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Evaluation of Anti Candida Effect of *Melaleuca alternifolia* on Heat Cured Acrylic Resin

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ABSTRACT

Objectives: *To evaluate the anti-candida properties of tea tree oil as an additive to heat cured acrylic resin.* ***Materials and methods:*** *Total 24 heat cured samples were prepared without the addition of oil (control), and 24 with additives (20% of pure natural tea tree oil). These disks were inoculated with 0.1 mL of Candida albicans standard inoculum and were rinsed with 0.9% NaCl to remove the loosely attached cells from the surface of the discs. Sabouraud's dextrose agar plate was used for measuring the attached yeast. The control and treated disks have been placed in distilled water for 1 day, 21 days and 42 days and washed daily with wet cotton.* ***Results:*** *CFU the mean colony forming units for control disks were placed in water and cleaned with wet cotton for 1 day, 21 days and 42 days was 1.5000, 5.0000, 1.0000, respectively and CFU for disks with tea tree oil decreased to 0.8750, 1.6250 and 0.3750 after 1 day, 21 days and 42 days. Tea tree oil incorporated specimens were effective in reducing the growth of C. albicans after storage for 42 days in distilled water.* ***Conclusion:*** *There was a significant reduction in the growth of C. albicans after the addition of oil to heat cured acrylic resin which suggests a new oral topical treatment for denture stomatitis.*

Keywords: Tea tree oil, *C. albicans*, Heat cured acrylic resin

INTRODUCTION

Wearing artificial prosthesis causes multiple changes in the oral cavity which may colonize with microorganisms. Denture wearers are highly prone to develop denture stomatitis which is mainly characterized by the presence of *C. albicans* and play a basic role in the development of candidiasis [1,2]. In spite of the etiology of denture stomatitis which is multiple factor, the component of bio-film that occur on the denture, such as *C. albicans* play a requisite role in the development of candidiasis [3].

Traditional treatment modalities of denture stomatitis include the prescribing of antifungal drug where the most widely used antifungal agent was nystatin, which was adjusted to the prosthesis to receive a denture liner [4,5]. Nowadays medicinal plants extracts are used within biomaterials as an effective natural alternative with excellent antifungal and antibacterial properties [6], thereby it presents a reasonable alternative to antifungal agents and overcomes its limitation of action, these are mainly their penetration and chemical reaction into biofilm, the extracellular polymeric material [7]. Tea tree oil is an essential oil which is produced from the terminal branches of *Melaleuca alternifolia* Australian native plants by steam distillation and is a new multi-purpose herb [6]. Tea tree oil is composed of more than 100 different compounds; the most abundant of these isterpenes (mainly monoterpenes and sesquiterpenes). In medicine, Tea tree oil (TTO) is used in the treatment of many conditions relating primarily to its antimicrobial, anti-inflammatory and antifungal especially anticandidal properties [8-13]. Tea tree oil works against bacteria and microbes by disrupting the microorganism's cell membranes and disables the proteins within them, basically, the microorganisms are deactivated and cannot multiply and cause health problems. Tea tree oil is added to a wide range of cosmetics in typical concentrations of their formulation, these are moisturizers, body lotions, conditioners and shampoos, face cleansing, mouthwashes, soaps, and hand washes. TTO is bactericidal against *S. aureus* and staphylococci which are commonly microorganisms associated with implant infections [14].

The main benefits to using the extract of the natural plant as antimicrobial agents are the safety and biocompatibility

without any adverse effects in addition to their low cost. Recent clinical data showed that the efficiency of the oil in the treatment of oral candidiasis presents a promising topical antifungal agent [7]. This study is taken to test the *in vitro* capacity of TTO incorporating in heat cured acrylic resin against *Candida albicans* growth.

PATIENTS AND METHODS

Total of 48 specimens was intended. The samples were divided into 2 groups, control heat cured resin without the addition of tea tree oil and a second group with the incorporation of tea tree oil to heat cured acrylic resin (experimental). Each group of specimens was subdivided into 3 groups (each group No. 8) according to the time of storage in distilled water (1 day, 21 days, 42 days). The heat cured acrylic resin (super acryl, Czech) was used. A pure tea tree oil (MASON, USA) was brought from the local market.

Specimen's Preparation

The plastic disks were constructed with dimensions of 50 mm × 2.5 mm width, thickness respectively according to ADA to produce a mold of stone. The conventional flasking technique for complete dentures was followed in the preparation of the mold. Specimens of a control group (24 samples) were prepared with (powder/liquid) ratio 44 gm ± 0.2/20 ml from heat cure acrylic resin according to producer's directions. While the experiment groups (24 specimens) were constructed with the addition of pure TTO to monomer (concentration of tea tree oil 20% by volume of monomer) [15]. The manufacturer's directions were followed for the curing process of acrylic. Later the samples have been placed in distilled water for different storage time (1 day, 21 days, and 42 days) and were cleaned gently for 1 minute each day with wet cotton.

Candida Growth Situation

Candida albicans (American type culture collection, ATCC 90028) was used in the experiments. The fungi were sub-cultured and propagated on the plates of Sabouraud's dextrose agar at 37°C for 24 hours [16]. Colonies were harvested, pendent in physiological sterile saline solution (0.9% NaCl) and the standard inoculum was obtained by diluting the fungal cells to an optical density of 0.284 (corresponding to a cell density of ~106 cells/mL) at wave length 530 nm (OD530) using a UV/visible spectrophotometer [16]. This inoculum was further used to grow biofilm on the previously prepared acrylic discs.

Biofilm Development on Acrylic Discs

To grow *Candida albicans* biofilms, the acrylic discs were previously disinfected for 15 min at 121°C with autoclave [17]. After sterilization, with the aid of sterile forceps, discs have been placed into the wells of 24-well cell culture plates containing 2 ml of Sabouraud's Dextrose broth and 5% sucrose. Then, wells containing discs were inoculated with 0.1 mL of *Candida albicans* standard inoculum [16]. Plates were placed at 37°C using orbital shaker incubator (75 rpm) to allow biofilms of microorganism's growth on the discs. This procedure had been repeated after 21 days and 42 days of specimens' storage in distilled water. Controls were also employed in this experiment using discs without tea tree oil and wells without *Candida* cells respectively.

Antifungal Susceptibility Test

The number of *Candida albicans* biofilm embedded cells grown on acrylic discs was determined using a previously described method with some modifications [18]. Briefly, after each incubation period, the discs of acrylic were removed from the wells and gently washed two times with 0.9% NaCl to remove the non-adherent cells from the surface of the discs. Then, the discs were transferred into a tube containing 10 ml of 0.9%, NaCl, vortexed for 10 min and homogenized with an ultrasonic homogenizer for 30 seconds to detach the adhered cells from these discs.

The obtained suspended samples were subjected to a 10-fold serial dilution, then 0.1 ml from each dilution were cultured on plates of Sabouraud's Dextrose agar at 37°C for 48 hours and the colony forming units per milliliter (CFU/mL) was determined considering plated containing 30-300 *Candida albicans* colonies. SPSS software version 18 was used to analyze the result.

RESULTS

The descriptive statistics for the antifungal action of tea tree oil on heat cured acrylic resin are shown in Table 1 which show that TTO treated acrylic resin samples at each time interval at 1 day, 21 days, 42 days in harber *C. albicans*

less than the untreated acrylic resin, *Candida* growth in TTO incorporated disks was lower in comparison to untreated disk.

Table 1 Descriptive Statistics data of candida growth after storage in distilled water at a different time interval (1 day, 21 days, 42 days)

Group	N	Mean	Std. Error	Std. Deviation
Control[1]	8	1.500	0.37796	1.06904
T.T.O[1]	8	0.875	0.29505	0.83452
Control [21]	8	5.000	0.37796	1.06904
T.T.O[21]	8	1.625	0.32390	0.91613
Control[42]	8	1.000	1.88980	0.53452
T.T.O[42]	8	0.375	1.82980	0.53452
*1, 21, 42 (storage time in distilled water)				

A t-test was made for comparisons between studied groups which showed no significant difference was found in *Candida* growth of control and experimental disks after placement in water for 1 day ($p>0.05$) (Table 2). While there was a significant difference between the TTO treated sample and untreated heat cured acrylic resin at 21 days and 42 days ($p<0.05$).

Table 2 T-test table between control and TTO for different storage duration (1 day, 21 days, 42 days)

Group	Paired difference		t	Sig. (2-tailed)
	95% confidence interval of the difference			
	Lower bound	Upper bound		
Control-T.T.O[1]	-0.26173	1.51173	1.667	0.140
Control-T.T.O[21]	2.75298	3.99702	12.830	0.000
Control-T.T.O[42]	0.19232	1.05768	3.416	0.011
*1, 21, 42 (storage time in distilled water)				

Statistically from one-way analysis of variance (ANOVA) results in Table 3, there was no significant difference found in *Candida* growth of tea tree oil treated disks following water storage up to 42 days.

Table 3 ANOVA table between TTO samples of 1 day, 21 days, and 42 days

Variables		Sum of Squares	df	Mean Square	F	Sig.
TTO samples of one day and 21 days	Between Groups	2.125	4	0.531	0.580	0.701
	Within Groups	2.750	3	0.917		
	Total	4.875	7	0.000		
TTO samples of one day and 42 days	Between Groups	1.008	1	1.008	1.565	0.258
	Within Groups	3.867	6	0.644		
	Total	4.875	7	0.000		
TTO samples of 21 days and 42 days	Between Groups	0.000	1	0.000	0.000	1.000
	Within Groups	10.000	6	1.667		
	Total	10.000	7	0.000		

DISCUSSION

Denture stomatitis is frequently seen in patients that wear a complete denture, which is mainly characterized by generalized inflammation of the palatal mucosa covered by the denture [19]. The prevalence of denture stomatitis in denture wearer is about 72% [20]. Candida-associated denture stomatitis should be treated even if it is associated with pain or not in order to avoid super infection and subsequent bone resorption. However, the treatment of denture stomatitis is complicated due to its high tendency of the infection to recurrence, due to the multifactorial etiology and the lack of antifungal drug efficiency [21-23]. Recently, there is an increase in the number of scientific researches demonstrating the therapeutic effects of essential oils. Generally, the useful effects of the natural plant are correlated to the presence of photochemicals and antioxidant substance in plant extract [24].

Tea tree oil is an essential oil produced from *Melaleuca alternifolia* Australian native plant, which has been largely used due to its antimicrobial and anti-inflammatory, and antifungal properties [25]. A strong antimicrobial and anti-

inflammatory property are due to its Terpinen-4-ol which is a major tea tree oil component. The mode of action of TTO against bacteria is similar to that of disinfectants as it destroys the cell membranes of microorganisms and prevents protein synthesis within it, basically, the microorganism is deactivated and cant multiplies and causes a health problem. This present study incorporated TTO into heat cured the acrylic resin and evaluated its effect against *C. albicans* growth. Results of current study revealed that acrylic disks treated with TTO showed a significant reduction in the growth of *Candida* compared to control disks up to 42 days, and this agrees with Al-Mashhadane who found that immersion of heat cure acrylic samples in 15% of TTO for 24-48 hours had significant effect against *C. albicans* [6]. The previous study immersed specimens in the oil instead of placement it in the acrylic itself. In the present study, 20% of TTO has been added into the heat cured acrylic resin so that it showed the antifungal effect which means that the oil is still present up to 42 days, avoiding other traditional methods of cleaning of denture [26].

Tea tree oil antifungal efficiency seemed to be the potential bioactive compound (s) Terpinen-4-ol have a distinct influence on *Candida* cell function, growth (Noumi Emira), which may alter membrane properties and permeability of fungal cells [27]. There were no significant changes in the growth of *Candida* on tea tree oil treated samples after the different time of storage in distilled water and this agrees with Pachava, et al., who added 15% of TTO in to soft liner and storage in distilled water up to 60 days [28].

CONCLUSIONS

Under limitation of this study, it was concluded that TTO has shown an antifungal effect up to 42 days on heat cured acrylic resin incorporated with it. Therefore, the results of this study reinforce the possible use of an extract of the natural plant as a denture cleaner and safe approach to topical treatment.

DECLARATIONS

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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