Antifungal Activity of Some Natural Oils on Heat Cured Acrylic and Tissue Conditioning Material

ABSTRACT

Aims of the Study: The current study aims to evaluate the effect of some natural products (olive oil, thymus oil,and grap seed oil) in relation to Protifex on disinfection of acrylic resin denture base and tissue conditioning materials (Heat Cured and GC extra soft denture liner). **Materials and method**: The total number of samples were 30 specimens , their dimensions were (10x10x2mm). 15 specimens were prepared from heat cured acrylic resin and 15 were prepared from GC extrasoft tissue conditioing material, for each group they were immersed for 8hrs in these oils after they had been infected with *Candida albicans* and incubated for 48hrs. This study compared antifungal efficiency of distilled water (Negative Control), effervescent Protifex tablet as a positive control, olive oil, thymus oil, and grape seed oil. The statistical tests used were one way analysis of variance test, Duncan multiple range test to compare the groups. **Results**: The results demonstrated that there were significant differences between all tested oils and D.W in relation to antifungal activity at (P=0.05). **Conclusions:** All the tested natural oils were effective as fungicidal agents and there is no significant difference among them

الهدف من الدراسة : تهدف الدراسة الحالية الى تقييم تأثير بعض المنتجات الطبيعية (زيت الزيتون. زيت الزعتر. زيت بذور العنب) (شركة العماد \ موصل \العراق) بالمقارنة مع الأقراص المطهرة للأطقم (البروتفكس) في تطهير قاعدة الطقم والمادة المبطنة للطقم.المواد وطرق البحث: تم تحضير ٥٠ عينة (١٠×١٠×٢ ملم), ٢٥ عينة محضرة من مادة قاعدة الطقم و٢٥ عينة من المادة المبطنة للطقم, كلا المجموعتين أغمست لمدة ٨ساعات في الزيوت قيد الدراسة بعد ان تم تلويث المواد بالمبيضات البيضاء بعد ذلك تم تحضينها لمدة ٤٨ ساعة . تم مقارنة التأثير المضاد للفطريات قبل وبعد المعاملة للمواد قيد الدراسة مع الماء المقطر والبروتفكس.النتائج : أوضحت نتائج الدراسة أن هناك فرق معنوي في التأثير المضاد للفطريات بين جميع الزيوت المستخدمة وبين الماء المقطر عند مستوى معنوية (٠,٠٥)الاستنتاجات والمناقشة : جميع الزيوت الطبيعية التي خضعت للدراسة لها تأثير قاتل للفطريات وان زيت بذور العنب كان أفضل زيت لكن دون فرق معنوي عن بقية الزيوت .

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Key Words: Natural oils, *Candida albicans*, Denture base, GC extrasoft liner.

Candida albicans, a dimorphic yeast-like fungus which resides as commensal in humans[1], can cause infections under altered physiological and pathological conditions. Candida infections of the skin and mucous membranes results from an interplay between a variety of Candida virulence factors (for example, hyphae formation, contact sensing, and lytic enzymes), and a variety of host defense mechanisms (including epidermal proliferation, T-cell immunity, phagocytosis, and immunoglobulins). Any factor that adversely affects normal

immune function may predispose a person to candidiasis[2].The most common oral form of candidiasis is thrush or pseudomembranous candidiasis. The prevalence and incidence of oral diseases, coupled with the resultant social and economic implications, has led to a constant striving to produce safer substances for the development of new natural antimicrobial agents[3]. In fact there is an overwhelming number of studies on the biological activities of plants and their natural product derivatives[4,5]. Essential oils and their derivatives are one such example[6,7].

Antimicrobial resistance is a common phenomenon in cell recovered from biofilms. The increased resistance of *Candida albican*s biofilms grow on denture acrylic to fluconazole, amphotericin B, nystatin, and chlorhexidine[8]. So, resistance to drugs as well as limiting toxic effect has stimulate the search for new groups of antimycotic agents, much attention was drawn to plant-derived fungicides, based on that plants have their own defense against fungal pathogens[9-11].

Many extracts of plants and isolated essential oils have demonstrated to exert biological activity in vivo and in vitro[12]. Natural products have been recently investigated more thoroughly as promising agents for the prevention of oral diseases. Plants can capable of sensing the presence of potential phytopathogens (fungi, bacteria and virus) and can produce antifungal compounds to protect themselves from biotic attack that could be essential for fungal infection resistance.

The Olive oil is an oil obtained from the olive a traditional tree crop of the Mediterranean Basin. It is commonly used in cooking, cosmetics, pharmaceuticals, and soaps and as a fuel for traditional oil lamps. Olive oil is used throughout the world, but especially in the Mediterranean[12] .

The saturated fats composed of Palmitic acid:7.5–20.0%, Stearic acid:0.5–5.0%, Arachidic acid: <0.8%, Behenic acid: <0.3%, Myristic acid: <0.1%, and Lignoceric acid: <1.0%. The monounsaturated fats composed of Oleic acid:55.0–83.0%, and Palmitoleic acid:0.3–3.5%. The polyunsaturated fats composed of Linoleic acid: 3.5–21.0 %, and Linolenic acid: <1.5%. The main phenolic compounds, hydroxytyrosol and oleuropein, which give olive oil its bitter, pungent taste, have powerful antioxidant activity both in vivo and in vitro. These compounds' possible beneficial effects are due to their antioxidant activity, anti-inflammatory and antimicrobial activity[8,10] .

Thymus (also known as 2-isopropyl-5-methylphenol), (IPMP) is a natural monoterpene phenol derivative of cymene, C10H14O, isomeric with carvacrol, found in oil of thyme, and extracted as a white crystalline substance of a pleasant aromatic odor and strong antiseptic properties. Thymus is only slightly soluble in water at neutral pH, but it is extremely soluble in alcohols and other organic solvents. It is also soluble in strongly alkaline aqueous solutions due to deprotonation of the phenol. It is also called "Isopropyl-m-cresol" and "hydroxy cymene"[11]. Thymus has been used to successfully control varroa mites and prevent fermentation and the growth of mould in bee colonies[11]. A tea made from the plant was also used to treat mouth and throat infections caused by dental caries and gingivitis[12], and thymus has been shown to be an effective fungicide, particularly against fluconazole-resistant strains. This is especially relevant given that opportunistic Candida (fungus) infections can cause severe systemic infections in immunocompromised patients and current treatments are highly toxic, often result in drug resistant Candida strains[13-18] .

Grape seed oil (also called grapeseed oil or grape oil) is a vegetable oil pressed from the seeds of various varieties of Vitis vinifera grapes, an abundant by-product of winemaking. Saturated fats composed of Palmitic: 7%, and Stearic: 4%, monounsaturated fats composed of

Oleic acid: 16-17%, and Palmitoleic acid<1%, the polyunsaturated fats composed of Omega-3 fatty acids (α-Linolenic) : <1%, and Omega-6 fatty acids (Linoleic 72%)[19-22].

The current study aims to evaluate the effect olive oil, thymus oil, and grape seed oil on disinfection of acrylic resin denture base and tissue conditioning materials.

MATERIALS AND METHODS

I-Sample preparation:

Heat cured acrylic denture base material and the GC extra soft denture lining material were used in this study.

The total number of specimens were thirty, their dimensions were (10x10x2mm) and divided into two equal groups for both heat cured and denture lining material.

The wax specimens plates were fabricated[23] and flasked according to Craige *et al*(1987)[24], wax elimination were done and the samples were prepared according to conventional heat curing technique[26,27] .

GC extra soft lining materials supplied as two pastes, using special gun for injection the material then mixed and applied into the created mould and allowed to set under press for ten minutes according to manufacturer instruction, then the flask was opened and the excess material was removed using sharp scalpel.

II-Sterilization of specimens :

After the samples were autoclaved the samples were immersed in distilled water at 37ºC and stored for seven days in incubator for conditioning[28-29].

III- Microbiological experiment:

All samples had been infected with *Candida albicans* by adding 1ml of 12hrs young candidial suspension without agitation and incubated for 48 hours and by using the standardized candidal cell suspensions ($600x10^6$ CFU\ml) which equal to Macfarland standard bacteriological solutions [30].

Then the infected samples were immersed for eight hours in the tested oils together with the protifex (control positive group) and distilled water (control negative group).

After incubation with tested materials for 8hrs at 37ºC, plating and counting was done for 0.01 ml of each tested material, the retaining cells were counted for CFU\ml of *Candida albicans.*

Dunnet -2sided-test, ANOVA followed by Duncans multiple range test were employed.

RESULLTS AND DISCUSSION

Over the past decades, herbal medicine has become a thing of global significance with medicinal and economic implications. Wide spread use of herbs throughout the global has raised serious concern over this quality, safety and efficacy. Thus exact scientific assessment has become a precondition for acceptance of herbal health claims. Aromatic herbal oils used for cooking and flavoring are increasingly claimed to have broad spectrum antifungal activities. The mean, number of samples and standard deviation were illustrated in Table (1).

Table (1): Descriptive statistic of the tested materials

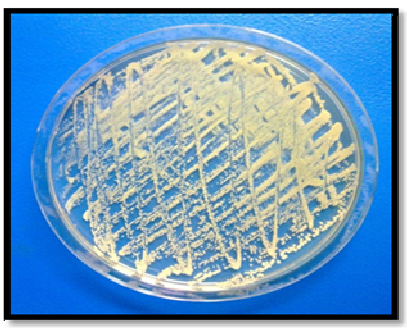
Treats	Materials	N	Mean	Std.deviation
Distilled water (Control –ve)	Heat cure acrylic	3	85661.00	5378.953
	GC soft liner	3	6433.33	802.081
	Total	6	46047	43530.880
Protifex (Control +ve)	Heat cure acrylic	3	.00	.000
	GC soft liner	3	.00	.000
	Total	6	.00	.000
Thymus oil	Heat cure acrylic	3	30.00	17.758
	GC soft liner	3	40.33	8.737
	Total	6	35.17	13.644
Olive oil	Heat cure acrylic	3	24.00	14.422
	GC soft liner	3	108.67	10.017
	Total	6	66.33	47.685
Grape seed oil	Heat cure acrylic	3	4.67	1.528
	GC soft liner	3	13.67	3.215
	Total	6	9.17	5.419

The antifungal activity of these oils on heat cured acrylic resin denture base and extra soft denture lining materials was demonstrated. The antifungal activity was better on heat cured denture base material (58.67 CFU) than denture lining material (162.67 CFU) this may be due to the fact that the denture lining material accommodate *Candida albicans* more than heat cured acrylic resin because the temporary soft lining material are not resistant to adhesion i.e the adherence of *Candida Albicans* to soft lining material is more than acrylic denture base[31] .

Multiple comparisons using Dunnet t-test (Table 2) revealed that there is a significant difference at (p=0.05) between all tested oils and the distilled water Figure (1) (control negative) as shown in Table (3).

Table (2): Multiple comparisons t-test

	Treats	Control -ve	90% confidence interval	
			Upper bound	Lower bound
Dunnett (2-sided) test	Protifex	Distilled water	-71667.67	-20426.67
	Thymus oil	Distilled water	-71632.50	-20391.50
	Olive oil	Distilled water	-71601.33	-20360.33
	Grape seed oil	Distilled water	-71658.50	-20417.50



Figure(1) Control –iv group

Table (3): Duncan s' multiple range test

Treatments	N	Subset for alpha =0.5	
		A	B
Protifex	6	.00	
Thymus oil	6	35.17	
Olive oil	6	66.33	
Grape seed oil	6	9.17	
Distilled water	6		46047.17
Sig.		.995	1.000

The essential oils were able to inhibited germ tube formation, an important virulent factor in *Candida albicans*, they act as fungicidal, promoting sever lesion on the plasma membrane. This suggest that the fungicidal effect result from direct damage to the cell membrane rather than from metabolic impairment leading to secondary plasma membrane damage[32].

Also these oils include considerable impairment of *Candida albicans* biosynthesis of ergosterol, the predominant sterol in fungi cells, that play an important role in membrane fluidity, permeability and on the activity of many membrane bounded enzymes. Another antifungal activity explanation is the mechanism of action of these oils may be due to its viscosity that act by pulling mechanism, saponification, and emulsification action, that may be caused by high content of polyunsaturated fatty acid[33-37].

The difference in the antifungal activity of the tested oils was explained by both strain susceptibility and different oil composition[32] as shown in Figures (2-4).

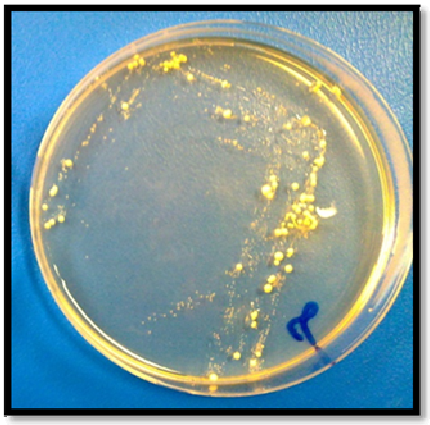
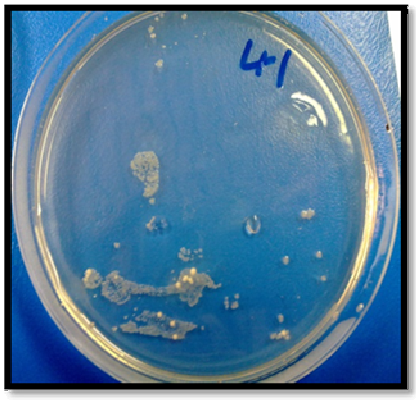


Figure (2) Thymus oil group



Figure(3) olive oil group

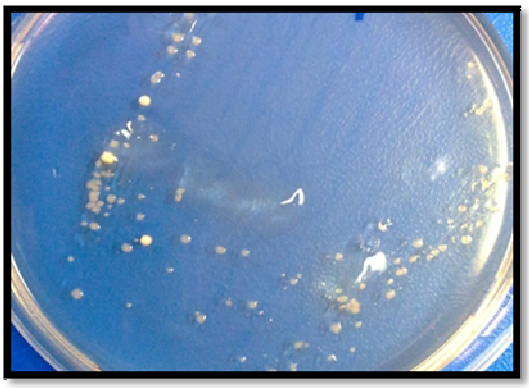


Fig (5) Grape seed oil

Although protifex (the control positive) showed the most antifungal activity as shown in Figure (5) but considering the importance of fungal infection, and the difficulties encountered in their treatment, as well as the increase in the resistance of antifungal drugs, many scientists have recently paid attention to extract an biologically active compounds isolated from plates species used in herbal medicine. A wide variety of essential oils are known to possess antifungal activity, also these oils are more available and cheaper.

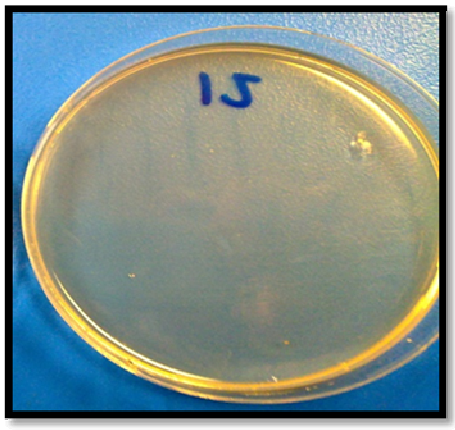


Figure (2) Control +ve group

CONCLUSIONS

All the materials have antifungal activity when comparing them with distilled water, but there are no significant difference among them.

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