The Fungicidal action of Some Natural Products (Denture cleansers) on Acrylic Denture Base Material

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Abstract:

Aim: To evaluate some natural products (soda+ vinegar, soda + thymol , saturated salt solution) compared with the commercial denture cleanser (Protifex), for disinfection of acrylic denture base material from *C. albican*s (*in vitro*). **Materials And Methods**: Thirty five samples, (10 x 10 x 2 mm length, width, and thickness respectively), were prepared from heat cured acrylic resin denture base material (Major heat cured acrylic resin), using MacFarland Standard Bacteriologica Solution technique, tube No.2., using a light microscope with camera connected to computer for testing anti fungal efficiency of these natural denture cleansers (soda, vinegar, thymol, saturated salt solution). This procedure was done after 1hour, 4hours, and 8 hours of immersion. in comparison with samples immersed in distilled water, (Control). **Results:** The results demonstrated that at P=0.05 there were significant differences between all prepared solutions and control. The best prepared natural solution was saturated salt solution. There were significant differences between all times of immersion, the best was 8hrs. **Conclusions:** All the prepared natural denture cleansers were considered as fungicidal disinfectants, and a proper disinfection of the acrylic denture base material requires 8hrs of immersion in the prepared disinfectant solutions. The best prepared natural solution was saturated salt solution.

الخلاصة

الهدف : تهدف هذه الدراسة لتقييم بعض المنتجات الطبيعية (صودا الخل + الصودا ثيمول + محلول الملح المشبع) مقارنة بالمنظف التجاري (Protifex)، للتطهير من المبيضة البيضاء لمادة قاعدة الطقم الاكريلي(وهي دراسة في المختبر) **المواد والأساليب** : خمس وثلاثون عينات، (١٠ × ١٠ × ٢ مم الطول والعرض، وسمك على التوالي) أعدت، من مادة الراتنج الاكريلي الحراري (Major heat cured acrylic resin)، وذلك باستخدام تقنية ماكفارلاند البكتريولوجي القياسي No.2 أنبوب ، واستخدام المجهر مع ضوء الكاميرا المتصلة بالكمبيوتر لاختبار كفاءة مكافحة هذه المطهرات الفطرية الطبيعية (الصودا, الخل, ثيمول، ومحلول الملح المشبع). وقد تم هذا الإجراء بعد، ١ساعة، ٤ ساعات و ٨ ساعات من الغمر. في مقارنة مع عينات مغمورة في الماء المقطر، (التحكم).**النتائج** : أظهرت النتائج أن P=0.05 هناك فروق ذات دلالة إحصائية بين جميع المحاليل التي تم تحضيرها مع نماذج التحكيم. وكان أفضل محلول تم تحضيره هو محلول الملح المشبع. وكانت هناك فروق ذات دلالة إحصائية بين جميع أوقات الغمر, وكان الأفضل هو ٨ ساعات. **الاستنتاجات**: جميع المنظفات الطبيعية تعتبر كمطهرات ومبيدة للفطريات، و تعقيم سليم للمادة الاكريليك لقاعدة الطقم يتطلب ٨ ساعات من الانغماس في المحلول المطهر . وكان أفضل محلول طبيعي هومحلول الملح المشبع .

Key words: Denture cleanser, anti fungal, acrylic resin.

The word "disinfection" signifies the elimination of hazards of infection, i.e. the removal or destruction of the infective agent, with the exception of bacterial spores [1-3].

Acrylic resin is the most employed material in the construction of removable complete denture [4–5].Chemical cleansing approach is recommended for plaque control [6-10] as an alternative to the mechanical approach in patients with lack of motor coordination[11]. Every surface in the oral cavity, natural, or synthetic, becomes covered within 30 minutes with 0.5-1.5 μ thickness precipitate of salivary glycoprotein, and immunoglobulin, that is termed "pellicle"[11]. The pellicle in turn provides a substrate to which oral debris; (mucin, food particles, desquamated epithelial cells, and microorganisms "bacteria, and fungi" readily adhere[12].

Many studies had shown that Candida species, and other microorganisms, including species of *Streptococcus, Staphylococcus, Lactobacillus bacteria,* and *Actinomyces* are associated with denture plaque [13]. The worldwide overuse of antibiotics has caused microorganisms to develop resistance to the current antibiotics, and to become virulent, therefore , antibiotic resistance is a global problem, and dentists must be involved in halting it[14].

The purpose of this study was to evaluate the effect of the natural denture cleansers disinfection of *C. albican*s for acrylic denture base material.

MATERIALS AND METHODS

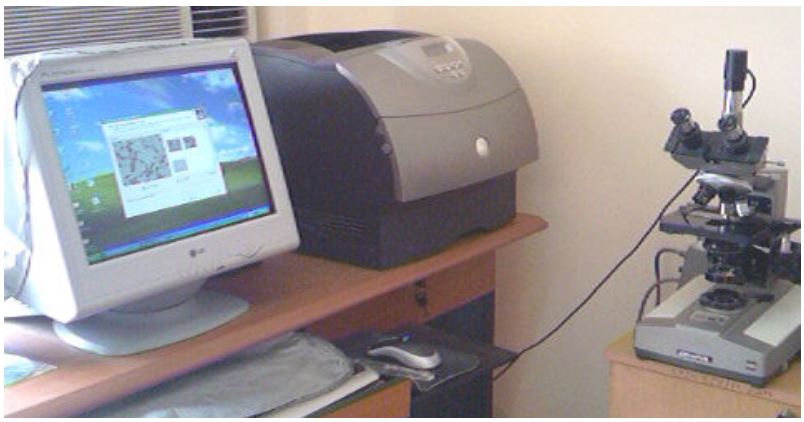
Thirty five specimen of acrylic denture base material used for testing antifungal efficiency of used denture cleansers (*In vitro* study) on *C. albicans*. Specimens are of (10 x 10 x 2mm length, width and thickness respectively) according to Webb *et al.* [1], Flasking was done by mixing in ratio of 28 -32 ml of water: 100 gm of stone, the procedure was done in the conventional method[1]. Packing and Curing were carried out by placing the clamped flask in the thermostatically controlled water bath for (1 hr at 74°C then 1/2 hr at 100°C), according to the manufacturer instructions. After the completion of curing, flasks were allowed to bench cool for 30minuts. The acrylic specimens were removed from their stone moulds.

By using MacFarland Standard Bacteriological Solution technique, tube No.2.,and using light microscope with camera, connected to computer (Figure 1). The culture media, and acrylic specimens were sterilized by using an autoclave at 15 pound / inch² at 121ºC for 15min., while glass Petri-dishes, screw cap bottles, and tweezers were sterilized by hot air oven at (160-180)°C for 1 hour [15]. This procedure was done after 1hour, 4hours , and 8 hours of immersion in the five prepared solutions. They were prepared by Khalil [16], and one commercial denture cleanser tablets (Protifex) for comparison, and distilled water as a control solution .Every solution was diluted in 100 ml of distilled water (Table 1)

For the identification of C. albicans the diagnostic laboratory tests used include: Culture characteristics: On Sabouraud's Dextrose Agar medium within (24-48)hrs at 37 ºC, Candida species produce soft creamy-coloured colonies with a yeast odor [14,17] Figure (2).

Microscopic Examination: The smears that had been obtained from the patients were examined by light microscopic using a gram's stain technique for pseudohyphae and budding [14,17] Figure (3), and Germs Tube Test: In this test, a loopfull was taken from each culture, incubated in test tubes containing human serum (0.5-1) ml for about 90 min intervals at 37ºC [17,18]. Microscopic observation made for a smear obtained from each test tube. The yeast cells of *C. albicans* will begin to form germ tubes or true hyphae after 30 min [19].

The procedure involved preparing the MacFarland Standard Bacteriological Solution (tube No.2 = 600*10 ^6 CFU/ml) that composed of 0.2 ml. Barium Chloride of 1% , and 9.8 ml. H2SO4 of 1%[20].(prepare new culture of pure *C. albicans* (so it will be fresh and in the active face), mix loop full *C. albicans* for several times with sterile distilled water to prepare a bacterial suspension matching MacFarland Standard Bacteriological Solution tube No.2. by using U.V. spectrophotometer (CECIL), then 1ml. of the prepared bacterial suspension was put in five screw capped bottles, then, one sterile wax specimen was immersed in each one, then incubated for 24hrs at 37ºC, where after incubation 0.01 ml. of the bacterial suspension was taken, and plated on Sabouroid agar for counting of *C. albicans* colonies after incubation for 24hrs at 37 ºC. (to check the count of viable species only).After that, each wax specimen was removed from their screw capped bottles by using sterile tweezers, then each one was placed in a screw capped bottle containing (1ml)of one of the five prepared solutions. then 0.01ml of solutions was taken from each screw capped bottle, and plated on sabouroid agar for counting of colonies as(CFU/ml) after the *C. albicans* was incubated for (24hrs at 37ºC). This procedure was done after 1hour, 4hours , and 8 hours of immersion in the five prepared solutions.

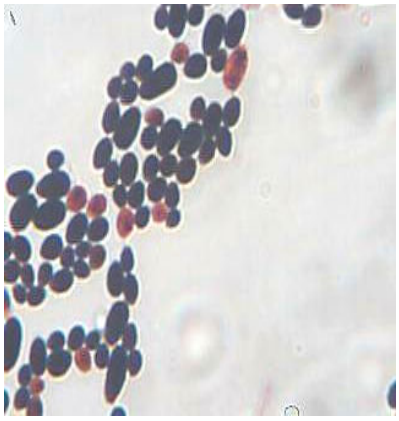


(Figure 1) Light microscope with camera connected to computer

Table (1) Solutions Preparation

Solution no.	Material 1	Weight or volume	Material 2	Weight or volume
1	**Soda**	7 g	Clear vinegar	5 ml
2	**Soda**	2 g	Thyme oil	3.57 g
3	**Saturated salt**	40 g		
4	**Distilled water**	100 ml		
5	**Protefix**	1 tab= 2.85		

(Figure 2) *C. albicans* on SDA



(Figure 3) *C.albicans*,microscopically

RESULLTS

The mean, standard error, and number of samples and 95% Confidence Interval for antifungal action on acrylic denture base are shown in (Table 2, and Figure 4), Duncan's multiple range test (Table 3) showed that at P=0.05, there were significant differences between all prepared solutions, and control. The best prepared natural solution was saturated salt solution. Duncan's multiple rang test (Table 4) showed that at P=0.05, there were significant differences between all times, the best was 8hrs.

Table (2) :Descriptive statistics for disinfection of acrylic denture base from *C. albicans*.

TREATS	Time of immersion	Mean (CFU/ml)	S. E	95% Confidence Interval	
				Lower Bound	Upper Bound
D.W	1 Hour	357500.000	3751.755	349503.323	365496.677
	4 Hours	404000.000	3751.755	396003.323	411996.677
	8 Hours	499000.000	3751.755	491003.323	506996.677
Salt	1 Hour	112000.000	3751.755	104003.323	119996.677
	4 Hours	57500.000	3751.755	49503.323	65496.677
	8 Hours	7000.000	3751.755	-996.677	14996.677
Protefix	1 Hour	4750.000	3751.755	-3246.677	12746.677
	4 Hours	2250.000	3751.755	-5746.677	10246.677
	8 Hours	100.000	3751.755	-7896.677	8096.677
S.+ving	1 Hour	307000.000	3751.755	299003.323	314996.677
	4 Hours	246000.000	3751.755	238003.323	253996.677
	8 Hours	95000.000	3751.755	87003.323	102996.677
S.+Thy	1 Hour	327500.000	3751.755	319503.323	335496.677
	4 Hours	287500.000	3751.755	279503.323	295496.677
	8 Hours	257000.000	3751.755	249003.323	264996.677

S.E: standard error

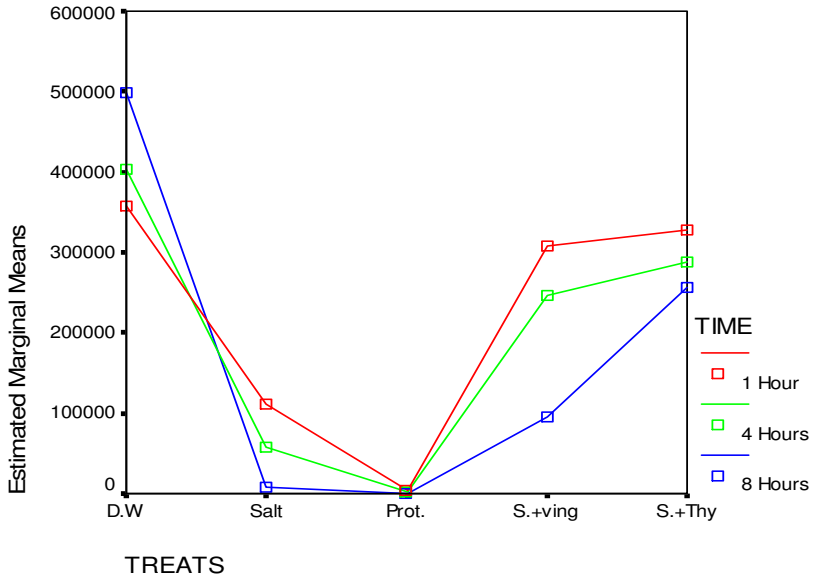


Figure (4) The mean for the disinfection of Acrylic denture base from *C. albicans*.

Table (3) Duncan multiple range test for disinfection of acrylic denture base material from *C. albicans*, between treats:

	TREATS	N	Subset				
			1	2	3	4	5
Duncan	**Protefix**	6	2366.6667				
	Salt	6		58833.3333			
	S.+ving	6			216000.0000		
	S.+Thy	6				290666.6667	
	D.W	6					420166.6667
	Sig.		1.000	1.000	1.000	1.000	1.000

N: number of samples

Table (4) Duncan multiple range test for disinfection of acrylic denture base material from *C. albicans*, between times:

	TIME	N	Subset		
			1	2	3
Duncan	8 Hours	10	171620.0000		
	4 Hours	10		199450.0000	
	1 Hour	10			221750.0000
	Sig.		1.000	1.000	1.000

N: number of samples

DISCUSSION

The results showed that there were significant differences between all treats, and the control. So, all prepared natural denture cleansers are effective in disinfection of acrylic denture

base material from *C. albicans*. The best prepared natural solution was saturated salt solution, and this agrees with Baltch *et al.* [21] where he stated that salt had antibacterial action against (*C. albicans*, *S. aureus*, *Pseudomonas aeruginosa*, and *Legionella pneumophila*) and that *C. albicans* was the most sensitive organism to salt. The best time of immersion was 8hours, this was in disagreement with Queisser Pharma [22] which stated that her product (Protefix) insured complete cleaning, and disinfection within 15 minutes only, and disagreed with Pavarina *et al.*[23] who concluded that immersion of denture in one of three solutions, 3.78% alkaline peroxide, 4% chlorhixidine gluconate, 1% sodium hypochlorite for about 10 minutes was effective in reducing microbial growth.

CONCLUSIONS

All the prepared natural denture cleansers were accepted, and considered as fungicidal disinfectant, and a proper disinfection of the acrylic denture base material requires 8hrs of immersion in the prepared disinfectant solutions.

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