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The Effect of Some Natural Products on Disinfection and Some Physical Properties of Modeling Wax

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Abstract: Aims: *evaluating some natural products (soda+ vinegar, soda +thymol , saturated salt solution) in relation to commercial denture cleansers(protifex), for disinfection of modeling wax from C. albicans, Streptococcus mutans, and Staphylococcus aureous and evaluate their effect on some physical properties of wax (dimensional accuracy, water sorption and color changes).* ***Methods****: The total number of samples were three hundred and fifteen (225 sample for the physical tests (dimensional accuracy test ,water sorption test and color changes), and microbiological tests :90 sample (C. albicans, Streptococcus mutans, and Staphylococcus aureous in an invitro study).* ***Results:*** *All the tested denture cleansers were accepted in the applied tests for modeling wax , except for soda+ thymol that show significant difference from the control in water sorption of modeling wax immersed for 1/2 hour and 8hours daily for one month ,and that the proper disinfection of the modeling wax require 8 hours of immersion in the prepared disinfectant solutions. Saturated salt solution was the best disinfectant, by having broad spectrum antibacterial and antifungal action, and it was the safest effect among all other solutions on modeling wax. Soda + vinegar and Soda + thymol were accepted in all tests of this study, and were the best prepared disinfectant of modeling wax from S. aureus when used for 8 hours.* ***Conclusions*** *:For the disinfection of modeling wax , all the prepared natural denture cleansers were effective and safe , except for soda +thymol which decrease water sorption of modeling wax , and the best and safest one was saturated salt solution.*

Keywords: Modeling wax, *C. albicans*, Streptococcus mutans, Staphylococcus aureous**.**

1. INTRODUCTION

Waxes have wide applicability in dentistry and many dental procedures involve wax containing material at some stage in the protocol [(1)]. Although a lot of importance is given to infection control in the dental clinic for all the instruments to be used and for the dentures, but it is usually overlooked in the laboratory and especially for the wax bites, where only a few studies of wax disinfection was done such as (Infection control manual ,2008) [(2)] suggested soaking wax bite plates in (1:10) dilution of Clorox or any other acceptable disinfectant for 10 minutes before using them and before sending them to the laboratory, (Bhat *et al.*, 2007) [(3)] stated that immersion disinfection may cause distortion to some items like wax bite so iodophor disinfection sprays should be used,(Guide lines on infection control in dental clinics,1993)stated that wax bites can be disinfected by immersion in 0.1% sodium hypochlorite for 10 minutes[(4)], and (USAF , 2004) stated that immersion disinfection of wax bite rims may cause distortion, so spray type is preferable[(5)].

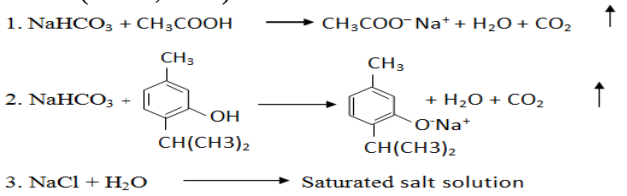
The worldwide overuse of antibiotics has caused microorganisms to develop resistance to the current antibiotics and to become virulent, therefore, antibiotic resistance is a global problem and dentists must be involved in halting it [(6)]. Microorganisms, however, do not appear to develop tolerance or resistance to the antibacterial effects of essential oils such as clove oil (Eugenol) and thyme oil (Thymol). Such oils provide an effective, powerful, and cost-effective means of infection control in the dental practice. Thyme oil is the most germicidal essential oil. Essential oils have been used since the 19th century for the root canal therapy, temporary fillings, and periodontal therapy. A component of thyme oil has been used in several medicaments. During the first half of the 20th century, thymol was used in alcohol solutions for cavity sterilization, but alcohol decreased the effectiveness of Essential Oils. Instead of eliminating the alcohol from the solution, practitioners mistakenly discontinued using thymol in dental procedures [(7)].

It is more accepted to use natural denture cleanser solutions such as powder or oils 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 [(8)]. To prevent bacterial cross-contamination among denture patients all dental prosthesis must be disinfected on entering and again on leaving the laboratory [(9**)**. There are only few researches on disinfection of modeling wax[(41,42)], while it is important and have multi uses in dentistry. So, this study aims to evaluate some natural products (soda+ vinegar, soda +thymol , saturated salt solution) in relation to the commercial denture cleansers(protifex), for disinfection of modeling wax , according to some ideal properties of denture cleanser (bactericidal, and fungicidal, , dimensional accuracy, , water sorption and color changes), to evaluate their effect on modeling wax.

2. METHODS

The total number of major wax samples were three hundred and fifteen (225 sample for the physical tests ,including 75 sample for each test**:** Dimensional accuracy test ,water sorption test and color changes, and microbiological tests :90 sample, including 30 samples for each type of bacterial species: *C. albicans*, *Streptococcus mutans*, and *Staphylococcus aureous* in an invitro study). **Solutions Preparation:** This study deals with three

experimentally prepared solutions by Khalil (2006) [10], 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). The following equations illustrate the preparation of the used solutions (Khalil, 2006) [10] :



The fresh solutions were prepared daily at the end of soaking trial (1/2 hr and 8 hrs) the solutions were removed, the beakers were cleaned and the specimens were immersed in distilled water till the next day, and so on until it simulates the immersion of 30 day.

Microbiological Study: ninety sample used in the total microbiological study on modeling wax, testing antifungal and antibacterial efficiency of used denture cleanser including 30 sample for each type of bacterial species: *C. albicans*, *Streptococcus mutans*, and *Staphylococcus aureous* in an invitro study. Specimens were of (10×10×2 mm length, width and thickness respectively) according to Webb *et al.*, (1998c) [11].

The identification of *C. albicans* by diagnostic laboratory test include cultural characteristics ,microscopic examination and germs tube test [12,13] (Figure 1 and 2). The identification of *Staphylococcus aureus* by diagnostic laboratory tests include cultural characteristics, microscopic examination (Figure 3and 4), and biochemical tests(coagulase test, catalase test , haemolysins test, mannitol fermentation test [12,14]. The identification of *Streptococcus mutans* by diagnostic laboratory tests include cultural characteristics, microscopic examination,(Figure 5and 6) and biochemical tests: that shows alpha type haemolysis and show negative results for the following tests: Bacitracin susceptibility, Bile solubility, Optochin susceptibility, and tolerance to 6.5%NaCl and positive result to the fermentation test of mannitol and sorbitol that produce acid[12,13,14].

Microbiological tests for disinfection of *C. albicans*, *Streptococcus mutans*, and *Staphylococcus aureous* on modeling wax were done by using **(**MacFarland Standard Bacteriological Solution technique ,tube No.2 = 600×10^{6} CFU/ml) that composed of 0.2 ml. Barium Chloride of 1% and 9.8 ml. H_2SO_4 of 1% according to Kazazoglu *et al.*, 2003[16].

Physical Tests on Modeling Wax: (225) specimens used in the total physical tests on modeling wax. For each test seventy five samples were divided into three groups: 25 specimens immersed for about 1/2 hr , 25 specimens immersed for about 8hrs,where each 5 specimens immersed in one of the five solutions throughout one month , third group of 25 specimens immersed for about 24hrs and 7days . Specimens were numbered, and a small hole was prepared in the midline of the upper part of the specimens to allow dispersion by a nylon dental floss in the solutions without contacting each other so that the specimen is surrounded by the solution only[17].

A- Dimensional Accuracy Test: Specimens for the dimensional accuracy test are of cylindrical shape(10×6 ± 0.1mm) according to ADA specification No.12., specimens preparation was done by placing a quantity of wax that broken into small pieces into metal pouring pan . The pan was then placed in water bath and the wax starts to melt and becomes fluid, the wax reaches (75± 5)°C and maintained at this temperature until pouring into mold. A thermometer is used to measure the temperature. The melted wax is then poured into a mold that has been lubricated with separating medium (separating film for acrylic resin), the mold consist of aluminum plate(6 ± 0.1mm thick),with a flat parallel top and bottom surfaces and containing a hole (10 ± 0.1mm in diameter) that was prepared by Al-Ubaidi (2008) [18]. Dimensional accuracy is measured by using electronic digital caliper, where marks was placed into the samples to allow taking four measurements around the circumstance (representing the length of the cylindrical sample) and one measurement in the center (representing the diameter of the cylindrical sample).

B- Color Changes Test: Specimens for the color changes test were of rectangular shape (45×10×1.5mm) length, width and thickness respectively according to ADA specification No.12. and in order to fit the cuvitte of the spectrophotometer machine. Color changes measured by using U.V. spectrophotometer (CECIL), after determination of the wavelength at which maximum absorption of major type of wax by using U.V spectrophotometer (SHIMADZU) which is 928.000 nanometer that is usually reported as (λmax).

C- Water Sorption Test : Specimens were of (50 ± 1mm in diameter and 0.5 ±0.05 mm in thickness)circular in shape, according to ADA specification No.12,specimens were dried in a desiccators containing freshly dried silica gel at 37°C ± 2°C for 24hrs, then removed to similar desiccators at room temperature for one hour and then weighed on a digital balance. This cycle was repeated until constant weight was attained. The weight loss for each specimen was not more than 0.5 mg in 24 hrs period. This weight was considered as "Conditioned weight ". Then the specimens were immersed in the prepared and tablets solutions according to the study plan. At the end of one month of immersion, They were then removed from the solutions with tweezers, wiped with clean dry hand towel until it became free from visible moisture, waved in air for about 15 seconds and weighed on a digital balance[19].

3. RESULTS

The goal of our study was to get the advantages of using natural products as denture cleansers include: safety and biocompatibility [10], has no chance to develop bacterial

resistance [7], effective as fungicidal and bactericidal agents, low cost and availability in mostly every house.

Disinfection of Major Wax from *C. albicans* :

The mean for the disinfection of major wax from *C. albicans* at 1hr, 4hrs, 8hrs of immersion in the prepared solutions were shown in Figure (7). Duncans multiple range test (Table 2) 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. Duncans multiple range test (Table 3) showed that at P=0.05, there were no significant differences between 4 and 8hrs, but there was significant differences between 4 and 8hrs in relation to 1hr.

Disinfection of Major Wax from *S. aureus*:

The mean for the disinfection of major wax from *S. aureus* at 1hr, 4hrs, 8hrs of immersion in the prepared solutions were shown in Figure (8). Duncans multiple range test (4) showed that at P=0.05, there were significant differences between all prepared solutions and control. The best prepared solutions were protefix then soda + vinegar and soda + thymol solutions equally. Duncans multiple range test (Table 5) showed that at P=0.05, there were significant differences between all times, the best was 8hrs.

Disinfection of Major Wax from *S. mutans*:

The mean for the disinfection of major wax from *S. mutans* at 1hr, 4hrs, 8hrs of immersion in the prepared solutions were shown in Figure (5) . Duncans multiple range test between treats (Table 6) showed that at P=0.05, there were significant differences between all prepared solutions and control. The best prepared solutions was saturated salt solution (there was no significant differences between saturated salt solution and protefix). Duncans multiple range test between times (Table 7) showed that at P=0.05, there were significant differences between all times, the best was 8hrs.

Physical Tests on Major Wax:

A- Water Sorption of Major Wax :

I- Water Sorption of Major Wax (one day versus seven days immersion in the prepared solutions): The mean and number of samples for the water sorption of major wax (one day versus seven days immersion in the prepared solutions) were shown in Figure (10) ,and it showed that there was increase in water sorption of major wax for seven days versus one day immersion in the prepared solutions. Duncans multiple range test (Table 8) showed that at P = 0.05 , there were no significant differences between the control, protefix, and saturated salt solutions. So the best prepared natural solution was saturated salt solution.

II- Water Sorption of Major Wax (1/2 hour versus 8hours immersion in the prepared solutions for one month): The mean and number of samples for the water sorption of major wax (between 1/2 hour versus 8hours immersion in the prepared solutions for one month) were shown in Figure (11) . Duncans multiple range test (Table 9) showed that at P = 0.05, there was significant differences between soda+thymol and all other prepared solutions, and that saturated salt solution was the best one.

Color of Major Wax:

I- Color of Major Wax (one day versus seven days immersion in the prepared solutions): The mean and number of samples for the optical density of major wax (one day versus seven days immersion in the natural denture cleansers) were shown in Table (4.28) and Figure (12). Duncans multiple range test (Table 10) showed that at P=0.05, there were no significant differences in optical density of major wax between all times (original, 1day, and seven days).

II- Color of Major Wax (1/2 hour versus 8hours immersion in the prepared solutions for one month) : The mean and number of samples for the optical density of major wax (between 1/2 hour versus 8hours immersion in the prepared solutions for one month) were shown in Figure (13). Dunnett (2-sided) t- test (Table 11) showed that at P = 0.05 and depending on the mean differences for the optical density of major wax between treats and control, there were no significant differences between all treats and the control.

Dimensional Accuracy of Major Wax :

I- **Dimensional accuracy of Major Wax:** length and width (one day versus seven days immersion in the prepared solutions):

A- Dimensional Accuracy of Length of Major Wax (one day versus seven days immersion in the prepared solutions): The mean and number of samples for the length of major wax (one day versus seven days immersion in the prepared solutions) were shown in Figure (14). Duncans multiple range test (Table 12) showed that at P = 0.05 , there were no significant differences in length of major wax between 1day and seven days.

B- Dimensional Accuracy of Width of Major Wax (one day versus seven days immersion in the prepared solutions): The mean and number of samples for the width of major wax (one day versus seven days immersion in the prepared solutions) were shown in Figure (15). Duncans multiple range test (Table 13) showed that at P=0.05, there were no significant differences in between protefix, saturated salt, and soda+thymol. Duncans multiple range test (Table 14) showed that at P = 0.05 , there were no significant differences in length of major wax between 1day and seven days.

II- Dimensional Accuracy of Major Wax : length and width (1/2 hour versus 8hours immersion in the prepared solutions for one month):

A- Dimensional Accuracy of Length of Major Wax (1/2 hour versus 8hours immersion in the prepared solutions for one month): The mean and number of samples for the length of major wax (between 1/2 hour versus 8hours immersion in the prepared solutions for one month) were shown in Figure (16). Duncans multiple range test (Table 15) showed that at P=0.05, there were no significant differences between saturated salt, potefix, and soda+vinegar solutions.

B- Dimensional Accuracy of Width of Major Wax (1/2 hour versus 8hours immersion in the prepared solutions for one month): The mean and number of samples for the width of major wax (between 1/2 hour versus 8hours immersion in the prepared solutions for one month) were shown in Figure (17). Dunnett (2-sided) t- test (Table 16) showed that at P = 0.05 and depending on the mean differences for the width of major wax between treats and control, there were no significant differences between all treats and the control.

4. DISCUSSION

Disinfection of Major Wax from C.albicans : Table (2&3) showed that there were significant differences between all treats and the control. So, all prepared natural denture cleansers are effective in disinfection of modeling wax from C. albicans. The best prepared natural solution was saturated salt solution, and this is agreed with Baltch (2007) [20] , and there were no significant differences between 4 and 8hours, but there was significant differences between 4 and 8hours in relation to 1hour , this was disagreed with Queisser Pharma (2008) [21] which stated that her product (protefix) insure complete cleaning and disinfection within 15 minute only, and disagreed with (Infection control manual, 2008) that suggested soaking wax bite plates in (1:10) dilution of Clorox or any other acceptable disinfectant for 10 minutes before using them and before sending them to the laboratory is enough, and disagreed with (Guide lines on infection control in dental clinics, 1993) [4] stated that wax bites can be disinfected by immersion in 0.1% sodium hypochlorite for 10 minutes.

Disinfection of Major Wax from S.aureus : Table (4&5) showed that there were significant differences between all treats and the control. So, all prepared natural denture cleansers are effective in disinfection of modeling wax from S.aureus. That the best prepared natural solution were soda +vinegar and soda +thymol solutions, this may be due to the antibacterial action of thymol oil ,especially on S.mutans as stated by (Azaz et al. ,2002; Azaz et al.,2005; Cavar et al.2008) [22-24], where the active group in thymol is the hydroxy(-OH) group (1974, د.فوزي قطب ; Hill, 2003) [25,26]. and the mechanism of action of thymol may be due to its viscosity that act by oil pulling mechanism, or saponification ,or emulsification action of thymol as stated by Asokan et al.,2008 ; Shanmugam , 2001 ; Sekino and Ramberg , 2005 ; Chung et al ., 2006) [8,27,29], or due to the solvent action of thymol [26], another mechanism of action of thymol as a phenol, where phenole even if diluted as low as 1/50,000 destroys many unwanted toxins. Some of these toxins include C. albicans, Staphylococcus [2] or result from the fact that thymol contain tannins and volatile oil that have a detergent action [25]. Or this may be due to the powerful cleaning and scrubbing action of sodium bicarbonate when mixed with clear vinegar that produce bubbling and carry's contaminants away from the denture surfaces,or due to the antibacterial action of vinegar [25,31,32].

The best time of immersion was 8hours , this was disagreed with Queisser Pharma (2008) [21] which stated that her product (protefix) insure complete cleaning and disinfection within 15 minute only, and disagreed with (Infection control manual, 2008) that suggested soaking wax bite plates in (1:10) dilution of Clorox or any other acceptable disinfectant for 10 minutes before using them and before sending them to the laboratory is enough[2].

Disinfection of Major Wax from S. mutans: Table (6&7) showed that there were significant differences between all treats and the control. So, all prepared natural denture cleansers are effective in disinfection of modeling wax from S. mutans. The best prepared natural solution was saturated salt solution, and this is agreed with Baltch (2007) [20], and that the best time of immersion was 8hrs, this was disagreed with Queisser Pharma (2008) [21] which stated that her product (protefix) insure complete cleaning and disinfection within 15 minute only, and disagreed with (Infection control manual, 2008) [2] that suggested soaking wax bite plates in (1:10) dilution of Clorox or any other acceptable disinfectant for 10 minutes before using them and before sending them to the laboratory is enough , and disagreed with (Guide lines on infection control in dental clinics,1993) [4] stated that wax bites can be disinfected by immersion in 0.1% sodium hypochlorite for 10 minutes.Finally, the results of this study showed that all the tested solutions are effective as a bactericidal and fungicidal agent, this agreed with(Lima et al.,2006) [33] that stated that when S. mutans <100 000 CFU /ml and yeast <1000 CFU /ml of saliva considered as no growth.

Water Sorption of Major Wax:

A- Water Sorption of Major Wax (one day versus seven days immersion in the prepared solutions): Table (8) showed that wax also showed water sorption like acrylic resin but in a fewer manner that was agreed with Gandar and Tanner (1976) [34] whom stated that coating acrylic resin with wax reduce water sorption from 2.5 to 0.9-1.2 , where wax coating reduce water absorbance ,but still absorbing water, and the fact that wax absorb oils from water and absorb water containing salt [35], and showed that there was an increase in water sorption of major wax for seven days versus one day immersion in the prepared solutions, and there were significant differences between the two times(immersion for one day and seven days in the prepared solutions). There were significant differences between all treats and the control except for saturated salt solutions and protefix . So, the best prepared natural solution would be saturated salt solution in relation to water sorption of major wax.

There were no previous studies on the water sorption of major wax to make direct comparison with their results.

B-Water Sorption of Major Wax (1/2 hour versus 8hours immersion in the prepared solutions for one

month): Table (9) showed that wax also showed water sorption like acrylic resin, but in a fewer manner that was agreed with Gandar and Tanner (1976) [(34)] who stated that coating acrylic resin with wax reduce water sorption from 2.5 to 0.9-1.2, where wax coating reduce water absorbance but still absorbing water, and the fact that wax absorb oils from water and absorb water containing salt [(35)], and showed that there were no significant differences between 1/2 hour and 8hours immersion in the prepared solutions for one month, there were no significant differences between all treats and the control except for soda+ thymol, in fact soda+ thymol was the only one causing decrease in weight may be due to dissolving action of thymol oil or soda on the wax , but saturated salt solution was the best one in relation to water sorption of major wax. There were no previous studies on the water sorption of major wax to make direct comparison with their results. But water sorption of major wax immersed in the four tested solutions is less than those immersed in water, the same phenomenon occur in acrylic resin may be due to the small size of water molecules that is more easily penetrate the material [(36)].

Color of Major Wax

A- Color of Major Wax(one day versus seven days immersion in the prepared solutions): Table(10) showed that there were no significant differences between all treats and the control .So , all the prepared natural solution would be accepted in relation to effect on color of major wax, and there were no significant differences in optical density of major wax between original and 1day, or original and seven days , there were no significant differences in optical density of major wax between all times (original, 1day, and seven days). There were no previous studies on the color of major wax to make direct comparison with their results.

B- Color of major wax (1/2 hour versus 8hours immersion in the prepared solutions for one month): Table (11) showed that there were no significant differences between 1/2 hour and 8hours immersion in the prepared solutions for one month, there were no significant differences in the optical density of major wax between all treats and the control . So, all the prepared natural solution would be accepted in relation to their effect on color of major wax for 1/2 hour and 8hours immersion in the prepared solutions for one month .There were no previous studies on the color of major wax to make direct comparison with their results.

Dimensional Accuracy of Major Wax:

A- Dimensional Accuracy of Major Wax: length and width (one day versus seven days immersion in the prepared solutions): Table (12-14) showed that there were no significant differences in length of wax between all treats and the control. So, all the prepared natural solution would be accepted in relation to effect on length of major wax. There were no significant differences in length of major wax between original and 1day. There were no significant differences in length of wax between 1day and seven days.

There were significant differences in width of major wax between all treats and the control, may occur due to relief of internal stresses that occur during cooling of wax after moulding, due to the low thermal conductivity of wax, that cause contraction ,but not immediately due to the high coefficient of thermal expansion [(37)], where the outer surface of wax solidify before the internal bulk resulting in internal stresses[(38)], there were no significant differences between soda +thymol, saturated salt solutions, and protefix, so these natural solutions has same effect as the commercial disinfectant. and that there were no significant differences in width of major wax between original and 1 day, but there are significant differences in width of major wax between original and seven days, this may be due to longer time of immersion ,or longer storage time and change in temperature [(39,18)]. There were no significant differences in width of major wax between 1 day and seven days.

B- Dimensional Accuracy of Major Wax: Length and Width (1/2 hour versus 8hours immersion in the prepared solutions for one month): Table (15&16) showed that there were no significant differences in length of major wax between the 1/2 hour and 8hours immersion in the prepared solutions for one month. There were no significant differences in length between all treats and the control except for saturated salt solution. There was no significant differences between saturated salt, potefix, and soda+ vinegar solutions. So, these natural solution has same effect as the commercial disinfectant and can be accepted.There were no significant differences in the width of major wax between the 1/2 hour and 8hours immersion in the prepared solutions for one month. There were no significant differences in the width of major wax between all treats and the control.

This phenomenon of the effect of saturated salt solution on length and not on width can be explained by the research of Fattore et al.(1984) [(40)] who stated that distortion of wax occurred more frequently in a vertical direction, followed by an anteroposterior direction. Also in general dimensional change of wax may occur due to relief of internal stresses [(38)].

5. CONCLUSIONS

According to the disinfectant of modeling wax from C. albicans , S. aureus, and S. mutans, all the prepared natural denture cleansers were accepted and considered bactericidal and fungicidal disinfectant, and a proper disinfection of modeling wax require 8hrs of immersion in the prepared disinfectant solutions. Except for disinfection of modeling wax from C. albicans, where there was no significant differences between 4 or 8hrs of immersion in the prepared disinfectant solutions.

Saturated salt solution was the best disinfectant, by having broad spectrum antibacterial and antifungal action, and it was the safest of all other solutions on

acrylic denture base material. Soda + vinegar and Soda + thymol were accepted in all tests of this study, and were the best prepared disinfectant of modeling wax from S. aureus when used for 8 hours.

According to the physical properties of modeling wax including water sorption, colour, and dimensional accuracy of modeling wax , all the prepared natural denture cleansers were accepted, except for soda+thymol which decrease water sorption of modeling wax immersed for 1/2 hour and 8hours daily for one month.

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Table (1) Solutions Preparation (Khalil 2006)

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		

Table (2) Duncan multiple range test for disinfection of major wax from *C. albicans*, between treats:

	TREATS	N	Subset				
			1	2	3	4	5
Duncan	Protefix	6	12633.3333				
	Salt	6		48333.3333			
	S.+ving	6			148666.6667		
	S.+Thy	6				220000.0000	
	D.W	6					392166.6667
	Sig.		1.000	1.000	1.000	1.000	1.000

Table (3) Duncan multiple range test for disinfection of major wax from *C. albicans*, between times:

	Time of immersion	N	Subset	
			1	2
Duncan	8 Hours	10	150330.0000	
	4 Hours	10	156350.0000	
	1 Hour	10		186400.0000
	Sig.		.195	1.000

Table (4) Duncan multiple range test for disinfection of major wax from *S. aureus*, between treats:

	TREATS	N	Subset			
			1	2	3	4
Duncan	Protefix	6	483.3333			
	S.+ving	6		10000.0000		
	S.+Thy	6		13750.0000		
	Salt	6			21666.6667	
	D.W	6				74000.0000
	Sig.		1.000	.113	1.000	1.000

Table (5) Duncan multiple range test for disinfection of major wax from *S. aureus*, between times:

	Time of immersion	N	Subset		
			1	2	3
Duncan	8 Hours	10	14010.0000		
	4 Hours	10		22670.0000	
	1 Hour	10			35260.0000
	Sig.		1.000	1.000	1.000

Table (6) Duncan multiple range test for disinfection of major wax from *S. mutans*, between treats:

	TREATS	N	Subset			
			1	2	3	4
Duncan	Protefix	6	5833.3333			
	Salt	6	8000.0000			
	S.+Thy	6		260833.3333		
	S.+ving	6			289000.0000	
	D.W	6				840166.6667
	Sig.		.253	1.000	1.000	1.000

Table (7) Duncan multiple range test for disinfection of major wax from *S. mutans*, between times:

	Time of immersion	N	Subset		
			1	2	3
Duncan	8 Hours	10	202700.0000		
	4 Hours	10		291800.0000	
	1 Hour	10			347800.0000
	Sig.		1.000	1.000	1.000

Table (8) Duncan multiple range test for water sorption of major wax (one day versus seven days immersion in the prepared solutions):

	TREATS	N	Subset		
			1	2	3
Duncan	D.W	10	.00004590		
	Protefix	10	.00006330		
	Salt	10	.00011940	.00011940	
	S.+ving	10		.00019890	
	S.+Thy	10			.00045890
	Sig.		.219	.160	1.000

Table (9) Duncan multiple range test for water sorption of major wax**:** wax (between 1/2 hour versus 8hours immersion in the prepared solutions for one month)

	TREATS	N	Subset	
			1	2

Duncan	S.+Thy	10	-.00068022	
	S.+ving	10		.00015100
	Protefix	10		.00019360
	D.W	10		.00029300
	Salt	10		.00033690
	Sig.		1.000	.093

Table (10) Duncan multiple range test for colour of major wax (one day versus seven days immersion in the prepared solutions)

	Time of immersion	**N**	**Subset**
			1
Duncan	7 Days	25	1.408520
	One Day	25	1.427520
	Original	25	1.438320
	Sig.		.074

Table (12) Duncan multiple range test for dimensional accuracy of length of major wax (one day versus seven days immersion in the prepared solutions)

	Time of immersion	**N**	**Subset**	
			1	**2**
Duncan	Original	25	5.872800	
	One Day	25	5.892400	5.892400
	7 Days	25		6.004000
	Sig.		.730	.053

Table (13) Duncan multiple range test for dimensional accuracy of width of major wax (one day versus seven days immersion in the prepared solutions, between treats):

	TREATS	**N**	**Subset**		
			1	**2**	**3**
Duncan	S.+Thy	15	9.750667		
	Salt	15	9.804000		
	Protefix	15	9.812667		
	S.+ving	15		9.922667	
	D.W	15			10.111333
	Sig.		.137	1.000	1.000

Table (14) Duncan multiple range test for dimensional accuracy of width of major wax (one day versus seven days immersion in the prepared solutions, between times)

	Time of immersion	**N**	**Subset**	
			1	**2**
Duncan	Original	25	9.838000	
	One Day	25	9.878000	9.878000
	7 Days	25		9.924800
	Sig.		.189	.125

Table (15) Duncan multiple range test for dimensional accuracy of length of major wax

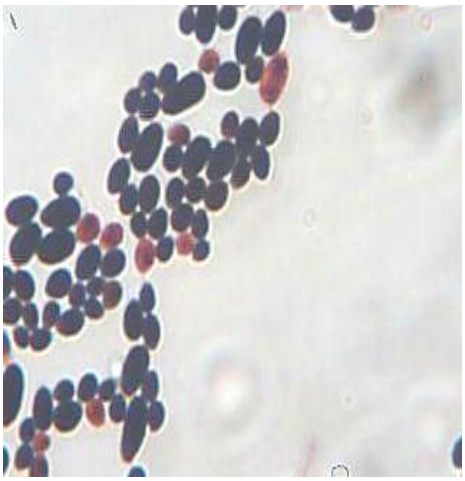
	TREATS	**N**	**Subset**	
			1	**2**
Duncan	D.W	10	5.8625	
	S.+Thy	10	5.9610	
	Prot.	10	6.0813	6.0813
	S.+ving	10	6.1400	6.1400
	Salt	10		6.3088
	Sig.		.051	.097

Table (16) Dunnett (2-sided) t- test for dimensional accuracy of width of major wax:

	(I) TREATS	(J) TREATS	Mean Difference (I-J) (mm)	S. E	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Dunnett t (2-sided)	Salt	D.W	.2000	.12516	.327	-.1211	.5211
	Protefix	D.W	.1438	.12516	.607	-.1773	.4648
	S.+ving	D.W	.1913	.12516	.365	-.1298	.5123
	S.+Thy	D.W	.2830	.11874	.075	-.0216	.5876

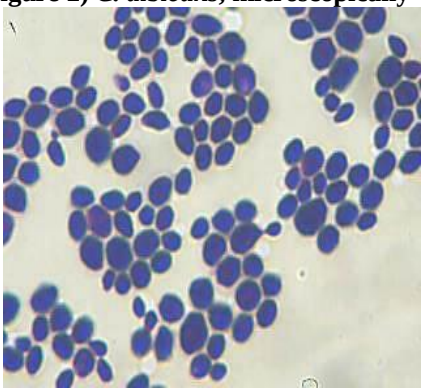
S.E: standard error

(Figure 1) *C. albicans* on SDA

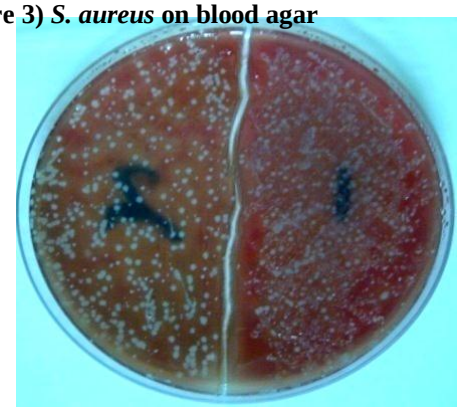


(Figure 2) *C. albicans*, microscopically

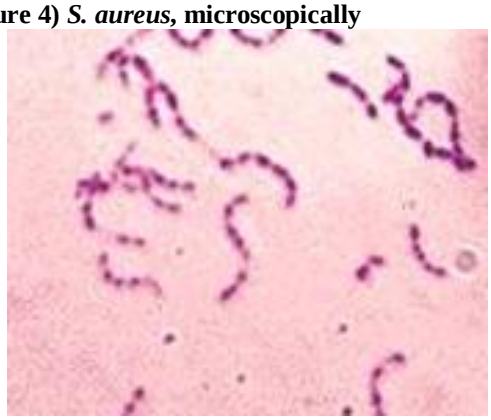
(Figure 3) *S. aureus* on blood agar



(Figure 4) *S. aureus*, microscopically



(Figure 5) *S. mutans* on blood agar



(Figure 6) *S. mutans*, microscopically

International Journal of Academic Health and Medical Research (IJAHMR)
ISSN: 2000-007X
Vol. 2 Issue 7, July – 2018, Pages: 1-12

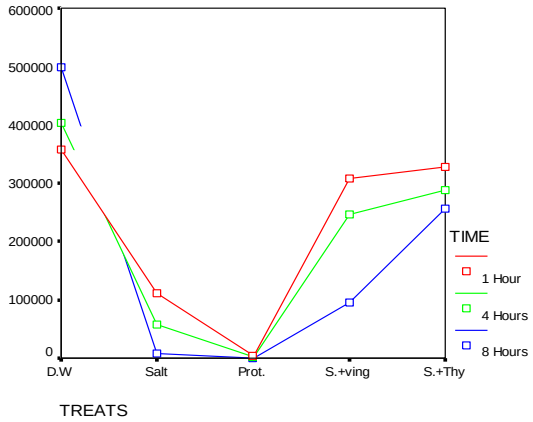


Figure (7) The mean for the disinfection of major wax from *C. albicans*

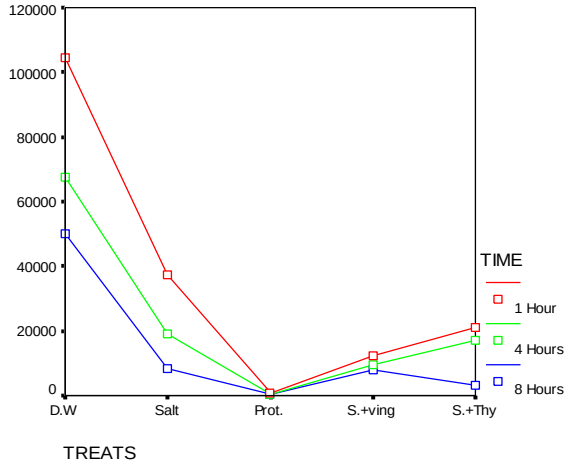


Figure (8) The mean for the disinfection of major wax from *S .aureus.*

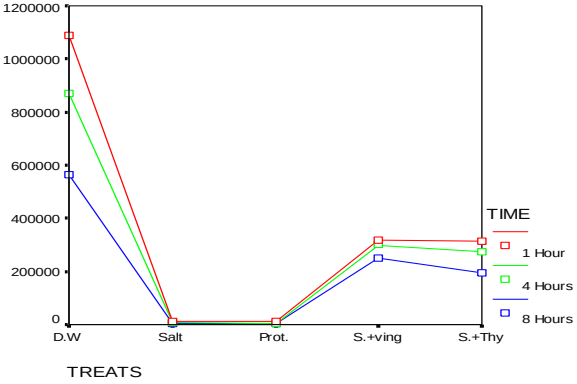


Figure (9) The mean for the disinfection of major wax from *S. mutans*

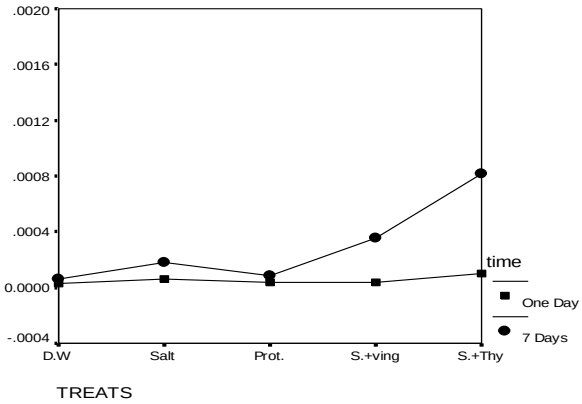


Figure (10) The mean for the water sorption of major wax (one day versus seven days immersion in the prepared solutions).

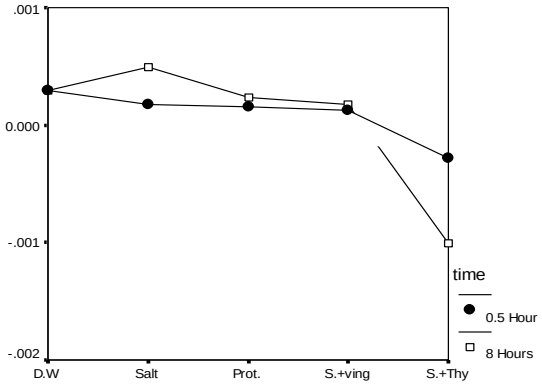


Figure (11) The mean for the water sorption of major wax (between 1/2 hour versus 8hours immersion in the prepared solutions for one month)

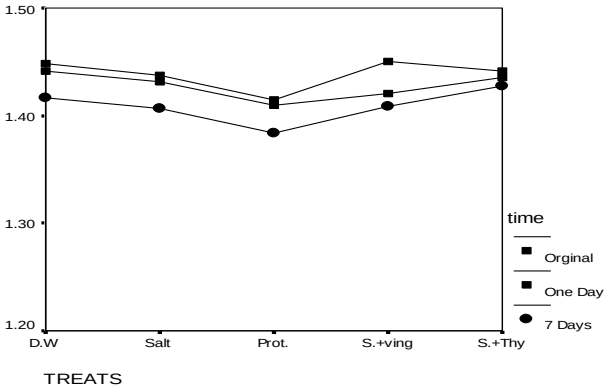


Figure (12) The mean for the Colour change of major wax (one day versus seven days immersion in the prepared solutions)

International Journal of Academic Health and Medical Research (IJAHMR)
ISSN: 2000-007X
Vol. 2 Issue 7, July – 2018, Pages: 1-12

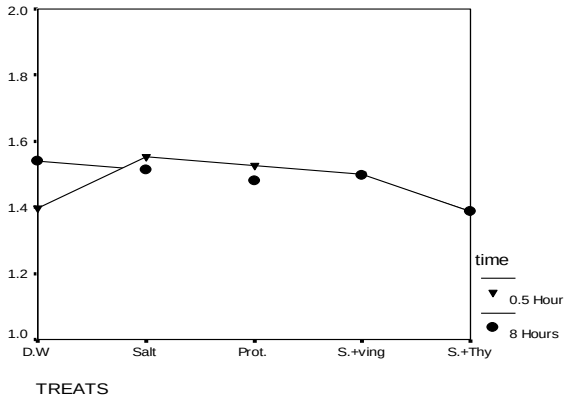


Figure (13) The mean for the colour change of major wax (between 1/2 hour versus 8hours immersion in the prepared solutions for one month).

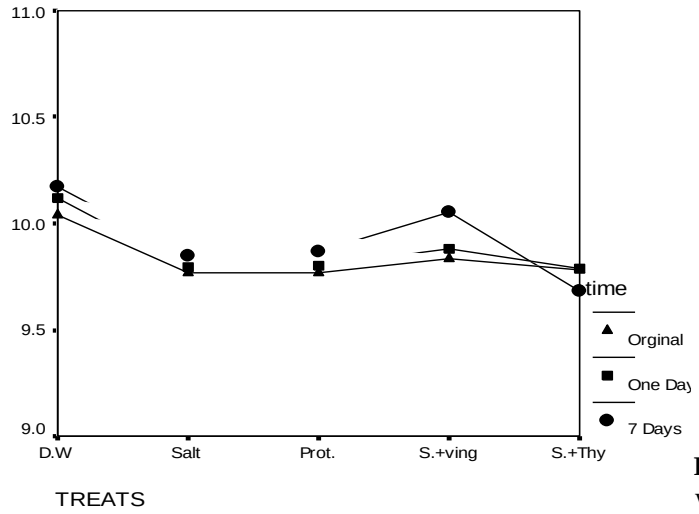


Figure (15) The mean for the dimensional accuracy of width of major wax (one day versus seven days immersion in the prepared solutions)

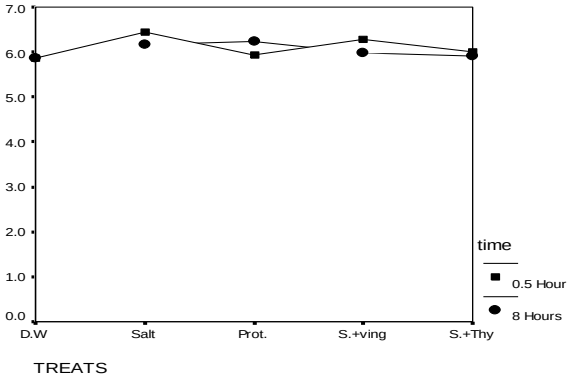


Figure (16) The mean for dimensional accuracy of major wax (length) between 1/2 hour versus

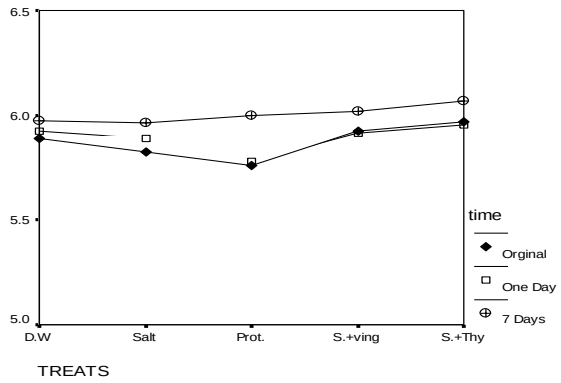


Figure (14) The mean for the dimensional accuracy of length of major wax (one day versus seven days immersion in the prepared solutions)

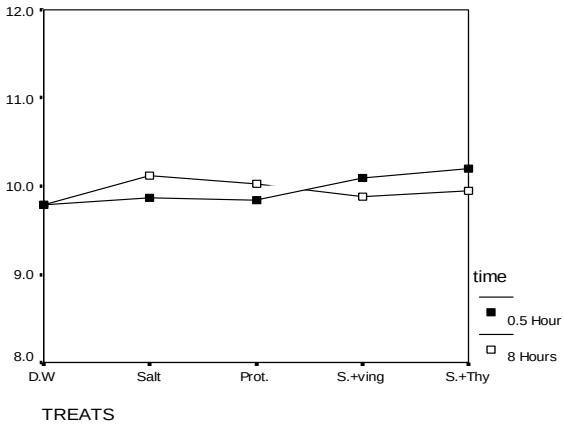


Figure (17) The mean for dimensional accuracy of major wax (width) between 1/2 hour versus 8hours immersion in the prepared solutions for one month.