Bergamot Essential Oil Effect against Candida Albicans Activity on Heat Cure Acrylic Denture Base

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Abstract

The aim of the current study is to evaluate the antifungal efficacy of several different percentages of Bergamot Essential Oil (BEO) incorporated into heat cure PMMA denture base material against Candida albicans.

Materials and Method: Eighty samples were divided into eight groups including six various percentages of BEO (2%,3%, 4% ,5%, 6% and 7% by volume) and 0% BEO as a control group, in addition to 1.4% by weight of nystatin which represent the positive control group. All these additives were incorporated into heat cure PMMA denture material. After 48h incubation in distilled water, all samples were assessed by Candida albicans colonies viable count test.

Results: For Candida albicans activity test; the experimental groups (2%,3%, 4%, 5%, 6% and 7% of BEO) showed highly significant decrease in the mean values of the viable count of Candida albicans when compared to the control group (0% BEO) ($p<0.01$). In contrast, a non-significant difference among experimental groups and 1.4% nystatin group.

Conclusion: Bergamot essential oil was successfully incorporated into heat cure PMMA denture base material and could act as potential antifungal agent with a drug delivery system against Candida albicans. It seemed that adding of 5% and 6% BEO was the most beneficial effects against the growth of fungi.

Key words: *Heat cure PMMA, Denture base, Bergamot essential oil, Nystatin, Candida albicans*

Introduction

Walter Wright discovered poly(methyl methacrylate) (PMMA) after publishing the results of his clinical evaluation of PMMA in 1937, since it is introduced has become most superior, popular and satisfactory of all polymeric denture base materials [1] Acrylic denture base is used in removable dentures fabrication, and its popularity and universal use has attributed to low cost, light weight, ease of processing , ease of reparability ,low water sorption and solubility, biocompatibility, satisfactory aesthetic properties [1,2] and accurate fit [3], in spite of that this material until now remain not ideal denture base material due to the presence of several drawbacks like susceptibility to microbial colonization and formation of biofilm on its surface and this make the denture a source of different infections because it harbor the microorganisms [4,5] in addition to lack of manual dexterity among the old age patients which make the effective biofilm removal not possible [6,7]. Denture stomatitis is a disease related to denture use, and considered chronic atrophic oral candidiasis, which affect up to 65% of denture wearer [8], its etiology is multifactorial, but Candida albicans considered the main pathological microorganism which is the mostly isolated type from the oral cavity in patient with denture stomatitis [9]. The most common line that is used in treatment of denture stomatitis is prescribing of topical antifungal medicines, but maintaining optimal oral drug dose and lacking of motor dexterity of geriatric patient who had impaired cognition, limit their use and make it challenging to get a maximum benefit of these topical drugs . To overcome these obstacles it is

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better to incorporate the antifungal drugs into denture base materials. Unfortunately fungal resistance and side effects of these drugs make it necessary to obtain naturally derived medicaments as a substitution to these synthetic drugs [10,11]. Herbal medicines are a powerful alternative treatment for microbial infections in the oral cavity which have less or no side effects; and this made a worldwide trend to make a lot of researches about them in order to find biologically safe herbal-based medicines with effective antifungal properties [11-13] Plants oils are herbal medicines and various researches, recently, have been done to test their antifungal efficiency against Candida albicans and they reported that these oils are considered a promising line of therapy which have effective antifungal properties and can be used for treatment of denture stomatitis [15,16]. One of the recent research was done through the incorporation of virgin coconut oil into heat cured acrylic-based denture soft lining and proved the antifungal efficiency of this oil against Candida albicans [17]. Bergamot is the popular name for Citrus bergamia Risso et Poiteau, Citrusbergamia is defined as "a hybrid between a sour orange (C. aurantium L.) and lemon (C. limon L. Burm.F.) or a mutation of the latter. Other authors considered it a hybrid between a sour orange and lime" [18]

Materials and Method

Regular- conventional heat cure acrylic resin denture base material (Vertex, Netherlands) was utilized. Bergamot essential oil (bergaptene-free) from (Aura Cacia pure essential oils, USA) was added to the liquid part of acrylic resin in six different percentages (2%, 3%, 4%, 5%, 6% and 7%), these percentages were selected to discover which one is as effective as 1.4% nystatin group (positive control group) . This proportion of nystatin was calculated by the conversion of International Units (U) to milligrams. Every 6079U corresponded to 1 mg of nystatin. Therefore, for this group (500,000U) the amount of nystatin used was 90 mg for every 6.6 g of acrylic powder[24]. In general, 500 000 units was the most common concentration used by many authors and result in decreased yeast count compared to control group [25]. Also this percentage of nystatin start to change the color of acrylic resin into orange so it is objectionable to use it above this percentage especially in aesthetic area.

Study design

Total of eighty samples were prepared, 10 samples for control group, 10 samples for each percentage of BEO groups (2%, 3%, 4%, 5%, 6% and 7% BEO) and 10 samples for positive control group. All samples were tested by viable count of Candida albicans colonies test at the same time and circumstances and they stored in distilled water for 48h prior to test procedure to reach the state of standardization.

Candida albicans colonies viable count test

Samples preparation

Plastic models were prepared with dimensions of 10×10×2.3 mm in length, width and thickness, respectively using laser cutting machine. Then, the models were invested in freshly mixed type IV extra hard dental die stone to create stone molds where the acrylic resin samples will be packed. For control group the acrylic resin denture base materials were proportionate and mixed as directed by the manufacturer instructions. Regarding BEO incorporated samples, the required amount of oil were measured by micropipette and subtracted from the volume of monomer, then mixed manually in dry clean glass beaker with monomer. Following which, the mixture was added to acrylic powder and mixed thoroughly.

Isolation and identification of Candida albicans

Candida albicans were obtained from oral cavity of patient with an indication of a denture induced stomatitis by using cotton swab which then cultured on the surface of a sabouraud dextrose agar SDA (Oxoid, United kingdom) plates and incubated at 37°C for 48 h. The identification of candida albicans was done according to the following order: Firstly, the colony morphology (macroscopic examination) in which the Candida albicans appear as creamy, pasty, smooth and convex colonies on SDA. Secondly, microscopic examination using Grams stain procedure , candida seen as small gram-positive oval or budding yeast cells. Thirdly , Germ tube formation. Fourthly, Biochemical identification using analytical profile index API Candida system (bioMérieux, France) and API 20 C AUX system.

Assessment of Candida albicans viable count

Candida albicans suspension of about 10^7CFU/mL which equal to 0.5 McFarland standards was prepared by diluting a small quantity of inoculums in a test tube containing normal saline and measuring this solution with McFarland densitometer device as shown in Figure 1(A). Then, 100µL of Candida suspension was added

to each tube contain 9.9 mL of sabouraud dextrose broth (Oxoid, England) to obtain 10 mL broth mixture this done by using micropipette. The samples after that immersed in tubes (Figure 1 (B)) and incubated at 37 °C for 24 h. After incubation, about 100µL was taken from all tubes of broth mixture and added to other tube contain 9.9 mL normal saline so tenfold dilution obtained. A second dilution made from this dilution by the same procedure as in Figure 1(C). About 100 µL taken from the second dilution and spreaded on SDA plates by using disposable inoculation loops (Figure 1 (D)). The SDA plates incubated aerobically at 37 ºC for 24 h. This dilution was chosen because it demonstrated a countable range of 30-300 CFU.

Following incubation, the Candida albicans colonies were visible on SDA plates, all these colonies counted and this viable count analyzed statistically so the material antifungal efficacy (AFE) was calculated using following formula (Equation 1):

$$\text{AFE } [\%] = \frac{Vc - Vt}{Vc} \times 100\ \% \qquad (1)$$

In this formula the number of viable colonies of control samples was represented by Vc and number of viable colonies of experimental samples was represented by Vt

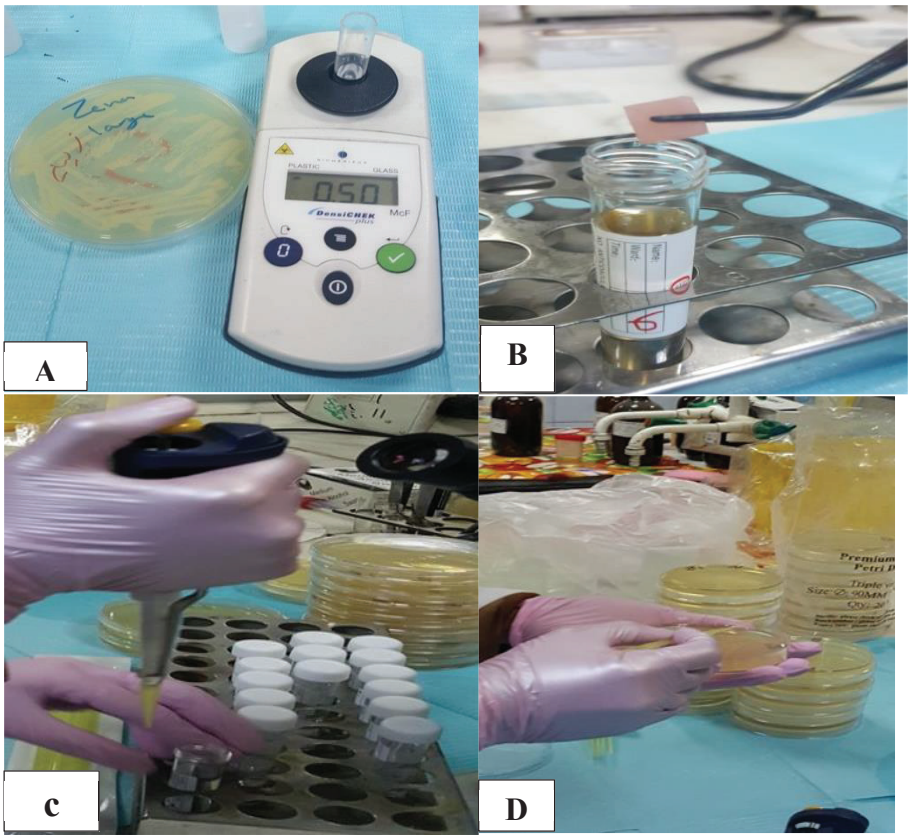

Figure 1 :A) McFarland densitometer; B) Placement of specimen in the broth ;C) Serial dilution; D) Spreading 100 µL of solution over SDA surface

Statistical analysis

The data of this research were collected and analyzed using SPSS (statistical package for social science – version 21) computer software. Descriptive statistics were made which include Means, Standard deviation, Minimum, Maximum. Inferential statistics include ANOVA (one-way ANALYSIS OF VARIANCE) test which was used to evaluate the significance of difference among the mean values of all groups, then multiple comparison using Dunnett t-tests which treat one group as a control, and compare all other groups against it was used.

Results

The viable count of Candida albicans colonies test was performed on six experimental groups (2%, 3%, 4%, 5%, 6% and 7% BEO) and two control groups (negative control 0% BEO group and positive control 1.4% nystatin group for comparison). After 48 h of samples incubation in distilled water, the results of viable counts of Candida albicans showed a reduction in the mean values for experimental groups in comparison to negative control group, So 5% and 6% groups showed the lowest mean value among all groups, while other experimental groups (2%, 3%, 4% and 7%) revealed higher mean values than positive control group. Antifungal efficiency (AFE) for the experimental groups are as follows: 55.53% for 2% BEO group, 67.21% for 3% BEO group, 68.64 % for 4% BEO group, 76.43% for 5% BEO group, 74.18 % for 6% BEO group, 66.80% for 7% BEO group and 69.87% for 1.4% nystatin group

Descriptive statistics (mean, standard deviation, maximum and minimum) and statistical test of viable count of C. albicans results using One- Way ANOVA for comparison of means of all studied groups listed in Table (1) the difference between groups was highly significant. For multiple comparison purpose the Dunnett t (2-sided) a test was used. In this test the 1.4% nystatin group taken as control group and all other groups compared against it. The 0% BEO group showed a highly significant difference while all other experimental groups showed a non-significant difference in comparison to 1.4% nystatin group as listed in Table (2).

Table (1): Descriptive statistics of viable count of Candida albicans, using One-Way ANOVA

Group	Mean (CFU/mL)	SD	Minimum	Maximum	F	p
Control	162.667	18.877	142.000	179.000	27.245	0.000 HS
2%	72.333	10.116	66.000	84.000		
3%	53.333	15.631	39.000	70.000		
4%	51.000	11.000	40.000	62.000		
5%	38.333	13.051	28.000	53.000		
6%	42.000	17.692	26.000	61.000		
7%	54.000	11.136	42.000	64.000		
1.4% Nystatin	49.000	4.583	44.000	53.000		

Table (2): Multiple comparisons test of Candida albicans (CFU/mL) among the groups using Dunnett t (2-sided)[a] test

Multiple Comparisons				
Dependent Variable: Canalb				
Dunnett t (2-sided)a				
(I) groups	**(J) groups**	**Mean Difference (I-J)**	**Sig.**	
control	1.4% Nystatin	113.66667*	.000	HS

Cont... **Table (2): Multiple comparisons test of Candida albicans (CFU/mL) among the groups using Dunnett t (2-sided)[a] test**

2%	1.4% Nystatin	23.33333	.214	NS
3%	1.4% Nystatin	4.33333	.999	
4%	1.4% Nystatin	2.00000	1.000	
5%	1.4% Nystatin	-10.66667	.867	
6%	1.4% Nystatin	-7.00000	.980	
7%	1.4% Nystatin	5.00000	.997	
*The mean difference is significant at the 0.05 level.				
a. Dunnett t-tests treat one group as a control, and compare all other groups agains it				

Figure 2: Viable counts of Candida albicans after 48 hours incubation of: a) Control samples; b) some experimental samples with BEO); c)positive control sample (1.4% nystatin). Medicinal Plants extracts are very good replacement to antimicrobial drugs with less or without side effects, as a result this encouraged the worldwide tendency towards herbal-based medicines and a lot of researches were done to obtain herbal medicine that is biologically safe and have excellent antifungal properties [10,12]. Essential oils are examples of naturally derived herbal medicaments which are concentrated, hydrophobic liquids extracted from plant and have wide spectrum of pharmacological activities, these essential oils considered as a promising therapeutic line for oral infections and in the last few decades a lot of kinds of oils have been tested for efficacy against Candida albicans, each one of them has special active component that affect the fungi in particular mechanism [16]. Bergamot essential oil has been used for its antiseptic, anti-inflammatory, diaphoretic, appetizing, and analgesic effect.

Conclusion

Within the limitations of the present study; the following conclusions can be obtained that Bergamot essential oil was incorporated successfully into heat cure acrylic resin denture base material and worked as a powerful antifungal herbal medicament against Candida albicans that is comparable with the effect of nystatin. Moreover, the samples with 5% and 6% BEO revealed a better antifungal efficiency compared to all other control and experimental groups.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Dentistry/ University of Baghdad, Iraq and all experiments were carried out in accordance with approved guidelines.

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