibitory concentration.

- Direct colony count test was carried out to certify the inhibition efficacy of the determined minimum inhibitory concentration. Mueller Hinton agar plates were inoculated with 100 μL aliquot removed from wells and diluted it in 10 mL of saline (1:1000 dilution). Then spread over the surface of media by a disposable loop, the plates were incubated for 18-24 h at $35\pm 2^{\circ}\text{C}$.

- Antimicrobial disk susceptibility test was carried out in accordance with the clinical laboratory and institute standards. Silicone duplicating material was utilized to make a duplication mold that produce dental stone disks measuring 3 mm height and 5 mm in diameter (de Paula Pereira et al., 2011). Dental stone manipulation was carried out in accordance with the manufacturer's instructions, duplication mold was gently vibrated to avoid air entrapments during dental stone pouring. After 45 min, the disks were separated from the mold, then the test was carried out.

Bacterial inoculum was prepared by direct colony suspension method from 18-24 h culture with reference to 0.5 McFarland standard to achieve an inoculum of $1-2 \times 10^8$ (CFU)/mL. Optimally, within 15 min following turbidity adjustment, mueller Hinton agar plates were inoculated with bacterial inoculum by a sterile cotton swab, the stone disks were applied onto the surface of the inoculated agar utilizing a sterile forceps, then the disks were pressed down to ensure complete contact with the agar surface (Cockerill et al., 2012). Plates were incubated for 18-24 h at $35\pm 2^{\circ}\text{C}$, after which inhibition zones were measured in millimeters, test was repeated thrice for each bacterial specimen (Figure 1).

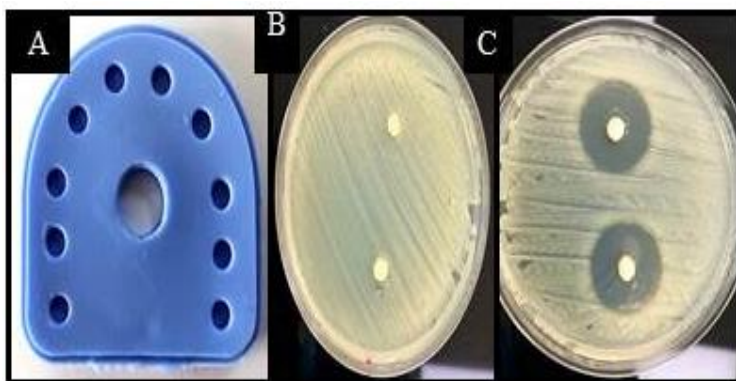


Figure 1: (A) Duplicating mold; antibacterial disk susceptibility test for (B) 0%; and (C) 8% concentrations of BEO

Regarding compressive strength and reproduction details tests, three concentrations of bergamot essential oil 0%, 8% and 10% were investigated for purposes of results comparisons.

- Compressive strength test was carried out in accordance with the revised ANSI/ADA standard No. 25-2015, split metal mold constructed from stainless steel material was utilized to construct

a total of 30 cylindrical specimens, 10 for each subgroup, each specimen measures 20 ± 0.2 mm in diameter and 40 ± 0.4 mm high. After coating the mold inner surfaces with a thin layer of non-reacting mold releasing agent, mixed dental stone was poured down the side of the inclined-placed mold until slightly overfilled while being gently vibrated for 30 sec in order to minimize air bubbles entrapment. Then the mix was leveled before loss of gloss in order to be flush with the top of the mold by placing a glass plate firmly into contact to produce equal and parallel flat specimens tops. About 45 ± 1 min later from start of mixing, mold halves were split and specimens released and stored in ambient air at $23 \pm 2^\circ\text{C}$ and relative humidity of $50 \pm 10\%$ until they were crushed 1 h after the start of mixing (wet strength). Specimens were tested utilizing Instron universal testing machine so that increasing load was applied until failure occurred at a rate of 5 ± 2 KN/min. The maximum load applied was utilized to calculate the value of compressive strength for each specimen involved from the formula as follows: $CS = F/A$, where F is the maximum load applied and A is the area of circle (πr^2). The data were analyzed using 1-way ANOVA then Tukey's honest significance test as a post-hoc test for comparison between either two of the concentrations utilized (Figure 2).

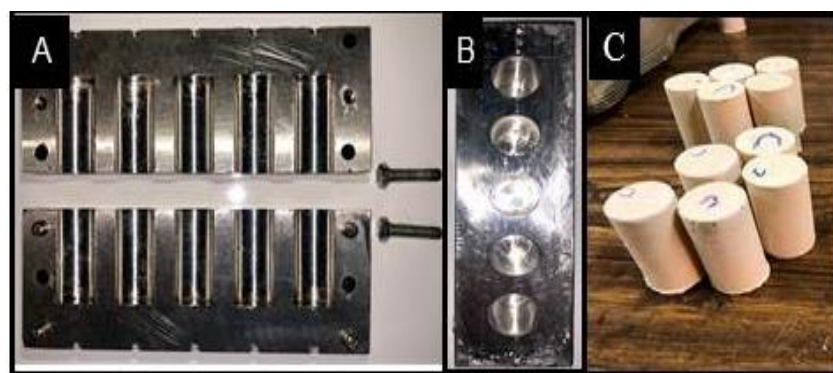


Figure 2: Split mold utilized for specimens preparation for compressive strength test (A) Split; (B) Assembled; and (C) Dental stone specimens

- Reproduction of details test was carried out in accordance with the revised ANSI/ADA standards No. 25-2015 by using custom made test equipment consist of a test block, a ring mold and a slit mold that were designed utilizing AutoCAD software in order to be printed by Max UV385, Asiga 3D printer to ensure accuracy. The block was printed with 3 straight grooves spaced 2.5 mm apart. These grooves differed in width $50 \pm 8 \mu\text{m}$, $20 \pm 4 \mu\text{m}$ and $75 \pm 8 \mu\text{m}$ but had the same peak angle of $90^\circ \pm 5^\circ$. Impressions for this test block were made using a silicone duplicating material poured into the ring mold. After adaptation of slit mold on the ring mold with set duplicating material, it will be overfilled with the mixed dental stone while being vibrated to prevent air bubbles entrapment. A total of 30 specimens were made, 10 for each subgroup. The set specimens were examined under optical microscope with 4X magnification to reveal whether a complete or incomplete $50 \mu\text{m}$ -wide groove reproduction has occurred in order to decide whether the specimen had fulfilled the test requirement.

Results

- For minimum inhibitory concentration test, the Concentrations greater or equal to 8% revealed clear wells with no turbidity when examined by the naked, unaided eye suggesting that the minimum inhibitory concentration is 8%.

- While for direct colony count test, the descriptive statistics and statistical test of direct colony count test results utilizing unpaired t-test for comparison of mean values of 0% and 8% concentrations revealed a highly significant difference between the groups ($P < 0.01$) as listed in Table 1.

Table 1: Descriptive statistics of direct colony count, utilizing unpaired t-test

Group	N	Mean (CFU/mL)	Minimum	Maximum	±SD	P-value
0% BEO	15	85.47	49.67	169	47.78	0.00 (HS)
8% BEO	15	11.53	5	21.67	6.75	
df=8 Levene's test =4.4						

- Regarding antimicrobial disk susceptibility test, the results of antimicrobial disk susceptibility test which utilized by unpaired t-test for comparison of mean values of 0% and 8% concentrations revealed a highly significant difference between the groups ($P < 0.01$) (Table 2).

Table 2: Descriptive statistics of disk diffusion test, utilizing unpaired t-test

Group	N	Mean (mm)	Minimum	Maximum	±SD	<i>P-value</i>
0% BEO	15	0	0	0	0	0.00 (HS)
8% BEO	15	19	16	22.33	2.32	
df=4 Levene's test =5.85						

- For compressive strength test, the results revealed that both experimental groups had a higher mean value in comparison to control specimens, whereas the specimens with 10% of BEO revealed the highest mean value (Table 3). The statistical difference among three groups and between two experimental groups was highly significant ($P < 0.01$).

Table 3: Descriptive and statistical test of UCS, utilizing 1-way ANOVA

Group	N	Mean (MPa)	Minimum	Maximum	±SD	<i>F</i>	<i>P-value</i>
0% BEO	10	27.89	26.67	28.96	0.78	119.72	0.00 (HS)
8% BEO	10	31.74	30.31	32.93	1.00		
10% BEO	10	33.73	32.19	34.81	0.77		
df=2 Leven's test=1.63							

- For reproduction of details test, the results revealed that all the specimens of the 0%, 8% and 10% concentrations had met the test requirement via reproducing a complete 50 µm-wide groove as shown in Figure 3.

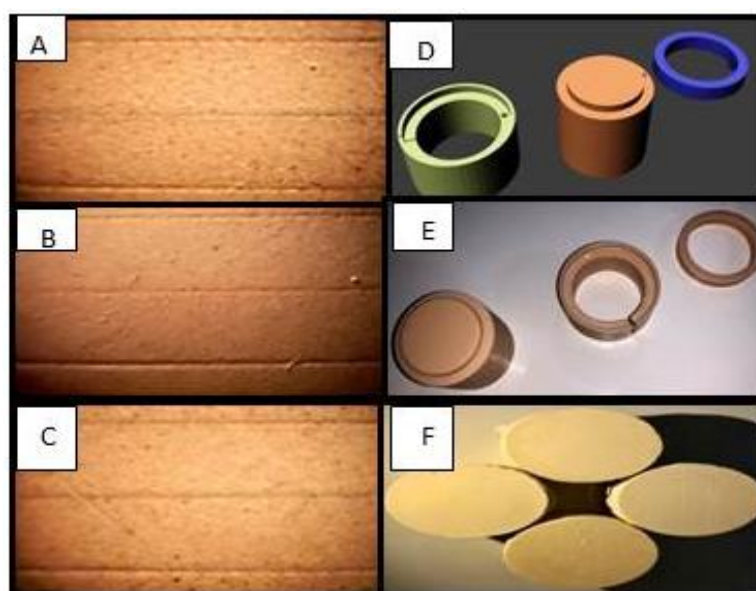


Figure 3: Reproduction of details specimens under optical microscope in case of (A) 0%; (B) 8%; (C) 10%; (D) The test equipment design in Auto CAD; (E) The printed test equipment; (F) Dental stone specimens

Discussions

As a result of the world-wide dissemination of the dangers of cross contamination, there is keen awareness of the needs for asepsis in all medical and dental practices. Infections pose a greater threat to health, most notably in health care personnel, hence the need effective sterilization and disinfection against microbial agents is growing (Sarma and Neiman, 1990). The microbiological efficiency of the bergamot essential oil has been established previously (Mawlood and Naji,

2020). In vitro, bergamot essential oil activity against *S. aureus* suggests its potential role in disinfection (Navarra et al., 2015). This antibacterial activity can be explained in general by the important characteristics of essential oils. The essential oil compounds sensitize the cell membrane, causing an increase in permeability and leakage of vital intracellular constituents, as well as the impairment of bacterial enzyme system and cell respiration (Singh et al., 2002; Moreira et al., 2005). Also this antibacterial activity is linked to well-recognized antimicrobial compounds which can be distinguished in volatile which constitute 4-7% and non-volatile compounds which constitute around 93-96% of bergamot essential oil composition (Zaynoun et al., 1997; Navarra et al., 2015). Some authors considered the major compounds of bergamot essential oil the base for its antimicrobial activity and the possible synergistic effect of these major compounds is another issue that can be taken into consideration (Pernice et al., 2009; Navarra et al., 2015; Salvo et al., 2015). These compounds include Linalyl acetate, limonene, linalool and γ -terpinene, which their antibacterial efficacy has been proven by many studies (Andoğan et al., 2002; Espina et al., 2015; Herman et al., 2016; Semeniuc et al., 2017). Hence it was suggested that bergamot essential oil incorporation into dental stone mixture would yield a biologically safe cast. However, addition of such substances may affect the mechanical and physical properties of dental stone.

In the present study, the findings of ultimate compressive strength are in agreement with Breault et al. (1998) who concluded that partial substitution of mixing liquid with 5.25% sodium hypochlorite significantly increase the compressive strength. Such an improvement may be attributed to the fact that the compressive strength is inversely proportional to L/P ratio of the mix, the less the liquid utilized to a certain point in the mixing process, the higher the compressive strength because of porosity reduction, also reduction in voids and porosity generally provides a denser dental stone structure, such an increase in density leads to a higher compressive strength value, however, the incorporation of 8% and 10% concentrations may have compensated for such liquid reduction, but such compensation appeared to be incompletely effective may be due to the volatile nature of almost 93-96% of essential oil compounds, as a result experimental groups revealed such an increase in compressive strength similar to that occurring in case of L/P ratio reduction. This finding is disagreement with Abdelaziz et al. (2002b) who concluded that partial substitution of mixing liquid with various disinfectants each at time result in reducing compressive strength value.

In this study, the findings of reproduction of details were in agreement with Al-khafaji et al. (2013) who concluded that the immersed or sprayed specimens with the SOLO and NaOCl disinfection solutions showed no signs of deterioration of surface details of type III, type IV and type IV extra hard dental stone. In addition, other studies were done by **Abdelaziz et al. (2002a)** and **Lucas et al. (2009)** revealed that there was no significant difference between the experimental and control group in the terms of detail reproduction. Such a finding can be attributed to the fact that fast, accurate and careful manipulation involving the incremental application of dental stone mixture into the slit mold while being vibrated in order to minimize air bubble entrapment and to ensure the highest possibility for the grooved surface to be reproduced accurately especially the 50 μ m-wide groove, in addition to the relatively small size of specimens. Additionally, the incorporation of 8% and 10% of BEO seems not been compensated by such incorporation due to the volatile nature of the essential oil compounds.

Conclusions

Within the limitations of this study; it can be concluded that the natural product of bergamot essential oil was successfully incorporated in the mixing liquid of type III dental stone have an antibacterial efficacy against *S. aureus*, whilst it revealed a significant increase in the compressive strength values of the material and had no effect in the reproduction of details quality for both experimented concentrations.

Conflict of interest

The authors have no conflicts of interests relevant to this article.

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