Research Article

The influence of lemongrass essential oil addition into heat-cured acrylic resin against Candida albicans adhesion

Seineen S. Al-Shammari [1*], Faiza M. Abdul-Ameer [2], Latifa R Bairam [3], Zeina Al-Salihi [4]

1 Master student, Department of.Prosthodontics Dentistry, College of Dentistry, University of Baghdad, Baghdad, Iraq.
2 Professor, Department of Prosthodontics Dentistry, College of Dentistry, University of Baghdad Bab-Almoadham, Baghdad, Iraq.
3 Clinical Associate Professor Director of EDI; Equity, Diversity and Inclusion Department of restorative Dentistry School of Dental Medicine University of New York at Buffalo, US.
4 Clinical Assistant Professor, Department of Prosthodontics, College of Dentistry University of Iowa, S421 DSB 801 Newton Road, Iowa City, IA, US.
* Corresponding author: sineensalam@gmail.com

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Abstract: Background: For decades, the use of naturally accessible materials in treating human disease has been widespread. The goal of this study was to determine the anti-fungal effectiveness /of the lemongrass essential oil (LGEO) versus Candida albicans (C. albicans) adhesion to polymethylmethacrylate (PMMA) materials. Material and methods: LGEO's anti-fungal activity was tested against C. albicans adhesion using the following concentration of LGEO in PMMA monomer (2.5 vol. %, 5 vol. % LGEO) selected from the pilot study as the best two effective concentrations. A total of 40 specimens were fabricated for the candida adherence test and were subdivided into four equal groups: negative control 0 vol. % addition, experimental with 2.5 vol. % and 5 vol. % of LGEO addition and positive control with 1.4 wt. % nystatin addition. The sterile PMMA specimens were incubated at room temperature for 1 hr in sterile tubes with a sabouraud dextrose broth (SDA) in which a small amount of the yeast was isolated and suspended; under the inverted light microscope, the examination was done. The data were evaluated using a one-way ANOVA test, which showed a significant result at $p< 0.05$. Results: The findings of the C. albicans adherence test exposed a considerable reduction in the number of C. albicans cells adhering to PMMA after adding 2.5 vol. % and 5 vol. % LGEO compared to specimens from the negative control and positive control groups at $p< 0.05$. Conclusion: Adding LGEO into a heat-cure acrylic material can result in a denture base material with anti-fungal properties versus C. albicans microorganisms. The experimental group 5 vol. % LGEO additive showed the best anti-fungal activity.

Keywords: Antifungal, *Candida albicans* adherence, Lemongrass essential oil, polymethylmethacrylate material.

Introduction

PMMA is the material that is most frequently used to fabricate denture bases because of its benefits, which include an appealing appearance, appropriate stability /in the oral environment, and affordability/ [1].Despite these advantages, it has drawbacks such as, susceptibility to microbial colonization [2] and its inferior mechanical and physical properties [3]. Denture stomatitis is a type of oral candidiasis that is caused by wearing dentures for a long time [4]; its etiology is numerous, although C. albicans is the most common pathogenic microbe [5]. Topical anti-fungal treatments are prescribed to treat denture stomatitis. However, maintaining an adequate oral drug dose and older patients' lack of motor skills make these topical drugs challenging. To manage these unfavorable situations, it is desirable to include anti-fungal

medications into denture base materials. Unfortunately, due to fungal resistance and drug side effects, it is necessary to obtain naturally derived remedies to replace these synthetic medicines [6, 7].

Researchers have recently tested herbal medicines and plant oils and found that these natural medication exhibited anti-fungal efficacy against C. albicans. They were considered as prospective line of therapy with effective anti-fungal abilities that can be prescribed to treat denture stomatitis [8, 9]. In tropical and subtropical climes, the herb Cymbopogon citratus often gets cultivated. The essential oil of Cymbopogon citratus, known as lemongrass essential oil (LGEO), is a common herb in tropical areas, particularly in Southeast Asia. Studies have shown that Cymbopogon citratus has antidiarrheal, antibacterial, anti-fungal, anti-filarial, and anti-inflammatory properties [10]. In this investigation, LGEO was added to heat-cured PMMA denture base material in order to generate PMMA denture base material with significant anti-fungal action, particularly against C. albicans, with the least possible impact on other material qualities.

Materials and Methods

Considering what the pilot study's findings showed in Table (1), the two concentrations of 2.5 vol. % LGEO and 5 vol. % LGEO were selected as the best two concentrations to be used in the main study since they had lower mean values than the negative control group, indicating a more favorable reduction of C. albicans adherence ability compared to the negative control group.

Table 1: The results of pilot study of the Candida albicans adherence test.

Specimens	0vol. %LGEO	2.5 vol. % LGEO	5 vol. % LGEO	7.5 vol. % LGEO
1	269	112	84	127
2	281	115	79	125
3	275	109	80	136
Mean	265	112	81	129.33

Preparation of the specimens

PMMA specimens were prepared using plastic (Disk-shaped) patterns with a diameter of 10mm and a thickness of 2mm. The plastic disks were embedded in the dental flask's lower section, which was filled with type IV extra hard dental die stone. Half of the plastic patterns were grandeur embedded; the other half was left above the stone level, and then the upper section of the flask was placed above the lower one and filled with dental stone. The flask was opened, and the plastic disks were removed when the stone's second layer had fully set, creating a space in the stone.

Proportioning and mixing of heat-cured acrylic resin, lemongrass essential oil, and nystatin

The amounts of polymer, monomer, LGEO, and nystatin powder percentage used in the main study were measured using an electronic balance (Worner lab company, China) with an accuracy of (0.001g) for powder weighing and a micropipette (dragon lab, China) for liquid measuring. The negative control, experimental, and positive control specimens were mixed according to the manufacturer's instructions (P/L ratio 22g: 10ml).

As for negative control heat-cured PMMA specimens, PMMA liquid and powder were measured according to product recommendations. In the case of LGEO addition specimens, a micropipette was used to measure the required amount of oil, which was then subtracted from the volume of the monomer. The mixture was then mixed with monomer using a tiny electric hand mixer (china) for around 20 seconds. This solution was then blended with acrylic powder, as previously stated.

While for the positive control nystatin incorporated specimens, The needed amount of nystatin powder was determined and deducted from the weight of acrylic powder before it was blended with acrylic powder for a minute with a small electric hand mixer to dissolve and thoroughly blend all of the solute particles in the solution. The required amount of monomer was then added to the mixture and mixed Table (2).

Table 2: The mixing ratios for lemongrass essential oil, nystatin powder, and polymethylmethacrylate powder and monomer.

Groups	Amount of LGEO	Amount of monomer	Amount of polymer powder
0 vol. % LGEO negative Control	0 %	10ml	22g
2.5 vol.% LGEO	2.5 %	9.75ml	22g
5 vol.% LGEO	5 %	9.5ml	22g
1.4 w.t % Nystatin	0 %	10ml	21.69g

Packing

Once the polymer reached the dough-like stage, it was packed in a pre-made mold. The upper and lower half's of the flasks were closed with a steady hydraulic pressure of 100kg/cm2, The flasks were then opened, and any excess materials were removed, and a separating medium was applied to the stone surface (Zhermack Italy). Flasks were placed and secured in the press machine in the standard way[11].

Acrylic resin curing

The short curing cycle was used for curing acrylic specimens. The curing process was performed according to the manufacturer's instructions. When the curing process was completed, all acrylic specimens were finished and polished. After deflasking, the specimen's dimensions, were verified with a digital Vernier (china) and polishing procedures were completed. All the specimens were immersed in water to reduce the amounts and duration of release of residual compounds and improve the mechanical properties of the PMMA [12]. The acrylic specimens were maintained in distilled water at 37°C for two days before the test procedure to achieve a condition of standardization [13].

Isolation of *Candida albicans*

Samples of C. albicans were isolated intraorally from three patients with denture stomatitis [14]. The lesions were gently scrubbed with a sterile cotton swab before being inoculated with sabouraud dextrose agar (a primary isolation medium) [15]. These swabs were cultivated and aerobically incubated for 48 hours at 37°C before being stored at 4°C for later use [16].

Identification of *Candida albicans*

1. Morphological examination: After 48 hrs at 37°C, creamy, pasty, and smooth candida colonies develop on SDA medium [17].

2. Microscopical examination: A little amount of a single isolated colony was taken and blended with a drop of normal saline on a glass slide to produce a solution that was evenly distributed; afterwards when, the slide was left to dry at room temperature before being stabilized by passing it over a bunsen burner flame multiple times, after that it was stained According to Gram's Method [18].

Under the light microscope, the slide was examined, and candida species appeared as Gram-positive round or oval yeast-like cells.

3. Germ tube formation: A lope inoculum of yeast cells was obtained/ to create a solution, and a small amount of the/ solution was placed on/ a transparent slide to be examined under/ a light microscope/ to observe the way germ tubes formed[19)].

4. Biochemical identification: applying the VITEK 2 device, the identity of the unknown microorganisms was verified via a Densi Chek instrument, and the final outcomes were then compared to the database.

Examination of the prepared specimens (negative control, experimental, and positive control) for *Candida albicans* adherence ability under the inverted light microscope were performed.

The created SDA was transferred into sterile tubes via a McFarland densitometer, and a tiny quantity of previously isolated yeast was suspended into the medium [(20)]. In the sterilized tubes, the sterile PMMA specimens were placed and cultivated for one hour within the already-prepared medium. The specimens were then removed from the suspension and carefully shaken over one minute using phosphate-buffered saline (PBS) solution in order to eliminate any non-adhered yeast cells prior being dried out via filter paper [(21)].

The attached candida cells on the PMMA specimens were fixed with methanol, stained with crystal violet for 60 seconds, rinsed with PBS solution for 30 seconds, then dried using filter paper and inspected under an inverted light microscope. The inverted light microscope was used for specimen examination with (x 40) magnification power; the microscope was connected to the computer. Four standardized fields were examined on each specimens [(8)]. To ensure optimum standardization of measurement for all specimens, filamentous form were not counted, and budding daughter cells were counted as a single yeast [(22)].

Results

Using an inverted light microscope, examine the stained specimens for each group with a magnification power of 40xwere examined; the negative control group shows the higher mean value (274.60 cells), while the lower mean values were (80.10 cells) for the experimental group, 5% by vol. of LGEO Descriptive statistics of candida adherence test results for all the studied groups listed in Table (3).

Table 3: Descriptive statistics of *Candida albicans* adherence test among groups.

Groups	Mean	±SD	±SE	Minimum	Maximum
0 vol. % LGEO (–ve Control)	274.600	4.006	1.267	269.000	281.000
2.5 vol.% LGEO	110.900	2.846	0.900	106.000	115.000
5 vol.% LGEO	80.100	2.025	0.640	77.000	84.000
1.4 w.t % Nystatin	122.500	2.014	0.637	120.000	126.000

Using the one-way ANOVA test, /comparison of the mean/ values among all groups' resulted in significant differences $p < 0.05$ and listed in Table (3).

Table 3: Statistical test of *Candida albicans* among groups using one-way analysis of variance (ANOVA).

	Sum of Squares	df	Mean Square	F	Effect size	*P* value
Between Groups	226608.275	3	75536.092	9354.315	0.999	0.000*
Within Groups	290.700	36	8.075			
Total	226898.975	39				

Levene p value=0.070**, *= significant at p<0.05, **= not significant at p>0.05.

Tukey's multiple comparisons test was used to compare mean values between each two different groups. The negative control group 0 vol. % LGEO revealed a significant difference compared to both experimental groups 2.5 vol. % LGEO and 5 vol. % LGEO and positive control group 1.4 w.t % nystatin at $p<0.05$ (Table 4).

Table 4: Multiple comparisons of *Candida albicans* between groups using Tukey's honestly significant difference (HSD).

Groups		**Mean Difference**	***p*-value**
0 vol. %LGEO (- ve Control)	2.5 vol.% LGEO	163.700	0.000*
	5 vol. % LGEO	194.500	0.000*
	1.4 wt. % nystatin (positve control)	152.100	0.000*
2.5 vol. % LGEO	5 vol.% LGEO	30.800	0.000*
	1.4 wt. % nystatin (positve control)	-11.600	0.000*
5 vol.% LGEO	1.4 wt. % nystatin (positve control)	-42.400	0.000*

*=significant at $p<0.05$.

Discussion

Polymethyl methacrylate acrylic resin (PMMA) has been the material of choice for fabricating denture bases since the twentieth century 36. This material has been modified to improve physical and mechanical properties and enhance the laboratory procedure using microwave polymerization, visible light curing, and vacuum pressure at low temperature curing systems 37. However, despite the advantages of this material, the porous surface was considered a disadvantage. Adding certain materials such as (oils) with the acrylic matrix could improve this drawback of PMMA [(23)].

The most common disease in elderly denture wearers is denture-induced candidiasis, with the principal causative agent being *C. albicans* [(24)]. various attempts to incorporate anti-fungal agents or antiseptics into denture base materials, tissue conditioners and denture reliners have been reported [(25, 26.)]. Medicinal plant extracts are a great alternative to antibacterial medications since they have fewer or no side effects [(7)].

Cymbopogon citratus LGEO is well known for its antibacterial qualities and has been frequently utilized as a medicinal herb [(27)]. Treatment for oral and vaginal candidiasis has proven to be quite successful when using Cymbopogon citratus oil. Some yeast, such Candida oleophila, are significantly inhibited from growing as a result of the citral in the oil [(28)]. Fungal conditions have been reported to respond well to citral and its oxidative metabolites, known as epoxides [(29)].

Several conjugated acid derivatives of citral had similar strong antibacterial activities and were significantly more powerful than common antibiotics chloramphenicol and nystatin [30]. The present study hypothesizes that this addition exhibits an anti-fungal efficiency. The addition of two different concentrations of LGEO of (2.5% vol. and 5vol. %) to heat cure PMMA denture base material resulted in a statistically significant decrease in the mean values of cell's number of *C. albicans* in comparison to the –ve control (0 vol. % LGEO) group as well as to the positve control group (1.4 wt. % nystatin. By estimating the minimum fungicidal concentration and the fractional inhibitory concentration index, respectively had the anti-fungal and anti-candidal potential of the citral component of LGEO has been evaluated.

When combined with fluconazole, citral has demonstrated strong synergistic action against a fluconazole-resistant strain of *C.albicans* [31]. It stopped *C. albicans* cells from growing by interfering with multiple cell cycle phases, most likely triggering apoptosis by interfering with the S phase of the cell cycle. Citral with other monoterpenes like eugenol, nerilidol, and alpha-terpineol produce irreversible ultra-structural changes in trichophyton mentagrophytes [32].

As a result of the production of citral and citronellol, the amides 5, 9-dimethyl-deca-2, 4, 8-trienoic acid, as well as 9-formyl-5-methyl-deca2, 4, 8-trienoic acid, are both powerful inhibitors of the bacterial efflux pump [33]. It seemed that adding of 2.5 vol. % LGEO and 5 vol. % LGEO was the most beneficial effect against the growth of fungi. It is important to remember that the comparison of the results for one study with other studies is tricky due to differences in assay technique, in addition to the differences in climate and environmental conditions, botanical source, harvesting time, and the methods of extraction which all can alter both composition and antimicrobial activity of essential oil.

The finding in this study agrees with the study of Sahal et al, in 2019, who stated that LGEO has a great promise as an anti-fungal and anti-biofilm agent for silicone rubber prostheses and medical devices where candida tropicalis biofilms can cause serious skin infections and limit the prosthesis' lifespan [27]. Also, this study agrees with Mat-Rani et al, in 2021who stated that LGEO also had a fungicidal effect on C. albicans biofilms that had been pre-established on silicone disks of various brands.

Furthermore, the oil's fungicidal efficacy against the mature fungal biofilm was dose-dependent [34]. This present study was also found to agree with the study of (, Srithavaj, and Thummawanit in 2021, who showed that Cinnamon and LGEO could remove pre-existing *C. albicans* biofilm and prevent fungal biofilm formation on heat-polymerized PMMA specimens. The minimal inhibitory doses of cinnamon and LGEO were established using agar disk diffusion and broth microdilution techniques (immersion). The effects of the essential oils studied were dosage and exposure or priming time-dependent [35].

Conclusion:

The effective addition of LGEO to heat-cured PMMA denture base material indicates that it possesses the ability for fighting C. albicans by functioning as an anti-fungal agent plus medication delivery system. Therefore, LGEO can be regarded as a solid anti-fungal substance. An improvement in the anti-fungal adherence ability of the heat-cured PMMA denture base material against C. albicans after the addition of 2.5 vol. % and 5 vol. % LGEO is comparable with the effect of positive control and negative control groups.

Conflict of interest

The authors have no conflicts of interest to declare.

Author contributions

FMA; study conception and design. SSA; data collection. FMA and ZI; Methodology. FMA and SSA; statistical analysis and interpretation of results. SSA; original draft manuscript preparation. FMA and LBA; Writing - review & editing. Supervision; LBA and FMA. All authors reviewed the results and approved the final version of the manuscript to be published.

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Informed consent

Informed consent was obtained from all individuals or their guardians included in this study.

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العنوان: تأثير إضافة زيت عشبه الليمون العطري و دمجها مع مادة راتنج الأكريليك المعالج بالحرارة ضد التصاق لمبيضات البيضاء

الباحثون: 1سينين سلام احمد , 2 فائزه محمد حسين عبد الأمير , 3 زينه اسماعيل

المستخلص:

لعدة قرون ، تم استخدام العناصر الطبيعيه في علاج الأمراض . تهدف الدراسة الحالية إلى تحديد الفعالية المضادة للفطريات لزيت عشبة الليمون العطري ضد التصاق المبيضات البيضاء بمادة قاعدة طقم ألاسنان.

المواد وطرق العمل: تم تقييم القدرة المضادة للفطريات لزيت عشبة الليمون ضد التصاق المبيضات البيضاءعن طريق إضافة زيت عشبه اليمون العطري إلى مونومر البولي مثيل ميثاكريليت بتركيز (2.5 حجم٪ ، **5** حجم٪) والتي تم اختيارها من الدراسة التجريبية كأفضل تركيزين فعالين. تم تصنيع ما مجموعه 40 عينة لاختبار الالتزام بالمبيضات وتم تقسيمها إلى أربع مجموعات متساوية: مجموعة السيطره 0 حجم ٪ إضافة ، تجريبية مع 2.5 حجم ٪ و 5 حجم ٪ من إضافة زيت عشبة الليمون العطري و مجموعه النستاتين مع 1.4 وزن. ٪ . تم وضع العينات في أنابيب معقمة تحتوي على مرق سكر العنب ، حيث تم تعليق كمية صغيرة من الخميرة المعزولة ، وحضانتها في درجة حرارة الغرفة لمدة ساعة واحده. ثم أزيلت العينات، وشطفت بمحلول ملحي مخزن بالفوسفات، وجففت، وثبتت بالميثانول، ولطخت باللون البنفسجي البلوري، وشوهدت تحت مجهر ضوئي مقلوب.

النتائج: كشفت نتائج اختبار التصاق المبيضات البيضاء عن انخفاض كبير في عدد خلايا المبيضات البيضاء الملتصقة بعينة البولي مثيل ميث اكريليت بعد إضافة 2.5 حجم. ٪ و 5 حجم٪ من زيت عشبة الليمون العطري مقارنة بالعينات من المجموعة السيطره 0 حجم٪ ومجموعة النستاتين 1.4 وزن٪ عند $p < 0.05$.