

Effect of Eucalyptol globulus oil on antifungal activity and some mechanical properties of conventional heat-cured acrylic resin (in vitro study)

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ABSTRACT

Background. Soft denture liners frequently experience bacterial and fungal colonization, particularly by *Candida albicans*, which can result in infection, discomfort, and failure. The use of herbal remedies to treat fungal resistance is growing, especially in the dental field.

Aim. The purpose of the study was to determine how well Eucalyptus globulus oil inhibited *Candida albicans* colonization in a heat-cured acrylic denture base material, as well as to evaluate the acrylic's hardness and impact resistance after oil incorporation.

Materials and methods. A total of 90 samples were created by adding Eucalyptus globulus oil (3% and 6% by volume) to heat-cured PMMA denture base material. The samples were separated into three groups based on the tests (30 samples for anti-*Candida albicans* activity, 30 for impact strength, and 30 for surface hardness). Each group was divided into three subgroups (control 0% without additive, experimental with 3% and 6% oil additive). All samples were evaluated after 48 hours of incubation in distilled water. Effect sizes were calculated to determine the magnitude of differences, and 95% confidence intervals were provided to quantify the precision of the estimates. Statistical analysis involved the use of SPSS version 26. One-way ANOVA and Tukey post hoc test at a significance level of $p = 0.05$ were conducted to determine the differences between groups.

Results. The experimental groups revealed a significant decrease in the viable *Candida albicans* cell count (5.49343 CFU/mL and 6.83862 CFU/mL for 3% and 6% groups respectively) compared to the control group (11.31420 CFU/mL). Impact strength was decreased significantly for both experimental groups especially for 6% experimental group (1.90919 kJ/m²). Shore D hardness was decreased non-significantly, with lowest value recorded for 6% experimental group (42.028) followed by 3% experimental group and the control group.

Conclusion. Achieving an optimal balance between antifungal efficacy and mechanical properties is fundamental for maximizing the performance of Eucalyptol globulus oil reinforced denture bases in dental applications.

Keywords: acrylic denture base, *Candida albicans*, denture base materials, Eucalyptus globulus

INTRODUCTION

Because of its numerous advantages, including good aesthetics, simplicity of manipulation in clinical and laboratory settings, suitable fitness and stability in the oral environment, and low-cost equipment, polymethyl methacrylate is the material most commonly used to create denture bases [1]. The

denture is a source of many infections, though, because of a number of problems, such as microbial colonization and biofilm formation on its surface, which makes it difficult to remove the biofilm effectively, especially in older patients with weakened manual dexterity [2]. Up to 65% of denture wearers may develop denture stomatitis, a condition associated with denture usage that is classified as chronic

atrophic oral candidiasis. The etiology of denture stomatitis is multifaceted, but candida albicans is thought to be the cause [3].

The most popular treatment for denture stomatitis is the prescription of topical antifungal medications; however, the use of these medications is restricted, and it can be difficult to maximize their benefits in elderly patients with impaired cognitive function due to their lack of motor dexterity and inability to maintain an optimal oral drug dose. It is preferable to include the antifungal medications into the denture base materials in order to get over these challenges. Regretfully, the side effects and fungus resistance of these medications force the need for organically produced medications to replace these manufactured ones [4].

Many studies that are testing the antifungal efficacy of plant oils and herbal remedies against Candida albicans have recently been conducted, and the results indicate that these oils are regarded as a promising therapeutic option with potent antifungal qualities that can be used to treat denture stomatitis [5-6].

The Myrtaceae family includes the species Eucalyptus globulus. The essential oil of this plant is used extensively across the world for a number of purposes, including as perfumes, medicines, culinary products, and cosmetics. The oil has been shown to have antibacterial, anti-inflammatory, and antioxidant properties [7]. Its antibacterial efficacy against a wide range of germs has been attributed to a number of factors, including the ability to destroy cell walls and membranes and induce ATP and metabolite leaks, even if the exact mechanism of action is

unknown. Furthermore, the oil's hydrophobic properties promote greater cell permeability, which results in the leaking of bacterial cells [8]. But research on this topic is still lacking, so it's uncertain how effective it is against Candida albicans.

MATERIALS AND METHODS

Materials

The materials used in the study are presented in Table 1.

TABLE 1. Materials used in the study

Material	Manufacturer
Regular – conventional heat curing denture base material (powder and liquid)	Vertex, Netherlands
Eucalyptus globulus	USA

Study design

Ninety (90) samples were prepared and split into three groups for each test based on the concentrations of oil to be added to the acrylic (control group: 0%, 3%, and 6%). These groups were used for the various tests (Vickers hardness, impact strength, and Candida adherence test) that were carried out as part of this investigation.

Sample fabrication

Molds for acrylic specimens were prepared using three distinct plastic designs. For antifungal activity test, plates measuring of 10×10×2 mm length, width and thickness respectively. For impact test, 80mm in length, 10mm in width, and 4mm in thickness plates were used. Finally, for shore A hardness,

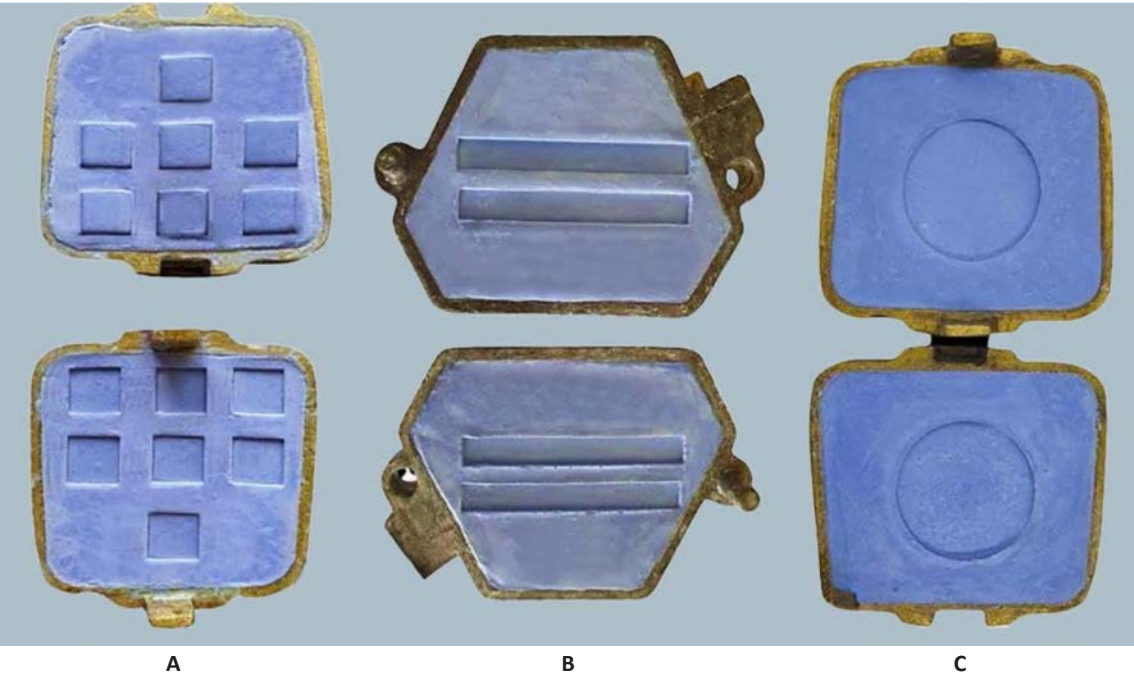


FIGURE 1. Mold spaces for packing the PMMA. A. Antifungal test; B. Impact strength; C. Shore D hardness

40mm in diameter and 4mm in thickness plates were used.

In order to create these plastic patterns, plastic plate was laser-cut into the required shape and size in accordance with the test specifications [9]. The processing method for acrylic specimens and complete dentures is the same. The plastic patterns are coated with the separating media (Shanghai New Century Dental, Materials Co., Ltd, China), which is then let to dry. After preparing the dental stone as directed by the manufacturer (100g powder to 20 ml water), it was vibrated on a vibrator (Bego, Germany) and then added to the lower half of the flask (HANUA, Engineering Crop., USA) and vibrated to eliminate any air bubbles. After reducing the depth of the plastic designs to roughly half, the stone was allowed to set. The lower half is then covered with separating media and allowed to dry, before the upper part is put and filled with stone, vibrated, and the flask lid is placed and left to set. Following the stone setting, the two halves are split, and the plastic patterns are removed to create a mold hole for the acrylic specimen. The separating media is then added and allowed to dry, and the flask is ready for packing the acrylic specimen as in Figure 1.

In order to create control heat-cure PMMA samples, the necessary quantity of liquid and powder acrylic was poured to a dry, clean glass beaker, sealed, and allowed to reach the dough stage for 30 minutes. The acrylic was removed from the mixing jar and poured into the cavity of the previously coated mold. The two halves of the flask were then sealed, and the sealed flask was placed under the hydraulic press. The flask was then clamped using flask clamps (HANUA, Engineering Crop., USA) and allowed to cure. The flask was tightly closed at 100 psi. When the water bath reaches a temperature of 100 degrees Celsius, the clamped flasks are placed in it. The water bath (Mettmert, Germany) is then turned off for 15 minutes, opened for 20 minutes, and the clamp is kept in the water until it cools. Lastly, the flask is carefully opened, and the acrylic specimens are extracted.

In respect to the Eucalyptol-incorporated samples, the necessary oil was measured using a micropipette, subtracted from the monomer volume, and then manually mixed with the monomer in a dry, clean glass beaker. After that, this mixture was added to the acrylic powder and mixed as described before.

All acrylic resin specimens were finished using finishing burs and polished on a lathe polishing machine (Bego, Germany). To prevent overheating and distortion of the specimens, the polishing machine was set to 1500 rpm, and the polishing was done with a rage wheel until a glossy finish was attained using pumice mixed with water.

Isolation of *Candida albicans*

Candida albicans isolates were collected from the oral cavity of 20 male patients aged 60 to 65 years old who wore full dentures and showed evidence of denture stomatitis. Sterile cotton swabs were used to gently treat the intraoral lesions. Sabouraud dextrose agar media were made by dissolving 65 grams of Sabouraud dextrose agar powder (Oxoid, United Kingdom) in 1000 mL of distilled water. The mixture was autoclaved in an autoclave (Tuttnauer, USA) for 15 minutes at 121°C. After cooling for 30 minutes, the antibiotic chloramphenicol (Brawn, India) (2 grams per 1000 mL of medium) was applied. The medium was placed into petri dishes (JRZ plastilab, Lebanon), solidified, and then aerobically incubated in an incubator (Mettmert, Germany) at 37°C for 24 hours before being refrigerated (Samsung, Korea) at 4°C.

Identification of *Candida albicans*

Candida albicans colonies on Sabouraud dextrose agar looked convex, smooth, creamy, and pasty. A little amount of colony was emulsified in a drop of saline on a glass slide. The suspension was distributed and stabilized by running the slide through a Bunsen burner flame. Gram staining was done with a Gram staining kit (Hardy Diagnostics, USA). The slide was stained with crystal violet stain for 1 minute, then washed with distilled water. Gram's iodine stain was applied for one minute, then washed. Acetone alcohol was used to decolorize the solution until it was colorless, after which it was rinsed with water. Safranin was used to counterstain for 1 minute before being washed and dried. *Candida albicans* emerged as Gram-positive round or oval yeast-like cells under a light microscope.

Fungal adherence test

Sabouraud dextrose broth was made as directed by the manufacturer by dissolving 30 grams of Sabouraud dextrose broth powder in 1000 mL of distilled water. The media were sterilized in an autoclave at 121°C/15 pressure for 15 minutes, then cooled for around 30 minutes. Chloramphenicol (2 grams per 1000 mL of medium) was added, and the mixture was put into sterilized glass tubes. Isolated *Candida albicans* were suspended in the Sabouraud dextrose broth. The suspension's concentration was correctly determined using a McFarland densitometer, which is comparable to 0.5 McFarland standards.

To assess the antifungal efficiency of Eucalyptus globulus loaded materials, a viable count test was conducted using a *Candida albicans* solution of about 107CFU/mL, equivalent to 0.5 McFarland

standards. This was accomplished by diluting a small number of inoculums in a test tube containing normal saline and measuring the solution with a McFarland densitometer device, and then adding more normal saline or inoculum to decrease or increase the density of the solution until 0.5 McFarland was obtained.

To prepare a 10 mL broth combination with a final cell concentration of 105 CFU/mL, add a suspension to each tube. The samples were submerged in tubes and incubated for 24 hours at 37°C. After incubation, use a Vortex shaker (Janke & Kunkel, Germany) to create a homogeneous mixture. Take 100µl of this mixture and add it to another tube with 9.9 mL of normal saline to achieve a tenfold dilution. A second dilution is created using the same technique as the first.

Approximately 100 µL of the second dilution was distributed on SDA plates using disposable inoculation loops in a zigzag pattern to count candida colonies. The SDA plates were incubated aerobically at 37°C for 24 hours. This dilution was chosen for the countable range of 30-300 CFU [10]. After incubation, *Candida albicans* colonies on SDA plates were enumerated and evaluated statistically to determine the material's antifungal effectiveness (AFE) using the following formula:

$$AFE\% = \frac{C-T}{C} * 100 \quad (1)$$

Where C is the number of colonies in the control (untreated) sample. T is the number of colonies in the treated sample.

To ensure accuracy, the counting technique involved flipping closed petri dishes upside down and using a black marker to check out each colony counted. The plates were restored to their original location, and the overlapping colonies were counted and added to the first viable count to get the final count.

Impact strength test

Acrylic resin specimens of 80mm in length, 10mm in width, and 4mm in thickness were used to test impact strength (ISO 179, 2000). The impact strength test was carried out using the technique indicated by (ISO No. 179,2000) and the impact testing apparatus shown in Figure 2. Each specimen was horizontally supported at one end and held in place by a 2 Joule swinging pendulum. The scale displays the amount of impact energy in joules. The Charpy impact strength was determined in kilojoules per square meter using the following equation:

$$\text{Impact strength} = \frac{E}{b.d} * 10^3 \quad (2)$$

where E is impact energy measured in joules, b means the width in millimeters and d is the thickness of samples in millimeters

Hardness

The test was performed according to ASTM D2240, by using a durometer hardness tester (shore D hardness) shown in Figure 2. The instrument indented the surface of the specimen at load applied equal to 50N and depressing time equal to 15sec.

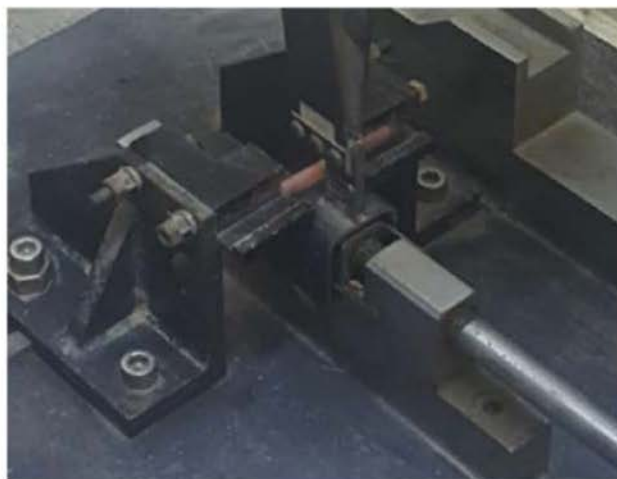


FIGURE 2. Charpys impact strength tester

The hardness value is displayed on the device's dial scale, which is graduated from 0 to 100. Five standardized points were selected for each specimen with 6mm apart from each other and the average reading of these five points was recorded as presented in Figure 3.



FIGURE 3. Shore D hardness durometer tester

TABLE 2. Statistical tests for candida adherence test

Candida albicans adherence test CFU/mL					ANOVA		Tukey HSD	
Group	Min	Max	Mean	±SD	F	P value	Groups	P value
Control group	121.00	164.00	141.7000	11.31420	435.364	0.000	Control group/ 3% group	0.000
3% group	43.00	66.00	51.1000	6.83862			Control group/ 6% group	0.000
6 % group	34.00	56.00	43.8000	5.49343			3% group/ 6% group	0.138
Levene statistics=1.717, p value=.199 [NS]								

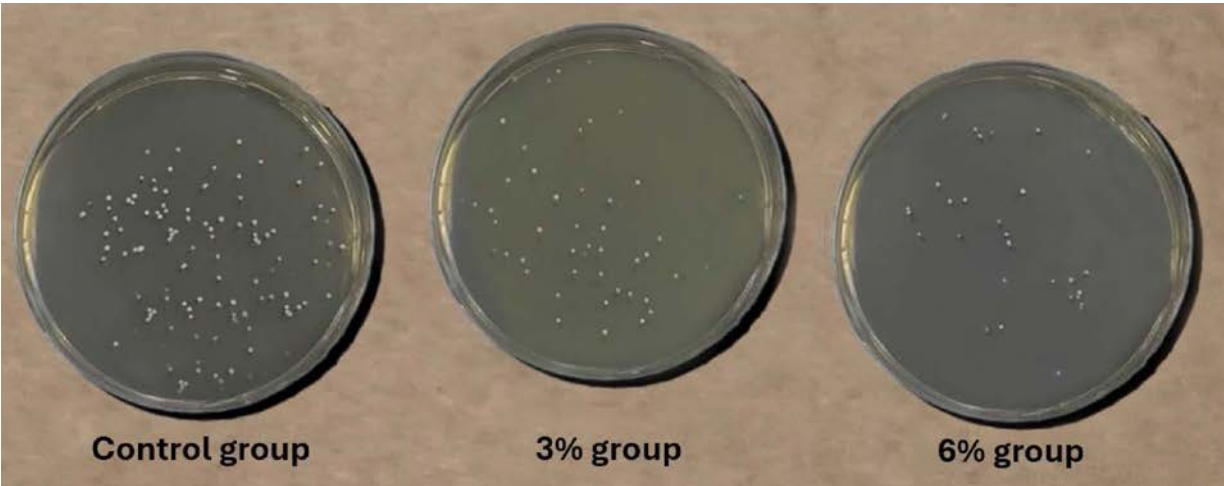


FIGURE 4. Viable count of *Candida albicans*

RESULTS

Candida albicans adherence test

After 48 hours of incubation in distilled water, the viable counts of *Candida albicans* in both experimental groups (3% and 6%) were lower than the control group. The 6% group had the lowest mean value of 43.8 CFU/mL (Table 2) and (Figure 4). There were statistically highly significant differences between control group and the experimental groups ($P < 0.01$); however, there was non-specific difference between 3% group and 6% group ($P > 0.05$).

Impact strength test

After 48 hours of incubation in distilled water, impact strength tests revealed that both experimental groups (3% and 6%) had lower mean values than the control group, with the 6% group having the lowest mean value of 8.35 Kj/m² as shown in Table 3. There was a significant difference only between control group and 6% group.

Shore D hardness

Surface hardness tests after 48 hours of incubation in distilled water revealed that both experimental groups (3% and 6% groups) had lower mean values than the control group, with the experimental group with 6% having the lowest mean value of 42.028 as indicated in Table 4. However, there as

TABLE 3. Statistical tests for impact strength test

Impact strength kJ/m²					ANOVA		Tukey HSD	
Group	Min	Max	Mean	±SD	F	P value	Groups	P value
Control group	7.51	16.00	11.5510	2.94664	4.376	0.023	Control group/ 3% group	0.098
3% group	5.00	13.00	9.1200	2.60973			Control group/ 6% group	0.023
6 % group	5.00	11.00	8.3500	1.90919			3% group/ 6% group	0.776
Levene statistics=1.443, p value=.254 [NS]								

TABLE 4. Statistical tests for shore D hardness test

Shore D hardness test					ANOVA	
Group	Min	Max	Mean	±SD	F	P value
Control group	40.00	49.00	45.1420	3.11795	2.146	0.136
3% group	40.00	49.10	44.5100	3.04428		
6 % group	35.00	49.28	42.0280	4.34601		
Levene statistics = 0.765, p value = 0.475 [NS]						

non-significant differences between the three groups ($P > 0.05$).

DISCUSSION

Achieving an optimal balance between antifungal efficacy and mechanical properties is fundamental for maximizing the performance of Eucalyptus globulus oil-reinforced denture bases in dental applications. The increase in antifungal property after the addition of Eucalyptus globulus can be related to its components such as eucalyptol (1,8 cineole), alpha-pinene and limonene. These components have strong antifungal properties and can have an effect on several fungal mechanisms that cause disruption in the integrity of the fungi [11]. Eucalyptol can incorporate itself into the lipid bilayer of the fungal cell membrane and disrupts the permeability causing leakage of cellular contents such as metabolites and ions [12]. In addition, the hydrophobic property of eucalyptol and alpha-pinene can also disrupt the membrane integrity of the fungal cell and can disrupt energy dependent processes and impairing important metabolic mechanisms [13]. Another factor is that eucalyptol and limonene have the ability to inhibit the synthesis or directly interact with complex cell wall structures such as glucans and chitin. This effect weakens the cell and make it more susceptible to mechanical damage by osmotic pressure which leads to lysis of the cell and ultimately death [14].

Furthermore, these essential oils interrupt with the normal metabolic performance of Candida cells. Eucalyptol and limonene can inhibit the development of an important element fungal membrane which is Ergosterol thus can damage the integrity of the cell membrane [15]. Moreover, Eucalyptol and limonene have the ability to restrain cell division

and proliferation by interfering with the enzymes actions that are vital to fungal reproduction. Such interaction with structural and functional mechanisms of fungal cells, these oils efficiently inhibit Candida albicans growth and survival [16].

The effects of Eucalyptus globulus are concentration dependent. At low concentrations, such as 3%, the essential oil has antifungal properties through interrupting cell membranes and metabolic activities, however the amount of damage is limited. However, at higher concentrations, the increased quantity of antifungal agents can result in considerable damage to cell structure of the *Candida albicans*. This higher number of essential oils means that more fungal cells are subjected to lethal amounts of antifungal agents which results in a substantial decline in fungal growth. The higher dose improves the overall antifungal activity by causing more damage that results in substantial cellular permeability and metabolic disturbance [17].

Adding Eucalyptus globulus to heat-polymerized polymethyl methacrylate enhances the antifungal effects while also affecting its mechanical properties, especially decreasing impact strength and slightly increasing Shore D hardness. The decrease in impact strength, specifically at higher concentrations such as 6%, can be attributed to a variety of variables relating to the interaction of the essential oils with the PMMA polymer matrix. Essential oils including limonene, α-pinene, and eucalyptol have a substantial plasticizing impact. These oils serve as plasticizers, inserting themselves between polymer chains to reduce intermolecular pressures. While this initially enhances the material's flexibility, it also makes the PMMA matrix less stiff and more vulnerable to impact pressures, resulting in reduced impact strength [18,19].

The decrease in hardness can be attributed to the reduction in cross linking density of the PMMA by interfering with the formation of chemical links between polymer chains which can lead to a less rigid network [20]. In addition, the flexible nature of the components (eucalyptol and limonene) can act as internal plasticizer agents inside the matrix which increases the free volume and reduces the intermolecular forces between the polymer chains. Lastly, oil components can create regions of lower cohesion inside the polymer and reducing the overall strength of the material to indentation [21].

CONCLUSION

By interfering with metabolic processes and cell membrane permeability, eucalyptus globulus oil enhances PMMA's antifungal qualities. This improvement depends on concentration. Though remaining within clinically acceptable bounds, the rise in anti-

fungal properties is accompanied by a decrease in impact resistance and a minimal fall in hardness levels.

Study limitations and future studies

This study has certain limitations. For instance, the long-term durability of the reinforced denture bases under simulated oral conditions, including temperature fluctuations, pH variations, and mechanical stresses, remains unexplored. Additionally, the potential for leaching of eucalyptol oil and its impact on oral tissues over extended periods has not been thoroughly assessed. Future studies should focus on evaluating the biocompatibility and cytotoxicity of these materials over time, as well as their clinical performance in diverse patient populations.

Conflict of interest: none declared

Financial support: Self-funded study

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