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The Effect of Adding Coconut Oil on *Candida albicans* Activity and Shear Bond Strength of Acrylic Based Denture Soft Lining Material

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ABSTRACT

One of the most serious problems of soft denture lining materials during usage is the accumulation of microorganisms. This problem is presented as denture induced stomatitis, which caused by the fungal growth especially Candida albicans. Hence, the development of soft lining material with a drug delivery system becomes necessary.

Aim: The aim of the current study is to investigate the antifungal efficiency of various concentrations of virgin coconut oil incorporated into heat-cured soft denture liner against Candida albicans. And also to evaluate the shear bond strength to the denture base after this addition. Both investigations were assessed at different time intervals.

Material and Method: One hundred eighty samples were prepared by addition of 1.5% and 2.5% (by volume) of virgin coconut oil into heat cured acrylic-based soft denture lining material. The main study samples were divided into two groups (90 samples for each group) based on the conducted test; Candida albicans activity test and shear bond strength test. Then each group was subdivided into three subgroups (control 0%, 1.5% and 2.5%) based on the concentration of the added virgin coconut oil (n=10 samples for each subgroup). Each group was assessed at different periods of time (24 hours in distilled water, 2 and 4 weeks in artificial saliva), ten samples were used for each time interval. Fourier transform infrared analysis was conducted to determine if there is any chemical reaction between coconut oil and soft lining material.

Results: For Candida albicans activity test; the incorporation of 1.5% and 2.5% virgin coconut oil caused a highly significant decrease in the mean values of the viable count of Candida albicans when compared to the control group ($p<0.01$). In contrast, a gradual (non-significant) increase in the viable counts was obtained as the time interval increased. There was a non-significant reduction in shear bond strength values for 1.5% group whilst a significant reduction for 2.5% group compared to the control group. In variance, each experimental group showed a non-significant increase in the bond strength as the time interval increase.

Conclusion: Virgin coconut oil was successfully incorporated into the soft denture liner and act as a potential antifungal medicament with a continuous drug-delivery system against Candida albicans. It seemed that adding 1.5% coconut oil was the most beneficial effects against fungi, with less adverse effect on the shear bond strength.

Key words: Soft denture liner, Virgin coconut oil, *Candida albicans*, Shear bond strength

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INTRODUCTION

Natural soft rubbers have been early used in 1869 by Twichell to fabricate the first soft lining material in dentistry field [1]. From that point forward, a marked evolution has influenced dental materials result in the development of different types of soft lining materials; each has its own advantages and drawbacks [2].

In general, soft denture liners are resilient materials applied over the denture bearing surface to act as a cushion which absorbs the loads generated by the masticatory process and reduces its traumatic effects over the denture bearing area makes the denture wearer more comfortable [3]. At present, two common types of soft liners are available; acrylic based and silicone based [4]. Acrylic based soft liners contain plasticizers which are released over time and increase the hardness of the material, while silicone base soft liners have no plasticizers and stay soft for longer period of time [5].

To be ideal, soft denture liners should exhibit certain properties to insure a maximum benefits for denture wearers; among these properties are the biocompatibility, dimensional stability, good resiliency, color stability, low water solubility, sufficient bond strength to the underlying denture base and the ability to inhibit or reduce the microbial growth [6].

Microbial colonization is a serious problem that affects the service efficiency of soft denture liner, the most common clinical condition related to this problem is denture induced stomatitis which is a multifactorial pathological condition affecting the denture bearing mucosa [7]. Denture stomatitis affects approximately 72% of denture wearers and is mainly caused by *Candida albicans*; the most common fungi species responsible for oral infections [8].

Prescribing topical antifungal drugs is the most common line of managing denture induced stomatitis. Unfortunately, maintaining the optimal oral dose of the drug, lack of motor dexterity and impaired cognition of geriatric denture wearers make it challenging to get a maximum benefit of the topical drugs. To overcome these challenging factors, the idea to incorporate the antifungal drugs in soft lining materials comes up. Moreover, the fungal resistance to these drugs makes it necessary to search for naturally derived medicaments to be used as a substitution to synthetic drugs [8,9].

Herbal medicines are a potent alternative treatment for oral microbial infections with less or no side effects; this makes a worldwide trend to investigate them in order to find biologically safe herbal-based medicines with efficient antifungal properties [10]. Among these herbal medicines are plants oils. Recently, various researches have been conducted to investigate the antifungal effect of different kinds of oil against *Candida albicans* and they report that plants oils are considered a promising therapeutic line with efficient antifungal properties for treating denture induced stomatitis [11,12].

Virgin coconut oil (VCO) is a natural plant extracts derived from fresh coconut meat. VCO gained extra attention because of its bioactive components which is well known by its antipyretic, anti-inflammatory, antimicrobial and antioxidant properties [13]. It is mainly composed of medium chain fatty acids such as Lauric acid, Capric and Caprylic acids which are approved to act against fungi particularly *Candida albicans* [14].

MATERIALS AND METHODS

Pilot study

Heat cured acrylic based soft denture lining material (Vertex, Netherlands) was utilized. Virgin coconut oil (VIVA naturals, Philippine) was added to the liquid part of the soft liner in three different concentrations 2.5%, 5% and 7.5%. Then 1.5% was used. These concentrations were selected to discover the difference of the effect of a small and large amount of VCO without a potential adverse effect on the material properties. Mixing procedure was conducted as directed by the manufacturer instructions for the control group. While for the experimental group, the volume of VCO was subtracted from the volume of soft liner liquid to obtain accurate P/L ratio [15]. Two tests were conducted; viable count of *Candida albicans* and shear bond strength tests. Five samples were used for each test. The results demonstrated that the lower the concentration of the incorporated coconut oil the better the antifungal efficiency and the lesser the adverse effect on shear bond strength. Suitable concentrations (1.5% and 2.5%) were selected based on the results of the pilot study.

Design of the study

A total of 180 samples were prepared and divided into two groups (90 samples for each group) based on the conducted test; *Candida albicans* activity test and shear bond strength test. Then each group was subdivided into three subgroups (control 0%, 1.5% and 2.5%) based on the concentration of the added virgin coconut oil (n=10 samples for each subgroup). Each group was assessed at different periods of time (24 hours in distilled water, 2 and 4 weeks in artificial saliva), ten samples were used for each time interval. Klimek method was used to prepare artificial saliva [16].

Viable count test for *Candida albicans*

Sample preparation

Plastic molds with dimensions of 10 × 10 × 2.3 mm in length, width and thickness, respectively was prepared and invested in freshly mixed extra hard dental stone to simulate the final shape of soft liner samples. Soft lining materials were proportionate and mixed as directed by the manufacturer instructions. For experimental samples, The volume of VCO was subtracted from the volume of soft liner liquid to obtain accurate P/L ratio, VCO was mixed with the liquid part of soft lining material for 20 seconds [17] using probe sonication apparatus at 120 W and 60 KHz for complete homogeneity, the powder part was added and the mixture was mixed packed and processed as directed by manufacturer. After complete processing; samples were finished with sharp blade, polished with sand paper and stored in distilled water for 24 hours at 37°C before being tested to discard any residual monomer. Samples to be tested after 2 and weeks were stored in artificial saliva at 37°C to simulate the oral condition [18].

Isolation and identification of *Candida albicans*

A patient with an indication of a denture induced stomatitis was selected to isolate *Candida albicans* using cotton swab which was cultured on the surface of a sabouraud dextrose agar (Oxoid, England) plates and incubated at 37°C for 48 hours. *Candida albicans* identification was done in the following order: Firstly, macroscopic examination in which the *Candida albicans* had pearl-shape appearance which was pasty and creamy on sabouraud dextrose agar (SDA) [19]. Secondly, microscopic examination using Grams stain procedure [20]. Thirdly, Germ tube formation [21]. Fourthly, Biochemical identification using Vitek 2 system [22].

Assessment of *Candida albicans* viable count

Viable count test was done by making a *Candida albicans* suspension (using normal saline) of about 10^7 CFU/mL which equal to 0.5 McFarland standards. Then, 0.1% of

this suspension was taken using micropipette and added to a test tube containing 0.9% sabouraud dextrose broth (Oxoid, England), the sample was immersed in this tube and incubated at 37°C for 24 hours. After incubation, 0.1% of the broth mixture was taken and added to a test tube containing 0.9% normal saline and serial dilution was made. Around 0.1% was taken from the third dilution and spreaded over the surface of SDA using glass spreader and the plates was incubated at 37°C for 48 hours as shown in Figure 1. Third dilution was chosen because it demonstrated a countable range of 30-300 CFU [23]. All viable counts presented over SDA surface were counted and a statistical analysis was performed. Antifungal efficiency (AFE) was calculated using following formula (Equation 1):

$$AFE[\%] = \frac{Vc - Vt}{Vc} \times 100\% \quad (1)$$

in which number of viable colonies of control samples were represented by Vc and number of viable colonies of experimental samples were represented by Vt [24].

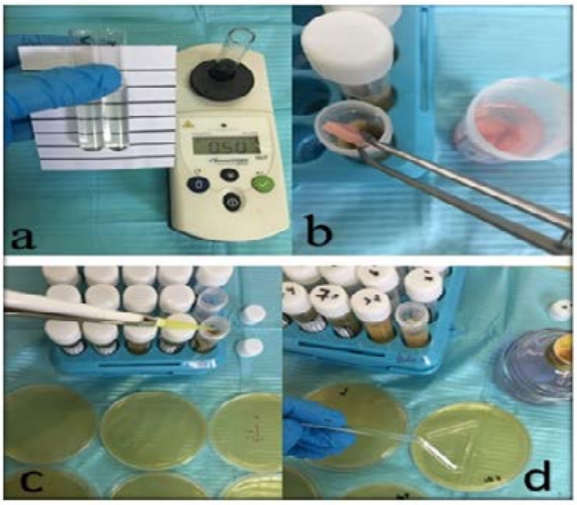

Figure 1: a) Preparation of *Candida* suspension equal to 0.5 McFarland standard; b) Sample placement inside test tube; c) Serial dilution; d) Spreading 0.1% of solution over SDA surface

Shear bond strength test

In order to evaluate the bond between the acrylic denture base and soft lining material; the shear bond strength test was performed using a special acrylic block design. Two heat cured acrylic blocks were required for each sample, the dimensions of each one was 5 × 25 × 75 mm depth, width and length respectively, along with 3 mm thickness stopper and 13 mm handle thickness. To fabricate one sample, two heat cured acrylic blocks were placed facing each other and creating an empty space between them for the placement of heat cured soft liner material, this space was 3 mm depth, 25 mm length and 25 mm width (Figure 2) [25].

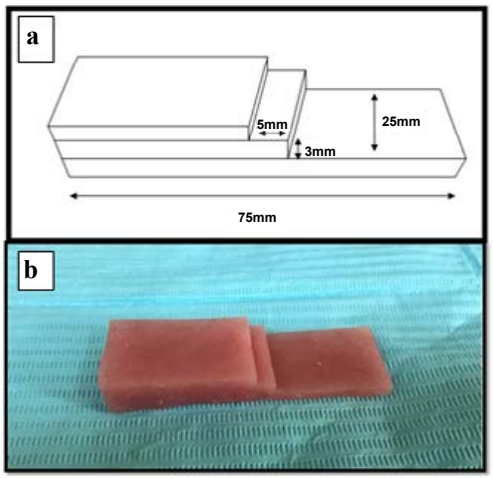

Figure 2: a) Model of acrylic block to use in testing tear strength; b) Fabricated acrylic block

The shear bond strength test was performed at load cell capacity of 100 Kg with cross head speed of 0.5 mm/min using Instron testing machine. Readings obtained from the machine represents the maximum load of failure. Bond strength was obtained by dividing the maximum load of failure by the cross section area of each sample (25 mm × 25 mm=625 mm) as directed by ASTM specification D-638, (1986) (Equation 2).

$$Bond\ strength\ \left(N/mm^2\right) = \frac{Maximum\ load}{Cross\ sectional\ area} = \frac{F}{A} \quad (2)$$

Statistical analysis

The results of this research were analyzed using SPSS (statistical package for social science–version 24) computer software. Descriptive statistics were made which include Means, Standard Deviation and Graphical presentation by bar-chart. Inferential statistics include; two way analysis of variance (ANOVA) was used to compare means among all groups and Tukey's multiple comparison test was used to show the significance among different groups.

RESULTS

Evaluating viable counts of *Candida albicans* (CFU/mL)

After 24 hours incubation of the samples in distilled water, the results of viable counts of *Candida albicans* of both experimental groups (1.5% and 2.5% coconut oil) revealed a lower mean values in comparison to the control group, the experimental group with 1.5% of coconut oil showed the lowest mean value during this period with 28.4 CFU/mL, as shown in Figure 3. Antifungal efficiency (AFE) for the experimental groups in the first incubation period was 81.1% and 60.3%, ordinarily (Table 1).

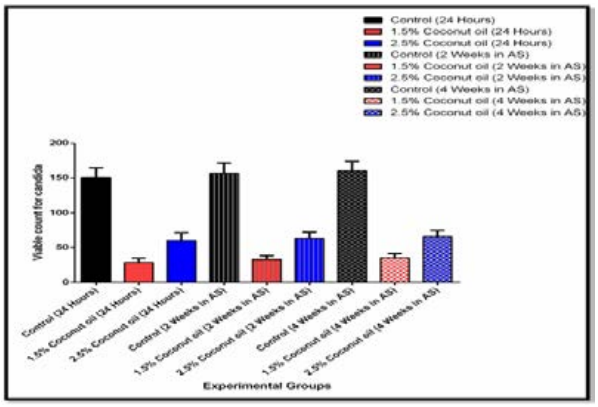

Figure 3: Bar chart showing mean values and standard deviation of viable count of *Candida albicans* for control and experimental groups at different periods of incubation

Table 1: Descriptive statistics of viable count of *Candida albicans*

Incubation period	Group	N	Mean ± S.D. (CFU/mL)
After 24 hours	Control	10	150.5 ± 14.02
	1.5% of coconut	10	28.4 ± 6.20
	2.5% of coconut	10	59.8 ± 11.26
After 2 weeks	Control	10	156.5 ± 15.13
	1.5% of coconut	10	33.1 ± 5.36
	2.5% of coconut	10	63.3 ± 8.84
After 4 weeks	Control	10	160 ± 13.74
	1.5% of coconut	10	35.2 ± 6.25
	2.5% of coconut	10	65.9 ± 8.62

At the second and third periods of evaluation (2 and 4 weeks of incubation in artificial saliva), the mean values of experimental groups (1.5% and 2.5% coconut oil) were less than mean values of the control group, also the lowest values were noticed in 1.5% coconut oil group after 2 and 4 weeks of incubation periods of 33.1 CFU/mL and 35.2 CFU/mL, respectively, while the mean values of the control group at the same periods were 156.5 CFU/mL and 160 CFU/mL ordinarily, as shown in Figure 3. In the second incubation period (2 week); AFE for the experimental groups (1.5% and 2.5% coconut oil) were 78.8% and 59.6%, respectively. Whilst the AFE for the experimental groups in the third incubation period (4 week) were 78% and 58.8%, respectively, as shown in Figure 4.

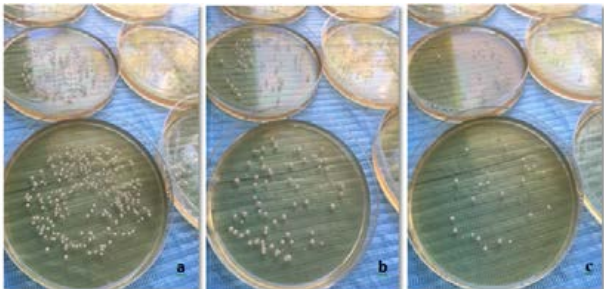

Figure 4: Viable counts of *Candida albicans* after 24 hours incubation of: a) Control samples; b) Experimental samples (1.5% coconut oil); c) Experimental samples (2.5% coconut oil)

Table 2: Comparison of average values of viable count test using two way ANOVA

Source	Sum of Squares	df.	Mean Square	F	Sig.	Partial Eta Squared
Concentration	247694.87	2	123847.43	1114.565	0	0.965
Incubation period	856.267	2	428.133	3.853	0.0252	0.087
Concentration Incubation period	35.267	4	8.817	0.079	0.9884	0.004

Table 3: Tukey's multiple comparisons test of different groups for viable count of *Candida albicans* test results

Period		Groups	Mean difference	P value	Sig.
After 24 hours	Control	1.5% (24 hours)	122.1	<0.0001	HS
		2.5% (24 hours)	90.7	<0.0001	HS
		Control (2 weeks)	-6	0.9426	NS
		Control (4 weeks)	-9.5	0.565	NS
	1.50%	2.5 % (24 hours)	84.6	<0.0001	HS
		1.5% (2 weeks)	-4.7	0.9868	NS
		1.5% (4 weeks)	-6.8	0.8888	NS
	2.50%	2.5% (2 weeks)	-3.5	0.9982	NS
		2.5% (4 weeks)	-6.1	0.9372	NS
		1.5% (2 weeks)	123.4	<0.0001	HS
After 2 weeks	Control	2.5% (2 weeks)	93.2	<0.0001	HS
		Control (4 weeks)	-3.5	0.9982	NS
	1.50%	2.5% (2 weeks)	-30.2	<0.0001	HS
		1.5% (4 weeks)	-2.1	>0.9999	NS
	2.50%	2.5% (4 weeks)	-2.6	0.9998	NS
After 4 weeks	Control	1.5% (4 weeks)	124.8	<0.0001	HS
		2.5% (4 weeks)	94.1	<0.0001	HS
	1.50%	2.5% (4 weeks)	-30.7	<0.0001	HS

N.S: No statistically significant difference between groups at p>0.05

HS: Highly significant difference between groups at p<0.01

In Table 2, two way ANOVA indicated a highly significant difference among concentrations of coconut oil addition ($p<0.01$), and among incubation periods the difference was significant ($p=0.0252$). While, a non-significant interaction was seen between concentrations and incubation periods ($p=0.9884$).

Tukey's multiple comparison tests was used to compare mean values of different groups. In first incubation period (After 24 hours), the control group showed a highly significant difference compared to both experimental groups in the same incubation period, while it was non-significantly different in comparison to both control groups in different incubation periods, 1.5% group showed a highly significant difference with 2.5% group in the same incubation period, but it showed non-significant difference when compared with the same concentration in 2 and 4 weeks, regarding 2.5% samples the difference was non-significant when compared with the same concentration after 2 and 4 weeks incubation period.

Control group of the second incubation period (2 weeks) showed a highly significant difference when compared to 1.5% and 2.5% samples for the same period, while showed a non-significant difference with the control group (4 weeks), 1.5% samples showed a highly significant difference when compared to 2.5% in the same period, and showed a non-significant difference with the same concentration after 4 weeks, 2.5% samples also showed a non-significant difference when compared with the same concentration in the third incubation period.

Control group of the third incubation period (4 weeks) showed a highly significant difference when compared to 1.5% and 2.5% (4 weeks), 1.5% samples in the third incubation period showed a highly significant difference when compared to 2.5% in the same period, as shown in Table 3.

Table 4: Descriptive statistics of shear bond strength test

Incubation period	Group	N	Mean ± S.D. (N/mm²)
After 24 hours	Control	10	0.4806 ± 0.0218
	1.5% of coconut	10	0.4637 ± 0.0276
	2.5% of coconut	10	0.4483 ± 0.0223
After 2 weeks	Control	10	0.4942 ± 0.0167
	1.5% of coconut	10	0.4765 ± 0.0208
	2.5% of coconut	10	0.4589 ± 0.0157
After 4 weeks	Control	10	0.5102 ± 0.0190
	1.5% of coconut	10	0.4904 ± 0.0245
	2.5% of coconut	10	0.4725 ± 0.0229

Shear bond strength test

Results of shear bond strength test after 24 hours of incubation in distilled water revealed that both experimental groups (1.5% and 2.5% coconut oil) had a lower mean values than the control group, the experimental group with 2.5% of coconut oil showed the lowest value of 0.4483 N/mm² in this period, followed by 1.5% samples of 0.4637 N/mm². At the second and third periods of evaluation (2 and 4 weeks of incubation

Table 5: Comparison of average values of shear bond strength test using two way ANOVA

Source of variation	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Concentration	0.019	2	0.009	19.859	0	0.329
Incubation period	0.011	2	0.005	11.591	0	0.223
Concentration*Incubation period	0.000077	4	0.0000193	0.041	0.9967	0.002

Table 6: Tukey's multiple comparisons test of different groups for shear bond strength test

Period	Groups		Mean difference	P value	Sig.
After 24 hours of incubation	Control	1.5% (24 hours)	0.01696	0.6544	NS
		2.5% (24 hours)	0.03232	0.0208	S
		Control (2 weeks)	-0.0136	0.8623	NS
		Control (4 weeks)	-0.0296	0.0478	S
	1.50%	2.5 % (24 hours)	0.01536	0.7632	NS
		1.5% (2 weeks)	-0.0128	0.8979	NS
		1.5% (4 weeks)	-0.02672	0.1053	NS
	2.50%	2.5% (2 weeks)	-0.01056	0.9646	NS
		2.5% (4 weeks)	-0.02416	0.195	NS
After 2 weeks of incubation	Control	1.5% (2 weeks)	0.01776	0.5964	NS
		2.5% (2 weeks)	0.03536	0.0076	HS
		Control (4 weeks)	-0.016	0.7213	NS
	1.50%	2.5% (2 weeks)	0.0176	0.6081	NS
		1.5% (4 weeks)	-0.01392	0.8463	NS
	2.50%	2.5% (4 weeks)	-0.0136	0.8623	NS
After 4 weeks of incubation	Control	1.5% (4 weeks)	0.01984	0.4455	NS
		2.5% (4 weeks)	0.03776	0.0032	HS
	1.50%	2.5% (4 weeks)	0.01792	0.5846	NS

N.S. No statistically significant difference between groups at $p>0.05$

S: Significant difference between groups at $p<0.05$

HS: Highly significant difference between groups at $p<0.01$

in artificial saliva), the mean values of the experimental groups (1.5% and 2.5% coconut oil) were less than mean values of the control group, also the lowest values were noticed in 2.5% coconut oil group (0.4589 N/mm^2 and 0.4725 N/mm^2, respectively), while mean values of the control group were 0.4942 N/mm^2 and 0.5102 N/mm^2, respectively as presented in Table 4 and Figure 5.

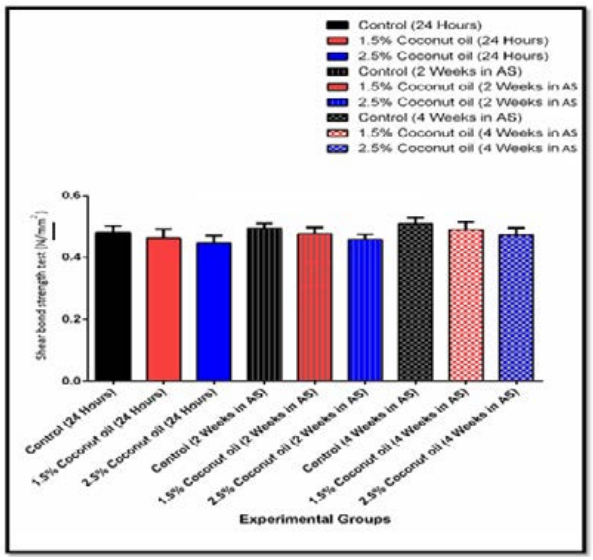

Figure 5: Bar chart showing mean values and standard deviation of shear bond strength for control and experimental groups at different periods of incubation

Two way ANOVA indicated a highly significant difference among concentrations of coconut addition ($p<0.01$), and among incubation periods ($p<0.01$). While, a non-significant interaction was seen between concentrations and incubation periods ($p=0.9967$) as listed in Table 5.

Tukey's multiple comparison tests was used to compare mean values of shear bond strength of different groups. In first incubation period, the control group showed a non-significant difference compared to 1.5% samples while it showed a significant difference compared to 2.5% samples in the same incubation period, also, it was non-significantly different in comparison to the control groups after 2 weeks incubation periods but it's significant when compared to the control groups after 4 weeks incubation periods, 1.5% samples showed a non-significant difference with 2.5% samples in the same incubation period, and it was non-significant difference also when compared to the same concentration after 2 and 4 weeks incubation periods. Regarding 2.5% samples, the difference was also non-significant when compared to the same concentration after 2 and 4 weeks incubation periods. Control group of the second incubation period showed a non-significant difference when compared to 1.5% samples for the same period, while showed a highly significant difference with 2.5% samples (2 weeks) and non-significant difference with the control group (4 weeks), 1.5% samples showed a non-significant difference when compared to both 2.5% samples (2 weeks) and 1.5% samples (4 weeks) in the same period, and showed a non-significant difference with 1.5% samples (4 weeks), 2.5% group also showed a non-significant difference with the same concentration in the third incubation period. The third control group after 4 weeks incubation period showed a non-significant difference when compared to 1.5% samples after 4 weeks incubation period and highly significant when compared to 2.5% samples after 4 weeks, 1.5% samples in the third incubation period showed a non-significant difference when compared to 2.5% samples in the same period, as shown in Table 6.

DISCUSSION

Medical plants extracts are considered as an excellent alternative to antimicrobial drugs with less or no side effects; this makes a worldwide tendency towards herbal-based medicines. Many researches in this respect have been done recently searching for biologically safe herbal medicine with potent antifungal properties [9,10].

Among the naturally derived herbal medicaments are the oils which are considered as a promising therapeutic line for oral microbial infections [12,26]. Virgin coconut oil is a medical plant extract consists of medium chain fatty acids (Lauric, Capric and Caprylic acids) which are approved to have a fungicidal effect particularly against *Candida albicans* beside other medical benefits [13,14].

The addition of different concentrations of virgin coconut oil (VCO) to the heat cured acrylic-based soft liner material in the present study revealed that; for all time intervals; the incorporation of 1.5% and 2.5% VCO caused a statistically highly significant decrease in the mean values of the viable counts of *Candida albicans* when compared to the 0% VCO group ($p<0.01$). This result can be explained by the ability of virgin coconut oil to disintegrate the cell membrane of fungi [27], in which the medium chain fatty acids found in VCO represented by Lauric acid, Capric acid and Caprylic acid are a bioactive components that have a strong antimicrobial effect; with the Lauric acid, which found in a high percentage in VCO, being the most important one as it is the precursor of monolaurin which in turn has a modulatory effect on the proliferation of immune cells and exhibit potent antifungal effect. This fact was also in agreement with another study [14], which reported that VCO is considered as a natural fighter for fungi which has the ability to destruct their cells membrane. Another recent study [28] investigated the antifungal effect of VCO incorporation into tissue conditioner and they found that specimens containing VCO interfered with *Candida albican*s growth and showed an inhibition zone on the surface of sabouraud dextrose agar and when compared to the control group.

The result also exhibited a highly significant decrease of the mean values of the viable counts of *Candida albicans* for 1.5% VCO when compared to that of 2.5% for all time intervals ($p<0.01$); this could be explained by the dynamic process that take place between the soluble components

of the soft liner material and the surrounding storage media; since soft lining material when stored in water or other aqueous solution, as artificial saliva, will be subjected to two responses: leaching out of plasticizer with other soluble contents, and fluid absorption such as water or saliva uptake. On the other hand, although adding oil to the sample will result in reduction of porosity, producing a more compact sample with less water micro bockets inside [29]; While another study stated that this fact is a concentration dependent [30], for example higher amount of VCO makes a state of instability inside the sample as more VCO will migrate to the sample surface result in more rapid loss of VCO during the first 24 hours of storage to reach a state of stability, this in turn decreases the amount of VCO presents inside the 2.5% samples in a faster rate than 1.5% samples, decreasing its antifungal potency.

For all concentration groups (0%, 1.5%, 2.5%), the results also demonstrated a gradual increase in the viable counts of *Candida albicans* as the time interval increased ($p>0.05$). After 4 weeks incubation period, the mean of viable counts of *Candida albican*s were exhibited the highest values. This observation could be attributed to the solubility-sorption behavior of soft lining material when stored in water or other aqueous solution. This behavior over a long period of time result in a rougher and more porous sample creating a suitable environment for *Candida albican*s colonization and proliferation [31]. Also this result can be explained by the high solubility of VCO medium chain triglycerides (MTC) in the biological fluids. These MTC's are bioactive substances with low molecular weight, this result is in good agreement with results of a study done on a similar material [32]; so as the samples were stored in artificial saliva; these bioactive substances had dissolved and released from the samples; this in turn will decrease the antifungal activity of the VCO as the time interval of samples storage increase.

Debonding between soft lining material and the underlying denture base is considered one of the most common failures noticed in clinical practice. Debonding does not only affect the quality of material surface; it also creates a potential environment for microbial colonization [33].

For all time intervals; the incorporation of 1.5% and 2.5% VCO caused a decrease in the shear bond strength values compared with those in the 0% VCO control group. This reduction was non-significant for the 1.5% group in all time intervals ($p>0.05$), while for the 2.5% group it was significant after 24 hours ($p<0.05$) and highly significant after 2 and 4 weeks incubation periods ($p<0.01$). This result could be attributed to the water diffusion into the bond interface in which area of stress concentration will be developed ending up with a hydrolytic destruction of the bond [34]. Another explanation could be related to the migration of some coconut oil contents to the superficial layer of the samples creating an oily layer that interferes with the bond between the two materials. The weak bond can be caused by any material present in the bonding layer before or during the processing [35]. Regarding the significant and highly significant differences that were exhibited in the 2.5% VCO group at different time intervals in comparison with the control group, this is might be regarded to the higher concentration of oil which result in sample instability and more oil diffusion into the surface. This also explains the lower mean values that obtained by the 2.5% group compared with those in the 1.5% group at all-time intervals.

For each concentration, the results revealed an increase in the shear bond strength values as the time interval increase, this increase was non-significant for all groups in the different intervals except for the control group after 4 weeks incubation in artificial saliva which demonstrate a significant difference compared to the control group after 24 hours incubation in distilled water. This could be attributed to leaching out of plasticizer from the material which results in an increase in the stiffness of the samples. This result was consistent with those reported by a study, which advocated that the tensile bond strength of the coconut oil incorporated tissue conditioner was increased as storage time in water increase due to the release of plasticizer components and increased material rigidity [28].

CONCLUSION

Within the limitations of the present study; the following conclusions can be obtained:

1. Virgin coconut oil was used as a potent antifungal herbal medicament and successfully incorporated into heat cured acrylic-based denture soft lining to obtain a material with a continuous natural drug delivery system against *Candida albican*s.
2. Samples with 1.5% VCO revealed a better antifungal efficiency compared to the control and 2.5% groups. Generally, the antifungal efficiency of VCO was reduced as the time interval of the incubation period is increased.
3. The addition of VCO into soft lining material resulted in a decreased shear bond strength of the material for both experimental groups compared to the control group; the 2.5% groups revealed the lowest shear bond strength values for all incubation periods.

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