Role of Some Natural products as a Disinfectant on Color of GC-Soft Liner

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Abstract

The study aims to evaluate the effect of some natural products (nigella sativa, sesame, flax and ginger oil) (Emad factory/mosul/Iraq) in relation to nystatin suspension on color of GC-Soft Liner Fifty Sample were prepared from GC-extra soft lining material, they were (10*10*2mm). Half of the samples for each group were immersed for 8hr in these oils after they had been infected with *Candida albicans* and incubated for 48hr, the other half of the samples immersed for 8hr daily for one month. This study compared antifungal efficiency before and after immersion in distilled water (Negative Control), nystatin suspension (100000 I.U/ml as a positive control), nigella sativa, sesame, flax and ginger oil. Color of samples tested by Vita – Easy Shade system, the statistical tests used were analysis of variance, Dunnet T-test. The results demonstrated that there were significant differences between all tested oils and D.W in relation to antifungal action, and no significant differences between all tested oils and D.W in relation to color of GC-soft lining material at (P=0.05). All the tested natural oils were effective antifungal agents and would be accepted and safe in relation to the color of soft liner.

تاثير بعض المنتجات الطبيعية كمطهرات على لون مادة تبطين الطقم

رنا ربيع الصميدعي

المستخلص

إن الغاية من هذه الدراسة هو تقييم بعض المنتجات الطبيعية كمطهرات لمادة تبطين الطقم, وتشمل: زيت حبة السوداء زيت السمسم، زيت الكتان،وزيت الزنجبيل (معمل عماد/موصل/عراق), مقارنة بمحلول النستاتين,وتقييم تأثيرها على لون مادة تبطين الطقم. بلغ العدد الكلي لعينات مادة تبطين الطقم (٥٠عينة) بابعاد(١٠×١٠×٢) ملم , نصف عدد العينات لكل مجموعة غمرت في هذه المحاليل لمدة ثمان ساعات بعد ان تم تحضينها مع خلايا المبيضات البيضاء لمدة ٤٨ ساعة ,والنصف الثاني للعينات لكل مجموعة غمرت في هذه المحاليل لمدة ثمان ساعات يوميا لمدة شهر كامل. اختبرت الدراسة فاعلية هذه المحاليل على القضاء على خلايا المبيضات البيضاء مقارنة بالنستاتين(100000وحدة دولية/مل) و الماء المقطر. تم استعمال جهاز (Vita – Easy Shade system) لقياس التغيير في لون العينات, وقد تم استعمال التحليلات الإحصائية الآتية (التحليل الوصفي و تحليل التباين(ANOVA) و Dunnet T-test) وذلك عند مستوى معنوية(٠.٠٥), حيث وجد فرق معنوي واضح بين الزيوت المستعملة والماء المقطر, وكانت هذه الزيوت اكثر فعالية من النستاتين. تستنتج من هذه الدراسة إن كل المحاليل المستعملة كمنظفات لمبطنات الطقم ناجحة في الاختبارات التي أجريت للقضاء على خلايا المبيضات البيضاء عند التغطيس لمدة ثمان ساعات, واختبار التغيير في لون مادة تبطين الطقم عند التغطيس لمدة ثمان ساعات يوميا في المحاليل خلال مدة شهر كامل.

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Introduction

For patients who cannot tolerate a hard denture base, soft liners are an important adjunct treatment to help the patients adapt to their new dentures [1]. The soft liners were often favored as an alternative to conventional hard relining materials [2]. A soft liner's assessment criteria as listed by many authors were, namely, resilience, tear resistance, biocompatibility, lack of odor and taste, adhesive bond strength, color stability, and resistance to abrasion [3-5]. Although some of the material types were reported to maintain these qualities for a longer time than the others, loss of bonding to denture base polymers and fungal colonization on and within the soft liners continue to be the main problems limiting their use [3-5]. Denture stomatitis is the most common infectious disease affecting the palatal mucosa and is highly prevalent in denture wearers, mainly characterized by the presence of *Candida albicans* [6,7]. Denture lining material support more Candidal growth than acrylic denture base materials [8,9]. Traditional treatment modalities of denture stomatitis include the use of antifungal agents and modification of the prosthesis to receive a denture liner, where the most widely used antifungal agent was nystatin [10,12]. It is more accepted to use natural denture cleanser solutions such as sunflower or sesame oil for their oil pulling action that prevent teeth decay, oral malodor, bleeding gum, dryness of throat and lips, and for strengthening of teeth, gums and jaws [13]. Recently many researchers improve that the essential natural oils has antifungal, antiviral, antibacterial and anti amoebic action, including nigella(nigella sativa), sesame(*Sesamum indicum*) , flax(*Linum usitatissimum*) and ginger oil(*Zingiber officinale)* [14-22] , and these were safe and biocompatible materials [23]. The specific aim of this study was to assess the antifungal action of these oils and their effect on color of GC soft liner.

Materials and Methods

Fifty Sample were prepared from GC-soft linning material (Japan), they were (10*10*2mm) according to Webb *et al.*[24]. Flasking was done in the conventional method, packing of GC Extra soft liner (auto polymerized material) where the material supplied as two pastes and need special gun, which allows mixing and injection of the material into created mould, according to the manufacture instruction. After the completion of curing flasks were opened and the samples were removed from their stone moulds. Any flashes of excess resin material were removed from the specimens with a sharp scalpel. Half of the samples for each group were immersed for 8hr in these products after they had been infected with *Candida albicans* and incubated for 48hr, the other half of the samples immersed for 8 hr per day in these products throughout one month. The procedure involve preparing the MacFarland Standard Bacteriological Solution (tube No.2 = 6×10^8 CFU/ml) that composed of 0.2 ml. Barium Chloride of 1% and 9.8 ml. H2SO4 of 1%[25]. Mix loop full *C. albicans* for several times with sterile distilled water to prepare a fresh *C. albicans* suspension matching MacFarland Standard Bacteriological Solution tube No.2. by using U.V.spectrophotometer (CECIL), then put 1 ml. of the prepared bacterial suspension in five screw capped bottles then immerse one sterile specimen in each one, then incubated for 24hrs at 37°C, where after incubation we take 0.01 ml. of the bacterial suspension and plated on Sabouroid agar for counting of *C. albicans* colonies after incubated for (24hrs at 37 °C) . (to check the count of viable species only).After that remove each specimen from their screw capped bottles by using sterile twizzer then place each one in a screw capped bottle containing (1ml) of one of these solutions for 8 hrs. Then take 0.01ml of solutions from each screw capped bottle and plated on sabouroid agar for counting of colonies as(CFU/ml) after *C. albicans* was incubated for (24hrs at 37°C). Then the color of samples was tested by using Vita – Easy Shade (VITA, U.S.A). When the easy shade is warming up, the bottom of the screen displays a "presets" selection box then the appropriate mode of

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measurement must be selected in the measurements, "tooth single mode" of operation was selected and the device is adjusted to display the results of a measurement as L (Lightness), C (Chroma) and H (Hue). Calibration is achieved by placing the 5 mm probe against a calibration block within the machine, according to the manufacturer's instructions. But the color of GC-extra soft liner samples were measured and not teeth, so a transparent calibration blocks were used during calibration.

Results and Discussion

This study attempted to assess the effectiveness of some available essential oils as antifungal agents and their effect on color of GC- soft liner .

1-For The Disinfection Test:

The mean ,number of samples, and standard deviation for the antifungal action of these oils on GC-soft lining material were shown in Table (1),the one way analysis of variance (Table 2) showed that at P=0.05 there were significant differences between treats, which came from the significant differences between all tested oils and D.W(control) shown in Dunnett t-test (Table 3). So, all tested oils were effective in disinfection of GC-soft lining material from *C. albicans*. That was agreed with different researchers for the antifungal action of these oils[26-28] , the mechanism of action of these oils may be due to its viscosity that act by oil pulling mechanism, saponification, or emulsification action [13,29], that may be caused by their high content of poly unsaturated fatty acids, palmitic acid, stearic acid , oleic acid , linoleic acid ,The triply unsaturated omega-3 fatty acid, α-linolenic acid [18,21]. Duncans multiple range test (Table 4) showed that at P=0.05, sesame and ginger oil had stronger antifungal action than nigella sativa and flax oil ,that may result from their content of gingerol , Sesamin, and sesamolin[18,21,29,30].

So, the results of this study showed that all the tested oils were effective as a fungicidal agent, even greatly better than nystatin which is the most widely used antifungal agent, this agreed with Lima [31] that stated that when yeast <1000 CFU /ml of saliva considered as no growth.

2- For The Color Test:

The mean ,number of samples, and standard deviation for the color of GC-soft lining material(Lightness, Chroma and Hue) after daily 8hrs of immersion in the tested disinfectant oils for one month were shown in Table (5). The one way analysis of variance (Table 6) showed that at P=0.05 there were no significant differences between treats in relation to color of GC-soft lining material(Lightness, Chroma and Hue), while Dunnett t-test (Table 7) showed that color changes with all the tested disinfectant oils were less than that for nystatin, that indicate that these are safe disinfectant in relation to color of GC-soft lining material. that can be explained by the fact that these oil having yellow color that can protect from U.V light[22].While in other studies ,some peroxide containing soft liner disinfectants, it cause significance differences in color of soft liner [31-35] .

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Table (1):- Descriptive statistics for disinfection of GC-soft lining material from *C. albicans*:

Treats	Mean(CFU/ml)	N	S. D
D.W	68224.00	5	5452.976
Nystatin	652.40	5	107.029
Nigella	15.20	5	8.786
Sesame	11.20	5	2.588
Flax	26.40	5	8.735
Ginger	10.60	5	2.881
Total	11489.97	30	25886.430

S.D: standard deviation , N: number of samples

Table (2):- Analysis of variance for disinfection of GC-soft lining material from *C. albicans*:

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	19314124817.767	5	3862824963.553	779.147	.000
Within Groups	118986293.200	24	4957762.217		
Total	19433111110.967	29			

df :degree of freedom

Table (3):- Dunnett (2-sided) t- test for disinfection of GC-soft lining material from *C. albicans*:

	(I) treats	(J) treats	Mean Difference (I-J)	S. E	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Dunnett	Nystatin	D.W	-67571.60	1408.228	.000	-71367.23	-63775.97
	Nigella	D.W	-68208.80	1408.228	.000	-72004.43	-64413.17
	Sesame	D.W	-68212.80	1408.228	.000	-72008.43	-64417.17
	Flax	D.W	-68197.60	1408.228	.000	-71993.23	-64401.97
	Ginger	D.W	-68213.40	1408.228	.000	-72009.03	-64417.77

S.E: standard error

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Table (4):- Duncan multiple range test for disinfection of GC-soft lining material from C. albicans

	Treats	N	Subset for alpha =0.05	
			1	2
Duncan	Ginger	5	10.60	
	Sesame	5	11.20	
	Nigella	5	15.20	
	Flax	5	26.40	
	Nystatin	5	652.40	
	D.W	5		68224.00
	Sig.	-	.688	1.000

Table (5):- Descriptive statistics for color of GC-soft lining material:

TREATS		LIGHTNES	CHROMA	HUE
D.W	Mean	66.0400	32.6400	29.8740
	N	5	5	5
	S. D	.59833	.87350	.41615
Nystatin	Mean	66.7800	31.4400	30.6600
	N	5	5	5
	S. D	.59330	1.91259	.24083
Nigella	Mean	66.0600	33.1600	30.5600
	N	5	5	5
	S. D	1.32023	1.24218	1.11041
Sesame	Mean	65.0800	32.2600	30.2220
	N	5	5	5
	S. D	.81976	3.09079	.38603
Flax	Mean	65.0200	31.9000	30.4600
	N	5	5	5
	S. D	1.85661	1.93778	.88204
Ginger	Mean	65.2000	35.1400	30.8800
	N	5	5	5
	S. D	.86313	1.06911	.73959
Total	Mean	65.6967	32.7567	30.4427
	N	30	30	30
	S. D	1.20071	2.07209	.71622

S.D: standard deviation, N: number of samples

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Table (6):- Analysis of variance for color of GC-soft lining material

		Sum of Squares	df	Mean Square	F	Sig.
LIGHTNES	Between Groups	12.542	5	2.508	2.057	.106
	Within Groups	29.268	24	1.220		
	Total	41.810	29			
CHROMA	Between Groups	42.854	5	8.571	2.519	.057
	Within Groups	81.660	24	3.403		
	Total	124.514	29			
HUE	Between Groups	3.123	5	.625	1.276	.307
	Within Groups	11.753	24	.490		
	Total	14.876	29			

df :degree of freedom

Table (7):- Dunnett (2-sided) t- test for color of GC-soft lining material:

	Treats	(I) Treats	(J) Treats	Mean Difference (I-J)	S. E	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Dunnett t	LIGHTNES	Nystatin	D.W	.7400	.69843	.736	-1.1425	2.6225
		Nigella	D.W	.0200	.69843	1.000	-1.8625	1.9025
		Sesame	D.W	-.9600	.69843	.521	-2.8425	.9225
		Flax	D.W	-1.0200	.69843	.465	-2.9025	.8625
		Ginger	D.W	-.8400	.69843	.638	-2.7225	1.0425
Dunnett t	CHROMA	Nystatin	D.W	-1.2000	1.16662	.756	-4.3444	1.9444
		Nigella	D.W	.5200	1.16662	.989	-2.6244	3.6644
		Sesame	D.W	-.3800	1.16662	.997	-3.5244	2.7644
		Flax	D.W	-.7400	1.16662	.952	-3.8844	2.4044
		Ginger	D.W	2.5000	1.16662	.153	-.6444	5.6444
Dunnett t	HUE	Nystatin	D.W	.7860	.44258	.290	-.4069	1.9789
		Nigella	D.W	.6860	.44258	.410	-.5069	1.8789
		Sesame	D.W	.3480	.44258	.894	-.8449	1.5409
		Flax	D.W	.5860	.44258	.555	-.6069	1.7789
		Ginger	D.W	1.0060	.44258	.119	-.1869	2.1989

S.E: standard error

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Conclusions

All the prepared natural solutions were effective as antifungal agents and would be accepted and safe in relation to the color of soft liner.

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