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The Effect of Adding Coconut Oil on *Candida Albicans* Activity and Some Properties of Acrylic Based Denture Soft Lining Material

A Thesis

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Dedication

To my parents who inspired me and guided me throughout my life…

To my lovely husband “Mohammed” who shared this journey with me and supported me along the way…

To the source of hope and joy my son “Mustafa”

With love

Bushra

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Abstract

Background: 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 is caused by the fungal growth especially *Candida albicans*. Hence, the development of soft lining material with a drug delivery system becomes necessary.

Aim of the study: The study investigates the antifungal efficiency of various concentrations (1.5% and 2.5%) of virgin coconut oil incorporated into heat-cured soft denture liner against *Candida albicans*. In addition, to evaluate the hardness, wettability and shear bond strength to the denture base after this addition. All investigations were assessed at different time intervals (24h in distilled water, 2 weeks and 4 weeks in artificial saliva).

Materials and method: Three hundred sixty samples were prepared by the addition of 1.5% and 2.5% (by volume) of virgin coconut oil into heat cured acrylic-based soft denture lining material. The study samples were divided into four groups (90 samples for each group) based on the conducted test; *candida albicans* activity test, hardness, shear bond strength and wettability tests. 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 ($p<0.01$). Shore A hardness test demonstrated a reduction in the mean value of hardness after adding 1.5% and 2.5% coconut oil in comparison to the control group, this reduction was highly significant after 24 hours incubation in distilled water. There was a non-significant reduction in shear bond strength values for 1.5% group whilst a significant reduction for 2.5% compared to the control group. Regarding the wettability test, the results revealed a reduction in the contact angles values after 24 hours and 2 weeks of incubation intervals, this reduction was highly significant for the 1.5% group ($p<0.01$).

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 better softness and wettability values and also less adverse effect on the shear bond strength.

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Abbreviations	Meanings
AFE	Antifungal efficiency
BMA	Butyl methacrylate
CDA	Chlorhexidine diacetate
CFU/mL	Colony forming unit per milliliter
°C	Degree centigrade
cm	Centimeter
FTIR	Fourier transform infrared spectroscopy analysis
h	Hour
ISO	International organization for standardization
IU	Indentation unit
Kg/ cm^2	Kilogram per square centimeter
KHz	Kilohertz
mL/g	Milliliter per gram
mm	Millimeter
µL	Microliter
MTC	Medium triglyceride chains
MMA	Methyl methacrylate
N	Newton
OH-	Hydroxyl group
PVC	Plasticized polyvinyl chloride
PMA	Poly methacrylate
P/L	Powder liquid ratio
psi	Pounds per square inch
RBD	Refined, bleached and deodorized
SDA	Sabouraud dextrose agar
SD	Standard deviation
SPSS	Statistical package for the social sciences
VCO	Virgin coconut oil
W	Watt
W/P	Water powder ratio
Al^{+3}, Fe^{+3}	Aluminum Ion, Iron Ion
ZnO, Al_2O_3, ZrO_2, SiO_2	Zinc oxide, aluminum oxide, zirconium oxide, silicone oxide.

Introduction

Natural soft rubbers have been early used in 1869 by Twichell to fabricate the first soft lining material in dentistry field **(Alaa'a, 2013)**. From that point forward, a marked evolution has influenced dental materials results in the development of different types of soft lining materials, each has its own advantages and drawbacks **(Haywood *et al*., 2003)**.

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 **(Demir *et al*., 2017)**.

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 with the underlying denture base and the ability to inhibit or reduce the microbial growth **(Pisani *et al*., 2012).**

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. Denture stomatitis affects approximately 72% of denture wearers and is mainly caused by *Candida albican*s; the most common fungi species responsible for oral infections **(Iqbal and Zafar, 2016)**.

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 make it necessary to search for naturally derived medicaments to be used as a substitution to synthetic drugs **(Iqbal and Zafar, 2016; Atai et al., 2017).**

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 a biologically safe herbal-based medicines with efficient antifungal properties **(Bakhshi *et al.*, 2012).**

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 albican*s and they reported that plants oils are considered a promising therapeutic line with efficient antifungal properties for treating denture induced stomatitis **(Khan et al., 2016; Perchyonok, 2017)**.

Virgin coconut oil (VCO) is a natural plant extracts derived from fresh coconut meat. VCO gained extra attention because of its bioactive components which are well known by their antipyretic, anti-inflammatory, antimicrobial and antioxidant properties **(Rajagopal and Rajeev, 2017)**. 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* (**Ogbolu *et al*., 2007)** .

Aim of study

The aim of the present study is to:

1. Evaluate the effect of adding 1.5% and 2.5% of Virgin Coconut Oil into heat cured acrylic-based soft denture lining material on:
   a) Antifungal efficiency against *Candida albicans*.
   b) Hardness.
   c) Shear bond strength.
   d) Wettability.
2. All investigations were assessed after 24 hours incubation of samples in distilled water and also after 2 and 4 weeks incubation intervals in artificial saliva.

Chapter one:

Review of Literature

1.1. Denture relining

Soft denture liner materials have been utilized in dentistry field for over one century. The first soft liner was utilized by Twichell in 1869, it was fabricated from natural soft rubber (**Qudah *et al*., 1990; Alaa'a, 2013)**.

A plasticized polyvinyl chloride (PVC) was then invented by Matthews in 1942 as one of the first denture lining materials based on synthetic resin and can be applied over acrylic resin dentures (**Morrell, 2012**). After that silicone based soft liner materials were developed in 1958 **(Matthews, 1945; El-Hadary and Drummond, 2000; Alaa'a, 2013).**

Relining is defined as "the technique used to resurface the tissue side of a removable dental prosthesis with new base material, thus producing an accurate adaptation to the denture foundation area" **(GPT9, 2017)**.

There are various factors that affect the success of complete and partial denture construction , one of the most important factors is the accuracy of denture base fitness to the underlying supporting tissue **(Hashem, 2015)**.

However, the anatomy of the supporting tissue composed of the residual alveolar ridge and its covering mucosa is not stable and keeps changing its morphology and topography during the long term use of the denture. After long period of edentulousim, the alveolar bone loose its function and start to resorb, the thickness of soft tissue covering alveolar bone is also get diminished with time. This leads to direct transmission of masticatory forces to the bone, as a result the harm to the alveolar bone will increase **(Hashem, 2015).**

These changes affect the adaptation of denture base to the underlying tissue leading to uneven distribution of the masticatory forces that results into discomfort, pain and soreness **(Chladek *et al*., 2014; Garg and Shenoy, 2016)**.

The aim of relining procedure is to restore the accurate relation between the denture base and underlying tissue, leading to an even homogenous distribution of the occlusal load over a wide area of residual ridge to create a more stable denture, improve patient mastication and chewing cycles and maximize the benefit for denture wearers **(Demir *et al*., 2017)**.

1.1.1. Indications of denture relining

The success or failure of denture relining process are related not only to the soft liner material properties; but the good understanding of the intended problems where soft liner materials can be successfully applied is also crucial; as explained by **(Zarb *et al*., 2013; Nallaswamy, 2017)**:

a) To restore the lost adaptation between the denture base and supporting tissue caused by residual ridge resorption.
b) Geriatric and chronically ill patient who can't adapt to a new denture because of physical or mental reasons.
c) Patient who has financial issues and can't afford the cost of a new denture.
d) After three to six months of immediate denture wearing when most of the residual ridge resorption takes place.
e) To minimize pain, discomfort and tissue damage where the denture has poor retention due to poor adaptation of denture base to the underlying tissue.

f) In case of free end extension partial denture, either due to loss of occlusal stop between upper and lower denture or between denture and natural teeth, or due to loss of tissue support which cause rotation of the denture.
g) In case of a recurrent ulcer or sore red lesion that develops frequently under denture base.

1.1.2. Contraindication of denture relining

If one of the following factors is present in addition to the poor fitting of the denture base, then rebasing or fabricating a new denture is indicated **(Zarb *et al*., 2013; Nallaswamy, 2017)**:

a) Severe residual ridge resorbtion.
b) Loose dentures causing trauma or pathologic changes for the underlying soft tissues.
c) When the aesthetic of the denture is unsatisfactory for the patient.
d) Denture that cause speech problems for the patient.
e) Undiagnosed and untreated temporo-mandibular joint problem.
f) Old dentures with unsatisfactory jaw relationship.
g) Excessive osseous undercuts.

1.2. Classification of materials used for denture relining process

Based on material consistency, **Van Noort in 2013** classified denture relining materials into:

1- Hard denture liners.
2- Soft denture liners.
3- Tissue conditioners.

Furthermore, soft denture liners are classified into **(Rodrigues *et al*., 2013)**:

1- Silicone based liners.
2- Resin based liners.

On the other hand, resilient denture liners products are classified as long- or short-term according to the ***International Organization for Standardization*** **(ISO 10139-1:1991)** who states that short-term denture liners, also named as tissue conditioning materials are the materials of choice to be used after surgical procedures to accommodate the mucosal tissue and aid in its healing, however, they are advised to be used for no more than one month. While **ISO 10139-2:1999** declared that long-term denture liners, maintain their resiliency for more than one month and can be used up to twelve months.

1.2.1. Hard denture lining materials

1.2.1.1. Auto-polymerizing hard denture lining materials

Direct hard reline materials can be used directly inside the mouth as a chair side procedure, using this material increases the fit and improves function of the denture base. In addition, this will allow the patients to receive their dentures immediately with no edentulous period, no further clinical visits and no extra laboratory costs **(Haywood *et al*., 2003; Murata *et al*., 2007)**.

Although auto-polymerizing hard reline materials are easy to use and time-saving, they exhibit some drawbacks, such as, unacceptable odor and strange taste, burning sensation caused either by heat generation during polymerization process or by excessive unreacted monomer, weak bond strength to acrylic denture base and porosity. Also, the amount of acrylic denture base need to be removed and

replaced by relining material can't be accurately controlled **(Hobkirk and Zarb, 2012; McCabe and Walls, 2013)**. Careful and fast manipulation of the material is required to prevent the resin from getting in the undercuts between the teeth and set; this will make the removal of the denture very difficult **(Murata *et al*., 2007)**.

Chair side liners are provided as powder and liquid that are mixed together and allowed to set at mouth temperature. Two types are available, type 1 and type 2, both of them have the same powder content which is poly(methyl methacrylate) (PMMA), but they differ in the liquid content. In type1, the liquid is composed of methyl methacrylate monomer (MMA) and in type 2, the liquid is composed of butyl methacrylate monomer (BMA) **(McCabe and Walls, 2013).**

1.2.1.2. Processed hard denture lining materials

Indirect hard reline materials involve using the denture as a tray to take an impression inside patient mouth and then transmits it to the laboratory where resin processing is completed **(Takahashi *et al*., 1997)**. Processed hard reline resins are heat activated materials and have similar components as that of denture base **(Sakaguchi and Powers, 2012).**

Water bath system is the most commonly utilized technique, It is a compression mold technique in which heat energy is applied and makes the initiator to analyze into free radicals that will initiate the polymerization process **(Sakaguchi and Powers, 2012)**.

Microwave polymerization technique is also reported to be used successfully. This method has several advantages such as being faster, easier, requires less equipment, more color stable, less un-polymerized monomers and

also insures equal heat distribution between the outer and inner layers of the material **(Schneider *et al*., 2002; Lai *et al*., 2004).**

Visible light cured resins is another method involves using photo initiator to start the polymerization reactions **(Hayakawa *et al*., 2003)**. They are quick, easily handled and show less heat release during polymerization. Moreover, the polymerization reaction is completed with no excess monomer **(Stipho and Talic, 2001)**.

1.2.2. Tissue conditioning materials

Tissue conditioners are resilient viscoelastic materials usually utilized on the fitting surface of the denture to act as cushion between the denture base and the abused soft tissue supporting it. They prevent the occlusal forces to be concentrated on one area of the mucosa by distributing the load all over the mucosal surface area available for denture support, this will aid in soft tissue healing **(Srivatstava *et al*.; 2013, Lee *et al.*, 2018)**.

In addition, they can be used as a functional impression material for a flappy ridge, short-term liners for immediate denture or as a diagnostic tool to verify the ability of the patient to bear a new occlusal vertical dimension **(Takakusaki *et al*., 2018).**

These materials are easily mixed and applied directly inside the denture and inserted in the patient mouth as a chair side procedure. To maximize the tissue recovery, 2 mm thickness is considered the best to be effective clinically. They are applied for short periods of time and need to be replaced every 3 to 4 days **(Rodrigues *et al*., 2013).**

Subsequently, dimensional instability, separation from a denture base, unpleasant odor, color change and loss of elasticity are factors that decrease the effectiveness of tissue conditioners usage **(Hashem, 2015)**.

Tissue conditioners are mainly composed of powder poly methacrylate (PMA) or other derivatives and liquid (aromatic ester-ethyl alcohols and plasticizer with no acrylic monomer) **(Dorocka-Bobkowska *et al*., 2017)**. Leaching of alcohol over time causes a major drawbacks such as stiffening of the material, increase surface roughness, injury to the soft tissue, microorganisms accumulation on the surface and bitter taste of alcohol. To overcome these problems; a tissue conditioner free from alcohol products were developed **(Murata *et al*., 2006)**.

1.2.3.Soft lining materials

Soft lining material is characterized as a soft viscoelastic material used to reline all or part of the fitting surface of dentures, its resilient characteristic allowed it to act as "shock absorber" between the denture base and the underlying soft tissue to absorb the force and energy created by masticatory and occlusal strokes **(Dayrell *et al*., 2012)**.

Currently, soft denture liners are generally utilized either to give comfort for the patient who cannot withstand the solid surface of the denture or to improve denture retention **(Pisani *et al*., 2012; Demir *et al*., 2017)**.

At present, two common types of soft liners are available; acrylic based and silicone based. Acrylic based soft liners contain plasticizers which are released over time and increase the hardness of the material, while silicone based soft liners have no plasticizers and stay soft for longer period of time **(Araújo and Basting, 2018).**

1.2.3.1. Requirements of ideal soft denture liners

For the soft liners to be ideal and sufficiently effective, they should exhibit some criteria which will insure the maximum benefit for the denture wearers **(Oshima *et al*., 2011; Pisani *et al*., 2012)**, such as:

1) Soft liner materials should be biocompatible with tasteless and odorless properties.
2) Dimensional stability of soft liners should be high.
3) Solubility in saliva should be low along with low water sorption.
4) Strong bond with the acrylic denture base with no adverse effect on its properties.
5) Sufficient tear resistance.
6) Resilience should be high and preserved when immersed in cleansers.
7) Easy to cope with and easy to clean.
8) Low cost and long shelf life.
9) Doesn't favor fungal or bacterial growth.

1.2.3.2. Uses of soft denture lining materials

As recently developed soft lining materials are time saving, easy to be used and highly biocompatible than before; soft lining materials can be widely used in the following different situations as long as the denture is considered clinically acceptable:

1. Patients suffering from atrophied severely resorbed or knife edge mandibular residual ridge, non-resilient thin mucosa, bruxism, sore or dry mouth and

sever osseous undercut when preprosthetic surgery is not indicated **(Chladek *et al*., 2014; Palla *et al*., 2015)**.

2. Soft lining materials are also used in lower complete denture when the patient is suffering from mandibular residual ridge with physiologic or anatomic defect or undercut **(Wright *et al*., 1998).**
3. Sometimes soft liners are used when dentures are opposed by natural dentition to act as a cushion and absorb the stresses generated by functional forces **(Oliveira *et al*., 2007)**.
4. Usually used for patients that can't manage to wear the traditional hard denture base **(Saraç *et al*., 2004)**.
5. Recently, soft liners are used to line obturators for patients with congenital or acquired maxillofacial defect such as oral cancer, to aid in the retention of over-dentures supported by implants or to act as a cushion for interim dentures used after implant placement **(Pavan *et al*., 2007; Hashem, 2015)**.
6. Frequently used to improve the retention of the denture and enhance the fit with the denture base **(Pavan *et al.*, 2007)**.
7. Utilized for removable partial denture with free end saddle such as Kennedy class I **(Wright *et al.*, 1998)**.

1.2.3.3. Types of soft lining materials

Soft denture liners are divided in two main categories: acrylic resin and silicone rubber, both of these types are further divided into heat-cured type and auto-cured type **(Rodrigues *et al*. 2013; Hashem, 2015)**.

A. Soft lining materials based on acrylic resin

Acrylic resin based soft liner materials are composed of powder and liquid. Both heat-cured and auto-cured materials share the same powder component but differ in the liquid component. The powder is mostly poly (methyl, ethyl or butyl) methacrylate or it can be copolymer in addition to peroxide initiator. The liquid of auto-cured type consist mainly of 2-ethyhexyl methacrylate added to tertiary amine and plasticizer, while this of heat-cured type consist of methyl methacrylate and plasticizer **(Mese and Guzel, 2008; Morrell, 2012)**.

Various types of plasticizers are reported to be used such as: butyl benzoyl phthalate, dibutyl phthalate, dibutyl sebacate, butyl benzoate, ethyl acetate **(Rodrigues *et al.*, 2013)**.

The heat-cured acrylic resin soft liners are laboratory processed and usually used for the newly fabricated denture. On the other hand the auto-polymerized type is used for the current denture and applied directly as a chair side technique **(Meşe, 2006).**

Two processes take place when acrylic resin based denture liners are submerged in water, the polymer component will absorb water, a process known as water imbibition, while the soluble components and plasticizers will leach out from the material. With time, these two processes will cause changes in the physical and mechanical properties of the soft liner **(Morrell, 2012; Jabbal and Datta, 2016).**

B. Soft lining materials based on silicone

Silicone-based soft liners are materials that resemble the silicone impression materials in their composition **(Banerjee and Shetty, 2015)**, they consist mainly of

a viscous liquid of polymer of di(methyl, ethyl or phenyl) siloxane polymers, added to this are different types of filler in different concentrations to control the material consistency such as fine silica particles **(M and Qiao, 2015; Jabbal and Datta, 2016)**. This material get hard by a cross linking reaction rather than polymerization reaction **(Anusavice *et al*., 2013)**. No plasticizers were needed in silicone-based soft liners, along with no component will leach out from the material, thus the material will be more stable and not change over a long period of time **(Banerjee and Shetty, 2015)**.

Regarding to the curing method, silicone based soft liners are divided into heat-cured type and auto-polymerized type. Moreover, the auto-polymerized type is two sorts, condensation curing silicone and addition curing silicone **(McCabe and Walls, 2013).**

These materials are considered as hydrophobic materials, this will decrease the affinity between the soft liner and the tissue beneath and decrease the ability of the material to absorb water **(Ergun and Nagas, 2007; Ahmadzade et al., 2016)**.

Silicone based soft liners don't have the ability to bond chemically with the underlying acrylic denture base; this is mainly related to the difference in their molecular construction along with their chemical composition; their bond is totally depend on interfacial adhesion, this is why bonding agent is usually required to improve this bond. This bonding system works by containing volatile solvents which have the ability to penetrate acrylic resin; unlike acrylic based soft liners which have a chemical composition similar to that of acrylic denture base and their bond is totally dependent on the ability of the monomer to penetrate the acrylic resin **(Kaur and Datta, 2015; Rajaganesh *et al.*, 2016)**.

1.2.3.4. Problems associated with soft denture lining materials

1) The most exhibited clinical failure is the separation between soft liner and denture base, this will create a suitable area for accumulation of microorganisms, plaque and calculus, thus the need for regular replacement of soft liner is necessary **(Braden *et al*., 1995)**.

2) It was reported that surface texture of soft lining materials in addition to their chemical and physical affinity play an important role in the attraction of *Candida albicans* and other microorganisms **(Chladek *et al*., 2012)**.

3) Water solubility and sorption are a major problems regarding soft lining materials, as time passed, some essential ingredients such as plasticizers of soft liner materials will leach out leaving a space within the material structure that may be occupied by other strange particles and water, this will change the chemical structure and physical properties of the soft liner **(Ahmadzade *et al*., 2016)**. As the plasticizer drains out, the modulus of elasticity of soft liner material will increase, subsequently it will lose one of its most important properties which is the resiliency. Moreover, some plasticizers such as phthalate ester will be released and may initiate an adverse reaction in the epithelial tissue. Finally, the absorbed water will weaken the bond between the soft liner and denture base **(Ergun and Nagas, 2007)**.

4) The need for regular substitution of soft liners will create a monetary issues, thus the cost of incorporating soft liner to an already existing denture base is more than fabricating a new denture base **(Liao, 2006; Shanmuganathan et al., 2012)**.

5) More complex procedures and laboratory techniques are required to fabricate a denture base with long term soft lining material than conventional denture base **(Liao, 2006)**.

6) Adding soft liner to the denture necessitates a thickness reduction and removal of layer from the denture base to create a space for the material, this will increase the risk of denture base fracture and will affect the longevity of the denture **(Anusavice *et al*., 2013)**.

7) Discoloration of soft lining materials especially after long term usage is one of the problems. The intrinsic pigments of the material undergo degradation as the material aged, in addition, water sorption, stain precipitation and leaching out of chemical ingredient, all are factors contribute to the color change of soft liners **(Ahmadzade *et al*., 2016; Nowakowska-Toporowska *et al*., 2016)**.

1.2.3.5. Some properties of soft denture liners

A- Hardness

In general, the resistance to permanent surface indentation or penetration is termed as hardness. Hardness also gives a hint about the finishing of the material and resistance against scratches and abrasion during the material service **(Rajaee *et al.*, 2014)**.

Hardness is a physical property measured by an instrument called Shore A durometer, this instrument is usually used in rubber dentistry to evaluate the hardness of elastomeric materials **(Hussein *et al*., 2009)**.

Moreover, hardness is considered as a simple method to evaluate the modulus of elasticity of a material **(Pavan *et al*., 2007)**, also, it provides a clue for material quality because as known the rigid material can't be used as a denture soft liner. Material with low hardness value is the best to be used as soft denture lining material **(Pavan *et al*., 2007; Mancuso *et al*., 2012)**.

Nevertheless, maintaining an ideal hardness value during the long term use of soft liner is very difficult, because soft liner material will lose some of its components and gain others, this will adversely affect its properties **(Pavan *et al*., 2007; Mancuso *et al*., 2012)**. In addition, the need to clean the denture base daily by submerging it in different kinds of denture cleansers and disinfectant solutions will affect the fundamental properties of soft lining materials **(Hussein *et al.*, 2009)**.

Polymerization process, chemical components, amount of monomer residues, way of manipulation and thickness of the material all are factors that affect the hardness value **(Rajaee *et al.*, 2014)**.

Moreover; nowadays; many different additives that have been added to soft liner materials to improve their antifungal properties, have a direct effect on material hardness. For example; an antifungal agent such as chlorhexidine diacetate (CDA) was added to an acrylic based soft liner material and showed a significant increase in the hardness values of experimental samples compared to the control samples; this was mainly related to CDA acting as a filler and affecting the ability of the plasticizer components to penetrate the polymeric chains **(Abraham and Abdul-Fattah, 2017)**.

On the other hand, adding antifungal drugs such as nystatin, miconazole and ketoconazole cause no significant change in hardness values when added to soft lining materials **(Bueno *et al*., 2017)**.

B- Bond strength

Bond strength is the force required to break the bond together with failure occurring in or close the adhesive-adherence interface **(GPT9, 2017)**.

Bond strength is one of the most important properties concerning resilient material. The bond between soft liner and denture base should be strong enough to prevent the early separation between these two materials during the service that can occur before the material lose its elastic properties. Debonding between these two materials is the most common noticed failure in clinical practice **(Mutluay and Ruyter, 2005; Goiato *et al*., 2015)**.

Detachment of the soft liner will create a potential environment that renders candida and bacteria to colonize this interface, plaque and calculus to accumulate over the surface and microleakage and water sorption to take place. Subsequently, the durability of the soft lining material will be compromised and a favorable property of the denture base will be lost **(Naik and Jabade, 2005; Meşe et al., 2005)**.

Many different ways are used to measure the bond strength between soft liner and denture base including shear, peel and tensile **(Naik and Jabade, 2005)**.

Taking into consideration that at least 4.5 kg/ cm^2 bond strength is needed to prevent the loss of the attachment **(Yasser, 2017)**. Many different factors are reported to play a role in bonding failure, i.e. type and chemical structure of lining material and denture base, water sorption, storage condition, adhesive use, surface

geometry, curing cycle, degree of polymerization and thickness of the lining material which applied over the acrylic surface **(Meşe *et al*., 2005; Goiato *et al*., 2015)**.

On the other hand, many different ways are reported to increase the bond strength. Heat cure acrylic resin exposure to plasma gases such as argon and oxygen result in significant increase in shear bond strength **(Abdullah *et al*., 2014)**. Adding net woven glass fibers and surface etching of the acrylic surface with chemicals as acetone, methylene chloride and MMA are also among the factors that enhance the bond strength effectively **(Mutluay and Ruyter, 2005; Hatamleh *et al*., 2010)**. Oxygen plasma treatment is a surface roughening method which recently used to improve the tensile bond strength between the soft liner and denture base beneath **(Zhang *et al*., 2010)**. Recently the laser is considered as safe surface treatment method to enhance the bond strength between the lining material and acrylic denture base **(Gundogdu *et al*., 2014)**.

The chemical bond is the type of bond presents between the acrylic-based soft lining material and the denture base beneath; since some monomer is released from the reacting polymer of the soft liner to interact with that of the nearby denture base, while this type of bond is not present in the silicone-based soft lining material because it owns different chemical structure than that of the acrylic **(Takahashi and Chai, 2001)**, this is why different kinds of adhesives are available to improve the bond strength of the silicone type, these adhesives should have the ability to react with the chemical composition of both the acrylic denture base and the silicon-based soft liner **(Goiato *et al*., 2015)**.

C- Wettability

Wettability is a fundamental requirement for a denture base, it has a great effect on the denture retention because it allows the saliva to spread smoothly and easily over the denture surface, this in turn increases the denture retention. Moreover, wettability plays a role in minimizing accumulation of candida on the denture surface by a cleansing action, the more the wettability the more the clean ability **(Muttagi and Subramanya, 2017)**. Also, by creating a lubricating layer over the denture surface; wettability enhances the patient comfort **(Jin *et al.*, 2009)**.

Contact angle is an essential parameter in the measurment of wettability of denture lining materials. Contact angle is the angle formed by a tangent to the drop of liquid and the solid surface **(GPT9, 2017)**. This angle is a unique feature for each substances because it is related to the surface energy of the solid substances and surface tensions of liquid substances. The highest is the contact angle the lowest is the wettability value **(Jin *et al*., 2009; GPT9, 2017)**.

Acrylic-based soft lining material has a chemical structure that considered hydrophilic in nature, although this property may cause a greater affinity to water and saliva to the surface of the lining material, but at the same time it will aid in providing an even layer of saliva to lubricate the oral mucosa and hence enhances the denture retention and patient wellbeing. Heat cured acrylic based soft liner material is considered the best choice for denture relining material since it shows a greater wettability value than other materials **(Jin *et al*., 2009)**.

Regarding silicone based soft lining material, hydrophobicity is a major drawback for this material, even though this will decrease the water sorption of the lining material, but simultaneously it will prevent the saliva from spreading all

over the reline surface creating a frictional force between the denture and underlying oral soft tissue end up with tissues soreness and patient discomfort especially if the patient has a low salivary flow rate **(Jin *et al.*, 2009)**.

1.3. *Candida albicans*

Candida is a type of fungi that has a variety of species, one of these species is the *Candida albicans* which is an opportunistic pathogen that can be existent in the oral cavity as well as different body systems as a part of the body's normal flora. It was discovered in the oral cavity of 45-65% of healthy individual and 60-100% of denture wearers **(Akpan and Morgan, 2002; Petrović *et al.*, 2014)**. *Candida albicans* are gram positive microorganism appears as oval or round structure under the microscope; lives in a relatively humid acidic condition of 6-6.5 pH and at a temperature of 37 °C **(Mayer *et al.*, 2013)**.

Under certain conditions such as reduced salivary flow, immunosuppression, diabetes mellitus or long term antibiotics use; *Candida albicans* can cause a variety of fungal infections range from mild to severe; hyperplastic candidiasis, erythematous candidiasis, angular chielitis and denture induced stomatitis are among the common fungal infections that can affect the oral cavity **(Farah *et al.*, 2010)**.

1.3.1. Virulence of *Candida*

The transformation of *Candida* from a normal commensal organism to a pathogenic one is a complicated multifactorial process; that usually end up with a significant change in the environment facilitates the way for the candida to express its pathogenic factors and act as an opportunistic pathogen **(Polke *et al.*, 2015)**.

For denture wearers, it is possible that the presence of the denture inside patient mouth makes the oral environment more acidic by reducing the salivary flow and oxygen diffusion to the underlying soft tissue; this condition encourages the candida to grow **(Dantas *et al*., 2014)**.

Various factors are responsible for *Candida* pathogenicity; they include adhesion to host cells or a surface of medical appliance, resisting host immune defense, construction of biofilm and hydrolytic enzymes release. Adhesion and building up a biofilm over the inner surface of the denture are very important in the development of the denture induced stomatitis, a superficial kind of oral fungal infection, that approximately affects 65% of people wearing a complete denture **(Pattanaik *et al.*, 2010)**.

1.3.2. Oral candidiasis

Oral candidiasis is a general term that dealing with a group of diseases caused by candidal infection. Till now it is the most dominant human fungal infection that has a wide range of clinical presentations and can affect healthy people as well as immunocompromised persons **(Akpan and Morgan, 2002)**.

This fungal infection may range from a topical infection that can be easily diagnosed and treated to an acute systemic disease that could be fatal. Some time, it is only a secondary infection indicating a more sever medical condition, such as AIDS and other immune diseases **(Farah *et al*., 2010; Mayer *et al*., 2013)**.

Oral candidiasis is classified as:

1. Primary candidal infection in which the lesion is confined in the oral cavity and surrounding tissues.

2. Secondary candidal infection in which the lesion is generalized and present in the oral cavity as well as other mucosal and cutaneous areas **(Samaranayake, 2011)**.

Other classification of oral candidiasis:

a) Acute pseudomembranous candidosis (thrush).
b) Acute atrophic candidosis.
c) Chronic hyperplastic candidosis.
d) Chronic atrophic candidosis known as candida induced denture stomatitis **(Scully *et al.*, 1994)**.

The presence of oral *Candidal* infection indicates a weakness in the host immune system; an increased persistent oral candidosis can some time discover a serious underlying medical condition such as AIDs. Recently, the excessive use of immunosuppressive medication and broad spectrum antibiotics in medical practice result in an increase in candidal infections, which represent the first signs inside the oral cavity **(Bokor-Bratić, 2008)**.

In denture wearers, oral candidal infection is most commonly presents as denture induced stomatitis **(Pattanaik *et al.*, 2010)**, this is mainly due to the time-related deteriorations of the denture base material presented by roughness and porosity which create an attractive environment for the *Candida albicans* and other microorganisms **(Emami *et al*., 2008)**.

1.4. Denture stomatitis

Denture induced stomatitis is an inflammatory condition that affects the mucosa under a complete or partial removable dentures. Regarding complete denture wearer patient, the maxillary arch is the most common site to be affected by denture stomatitis. Patients suffering from cleft palate who wear an obturator

and those with orthodontic appliances are not uncommon to be affected. Denture stomatitis can affect both males and females with the last showing a higher percentage **(Petrović *et al.*, 2014)**.

The most critical step in denture induced stomatitis is the *Candidal* adherence to the inner surface of the acrylic denture base.

Denture induced stomatitis can be classified into:

Type I: pinpoint simple inflammation confined to limited area.

Type II: simple inflammation diffused to all denture covered area.

Type III: diffused inflammation and granular nodules covering the center of the hard palate **(Newton, 1962; Pattanaik *et al*., 2010)**.

1.4.1. Etiology of denture induced stomatitis

Although the main etiology of denture induced stomatitis is yet known, however many predisposing factors are thought to be essential either in initiating or in aggravating this clinical condition **(Farah *et al.*, 2010)**.

A) Trauma

Ill-fitting prosthesis, incorrect vertical and horizontal jaw relationship, old worn dentures and traumatic occlusion, all these factors are reported to cause a trauma that will adversely affect the oral mucosa **(Emami *et al*., 2008)**.

Trauma of the soft tissue that supports the denture particularly the mucosa can initiate an inflammatory reaction, this in turn will make the mucosal epithelium

very weak, thin, and permeable to the toxic agents and soluble substances released by the *Candida*. Thus will make an easy path for the *Candida* to stick, penetrate and colonize the soft tissue. Although trauma has a role in the process of *Candidal* colonization, but it is only a co-factor that facilitates the path and supports the adhesion of the candida; while the main pathologic factor is the *Candida albicans* itself **(Salerno *et al*., 2011)**.

B) Denture factors

Old worn denture can be a predisposing factor for up growth of the denture stomatitis. The inner surface of an old denture undergoes many changes during the long time wearing and exposed to different oral environments; this makes the denture surface more porous, rough and consequently more attractive for pathogenic organisms **(Emami *et al*., 2008)**. Microorganisms present in denture micro-porosity are very difficult to be cleaned neither mechanically nor chemically. Poor fitting prosthesis and non-balanced occlusion are confirmed to play a role in denture induced stomatitis **(Pattanaik *et al*., 2010)**.

Continual day and bed time denture wearing leads to decrease oxygen supply to the soft tissue beneath, decreases the washing and immune effect of the saliva, prevents the tongue from cleaning the palatal mucosa, finally increases the susceptibility of the tissue to injuries and microbial colonization especially yeasts **(Akpan and Morgan, 2002)**.

Poor denture hygiene is one of the factors that enhances *Candidal* proliferation and colonization to the denture surface, as plaque accumulation and stagnation of food debris will provide a suitable environment for the microbes to grow. Carbohydrate rich diet, saliva composition and flow and

manual dexterity are all affecting the plaque pathogenicity and likewise increase the candidal proliferation **(Pattanaik *et al.*, 2010)**.

C) Saliva

A few researchers approved that the saliva has a role in decreasing the ability of *Candida* to stick to the denture surface, even though the importance of the saliva in the process of candidal colonization is still controversial, it can't be denied that the salivary immunogenic agents, i.e. lactoferring, lysozymes, calprotctin and immunoglobulin A prevent the candida to adhere to the prosthesis and the supporting tissues **(Farah *et al*., 2010; Valentijn-Benz *et al*., 2015).**

Moreover, patients with decreased or complete absence of salivary flow suffer from a disturbance in the balance of the normal oral micro-flora where some microorganisms will dominate over others **(Salerno *et al*., 2011)**.

D) Systemic factors

Different systemic conditions and medication can cause an increase in the prevalence of candidal infection especially oral candidiasis. Diabetes mellitus is one of these systemic conditions. Compared to a healthy denture wearer, diabetic patient wearing a denture shows a higher count of candidal colonies, this is mainly related to uncontrolled blood glycemic level leading to a glucose-rich saliva that supports yeast growth **(Dorocka-Bobkowska *et al*., 2010)**.

Weakness of host defense mechanism caused by immunodeficiency syndrome or malignancies that necessitate the treatment with radiotherapy or chemotherapy makes the candida more virulent and cause candidiasis **(Semlali *et al.*, 2014)**.

Malnutrition and vitamin deficiencies such as vitamin A, B12, C, iron and folic acid all can decrease body immunity and affect the integrity of epithelium tissue leading to a fungal infection, in addition to carbohydrate rich diet which is also considered as a risk factor **(Farah *et al*., 2010)**.

Many medications taken by older people are associated with reduction or alteration in salivary gland performance leading to dry mouth, as a side effect. Corticosteroids, antihypertensive drugs, antidepressant, anticholinergic and broad spectrum antibiotics are among these drugs. The subjective report of oral dryness is named xerostomia, which is a symptom, not a diagnosis or disease **(Farah *et al*., 2010; Semlali *et al*., 2014)**.

Age also has an effect in the development of candidal infection. As patient aged, the count of immune system cells will decrease, systemic diseases increase, along with differences in dietary intake and salivary component; all these make the old denture wearing patient more susceptible for denture induced stomatitis **(de Oliveira Mima *et al*., 2011)**.

E) Lining materials

Denture lining materials are known to undergo a significant deterioration over time, the surface of lining material becomes more hard and rough encouraging the *Candida* to attach and colonize the area, with time, the large amounts of toxins and metabolic by-products secreted by *Candida* initiate an inflammatory tissue reaction **(Pattanaik *et al*., 2010)**.

1.4.2. Clinical signs and symptoms of denture induced stomatitis

Although denture stomatitis can run without symptoms, sometimes the condition could be painful and causes dysphagia. Clinical signs may include redness, change of color, change in mucosal permeability and texture and dryness of soft tissue **(Lalla *et al*., 2013)**.

Moreover, oral candidiasis is some time considered as a source of discomfort, taste loss and food aversion. Mouth soreness and a wide area of erythema can be seen also with or without thrush **(Kharaidi *et al*., 2017)**.

In some patients, denture stomatitis is associated with the presence of angular chielitis as well as ulcers over the mouth corners; this in turn will make difficulty in mouth opening and so on difficulty in denture wearing **(AlTarawneh *et al*., 2013)**.

1.4.3. Prevention and treatment

As mentioned previously denture induced stomatitis is a multifactorial process related to many different factors based on patient, denture and dentist **(Dorocka-Bobkowska *et al*., 2010)**.

To prevent denture stomatitis, denture wearers must always be advised to clean their dentures thoroughly, massage their mucosa to stimulate blood circulation and remove their dentures at bed time to allow the mucosa to retain to its normal uncompressed form **(Emami *et al*., 2008; Sharma and Sharma, 2015)**.

In addition, denture wearers have to attend a periodic follow up dental visits to assess the denture quality, peripheries, occlusal status, the amount of ridge resorption and the need for a repair **(Pattanaik *et al*., 2010)**.

Recent studies approved that the placement of the denture in a diluted white vinegar to a ratio of one to twenty, 0.1 % hypoclourite or chlorhexidine solution two times a week for a quarter to half an hour is recommended **(Farah *et al*., 2000; Farah *et al*., 2010)**.

Treatment of *Candida* infection depends on the site of infection, immune response of the host and the severity of the disease. Treatment of denture induced stomatitis involves a thorough cleaning of the denture, avoid overnight denture wear habit, relining, rebasing or even replacing the denture, and prescription of topical and systemic antifungal medicaments. Usually, more than one method is needed for treating the fungal infection effectively **(Dangi *et al*., 2010)**.

Two main groups of antifungal medication are frequently used for the eradication of fungal disease, polyenes and azoles. Polyene group includes nystatin and amphotericin B both are used topically to treat the primary oral candidiasis. Polyenes have the advantage of not being absorbed by the gastrointestinal tract and this subsequently makes the possibility of drug resistance less. Nystatin drug becomes in contact with the ergosterol in the cell membrane and creates a holes inside this membrane, as a result, all cell contents including potassium storage will leak out end up with cell death. On the other hand, nystatin products have bad taste, can cause tissue irritation and sensitivity and if swallowed can induce nausea, vomiting and even diarrhea **(Farah *et al*., 2010; Pattanaik *et al*., 2010; Atai *et al*., 2017)**.

Azoles group are commonly used systemically for deep fungal infection. Ketoconazole and fluconazole are an examples, miconazole is an azole form that can be applied topically, it is available as a 2 % gel that applied two to three times a day for one to two weeks directly on a clean dry prosthesis and must be in direct contact with oral tissue **(Dangi *et al*., 2010; Bakhshi *et al*., 2012)**. The main disadvantage of azole when applied topically is the ability to be absorbed by the gastrointestinal tract this may increase fungal resistance to this drug **(Atai *et al.*, 2017)**. Fluconazole and ketoconazole are used only in refractory cases that failed to be treated with a topical medication **(Farah *et al*., 2010)**. In advanced extensive cases that show a papillary lesion; scalpel, laser, electro and cryosurgery can be used **(Akpan and Morgan, 2002)**.

1.5. Soft lining materials additives

As soft lining materials solve many problems for denture wearers and increase their comforts; unfortunately, they also cause many problems because they deteriorate over the long-term usage. Until now, no single type of these materials is considered to be ideal, since every type has its own advantages and disadvantages **(Yasser, 2017)**.

Over the last decades, many additives have been added to soft lining materials to improve their biological, mechanical and physical properties. Among these additives are:

1.5.1 Nanoparticles

Nanoparticles are defined as any particles of less than one hundred nanometers, they can be organic particles or inorganic particles. **(Zheng *et al*., 2003)**. Some of these nanoparticles are approved to have antimicrobial

properties **(Singh *et al*., 2014)**. Different studies have been done to approve the potential antimicrobial effect of zirconium nanoparticles and silver nanoparticles, both of them show an effective therapeutic ability and reduction of *Candida* colonies count **(Issa and Abdul-Fattah, 2015; Yasser, 2017)**.

1.5.2. Antifungal drugs

Many studies try to create a sustained drug delivery system by incorporating antifungal drugs such as miconazole, nystatin and ketoconazole as a therapeutic way to treat the denture stomatitis and a favorable results were obtained **(Jadhav *et al*., 2013; Rawat *et al*., 2017; Neppelenbroek *et al*., 2018).**

1.5.3. Chlorhexidine diacetate salt

Chlorhexidine is an antiseptic compound available in different forms; mouth wash solutions and gels are the most commonly used. Chlorhexidine diacetate salt exhibits a potent antimicrobial activity affecting both Gram negative and Gram positive microbes, it has anti-plaque and anti-gingivitis properties. In addition, it shows an anti-fungal activity by inhibiting the yeast colonization **(Siqueira and Sen, 2004)**.

1.5.4.Plants extracts and oils

As mentioned previously, treating denture induced stomatitis with antifungal drugs can cause a serious side effects such as resistance, sensitivity or recurrence. A new therapeutic line is followed recently by using the natural herbal substances as a substitutes for the chemical one to lessen the adverse side effects on humans **(Bakhshi *et al*., 2012; Petrović *et al*., 2014)**.

At 1998, the amount of the sold herbal medicines has increased significantly to reach four billion dollars in the United States of America; this gives a hint about the high tendency of the universe towards the herbal based medical products **(Marcos-Arias *et al*., 2011)**.

Various plants products have been used for many decades. However, accurate clinical trials are needed in order to evaluate their effectiveness in comparison with currently available chemical medicaments. Some plants extracts need special treatments or certain conditions to be biologically active **(Bakhshi *et al*., 2012)**.

Thus, recent therapeutic approach is necessary and the role of the natural medicaments is very essential as some of these products could be added to the composition of a mouth washes or tooth pastes **(Marcos-Arias *et al.*, 2011)**.

Garlic was one of the first plants that used to extract a natural medical products, it has a unique properties of giving support for human immune system as well as having a potential antimicrobial effect. Aqueous garlic extract is approved to be a good substitute for nystatin mouth rinse in treating denture stomatitis **(Bakhshi *et al*., 2012)**.

Chitosan is a naturally derived biocompatible polymer with antifungal and antibacterial properties which make it possible to be used in different medical fields. Chitosan oligomers interfere with the growth enzymes of *Candida albicans* and it is recommended to be applied over the oral mucosa. In addition, study showed that chitosan solution is comparable to nystatin in denture stomatitis treatment **(Atai *et al*., 2017)**.

Among the natural herbal based medicaments are the oils. Recently, they are considered as a promising therapeutic strategy for oral microbial infections. Oils are a complicated mix of different volatile substances derived from herbs; they can overcome a wide variety of pathogenic organisms with their antimicrobial and antioxidants properties **(Petrović *et al.*, 2014)**.

Over the few last decades, different types of oils have been tested to assess their antifungal activity against *Candida albicans* in denture wearers suffering from denture induced stomatitis **(Perchyonok, 2017)**.

Pelargonium graveolens and satureja hortensis are two types of plants, their extracted oils are used to treat *Candida* associated denture stomatitis, and one percent of gel composed from these oils extracts was approved to reduce the fungal growth and erythematous lesion in oral cavity. *S. hortensis* extract is even considered a broad spectrum antimicrobial agent that affect not only fungi but also yeast and bacteria **(Petrović *et al*., 2014)**.

Menthone, menthol, eugenol, linalool, tyrosol, carvacrol and farnesol are also nature-derived oils which have a potent effect against different types of fungi species. Besides that, carvacrol oil has a special powerful effect against *Candida albican* even those which resist fluconazole drug **(Vasconcelos *et al*., 2003; Marcos-Arias *et al*., 2011)**.

Tea tree oil is also known to have strong therapeutic power against *Candida*, a study showed that tea tree oil helps in the eradication of fluconazole resistant *Candida* in AIDs patients. In addition, 0.25 % of this oil inhibits the proliferation process of *Candida* by forming a germ tubes. Adding tea tree oil to soft liners and tissue conditioner helps in treating denture induced stomatitis

suggesting a new strategy for managing oral candidiasis **(Catalán *et al*., 2008; Pachava *et al*., 2015)**.

Seed oils, thyme and sesame oil are also among the naturally derived medicaments which have been added to soft liner materials or tissue conditioners and show a powerful activity against *Candida albicans* **(Muttagi and Subramanya, 2017).**

Although all of the above mentioned oils act against *Candida albicans*, but each of them has its own special active component which affects fungi in special mechanism. So, no wonder to have some discrepancies in the antifungal and antibacterial properties of the herbal extracts, this is more related to the differences between the plants themselves and their extracts compositions, variations between the method of extraction and processing, discrepancies between the genetic composition of the fungi and bacteria and whether the lasts are from the same or different species **(Petrović *et al*., 2014)**.

1.6. Virgin Coconut oil

Virgin coconut oil is an important herbal extract that showed an increasing popularity over the years as numerous researches investigated its benefits and pharmaceutical properties. It is an essential food oil and rich source of fat needed in human body **(Lukić *et al*., 2016)**.

1.6.1. Virgin coconut oil extraction

Coconut oil is obtained from the coconut meat by two different ways. Based on this fact, there are two types of coconut oil each has its unique properties;

virgin coconut oil (VCO) and refined, bleached and deodorized coconut oil (RBD) also named commercial coconut oil **(Marina *et al*., 2009b).**

RBD oil is extracted from dried stored coconut meat known as copra, the drying process which is usually made by sun or smoke and unclean storage make this oil unsafe to eat or use by human, therefore, a process of chemical refining, bleaching and deodorizing done under high temperature is important. However, this chemical process changes the oil nature, degrades some of the essential components and changes its color to yellow, pink or red orange **(Lukić *et al.*, 2016)**.

VCO oil is derived directly from fresh coconut meat by natural and mechanical methods with or without the aid of a controlled heat, no chemical processing is involved, no exposure to ultraviolet radiation from the sun during the drying process is included and no additives are added. All of this preserve the oil from changing its nature, keep the bioactive components intact, prevent the loss of the pro-vitamins and polyphenols and maintain its clear color in liquid state and white color in solid state **(Marina *et al*., 2009b; Lukić *et al*., 2016; Rajagopal and Rajeev, 2017).**

1.6.2. Chemical composition of virgin coconut oil

Virgin coconut oil is composed mainly from wide variety of medium chain fatty acids, each of them has its own effect **(Marina *et al.*, 2009a)**. Lauric acid is an essential fatty acid makes approximately 48 % of the oil contents **(Marina *et al*., 2009c; Lukić *et al*., 2016).** Myrestic acid share about 21 % of the contents, also Caprylic acid, Capric acid, Caporic acid, Palmitic acid and Stearic acid all are saturated fatty acids presents in VCO; While, Olic acid,

Linoleic acid and Linolenic acid are among the unsaturated fatty acids **(Carandang, 2008)**. Furthermore, VCO containd different types of vitamins and provitamins such as vitamin E, A and K and wide varaity of potent antioxidants such as tocopherol and phenolic compounds **(Rajagopal and Rajeev, 2017)**. (Figure 1-1)

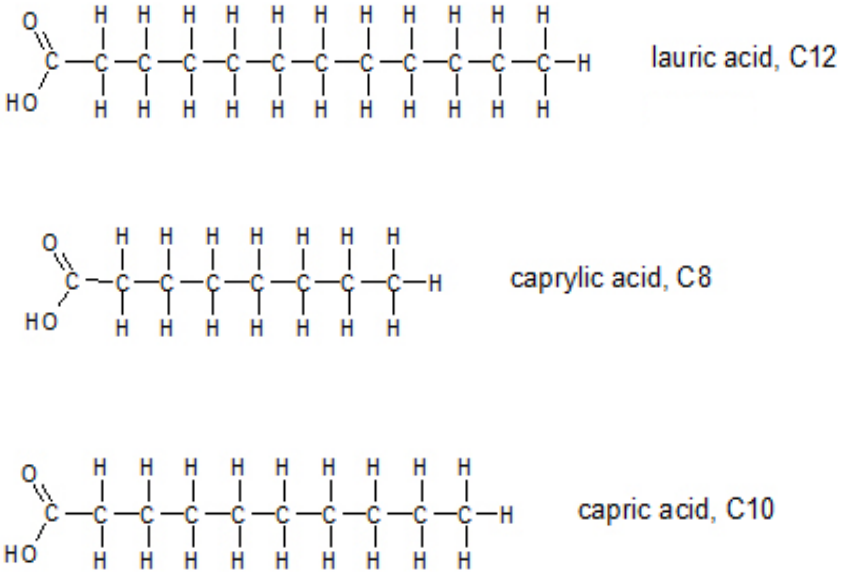


Figure (1-1): Some of the chemical compositions founds in virgin coconut oil.

1.6.3. General application and benefits of virgin coconut oil

Virgin coconut oil is an essential energy source in diet and takes a large part in the hair and skin care products. VCO is low calories oil which is easily digested and doesn't cause weight gain as it enhances the metabolic activity. It increases the vitamins uptake from the stomach, supports the defense mechanism of the body, minimizes the risk of atherosclerosis and coronary diseases and causes cancer agents inhibition **(Lukić *et al.*, 2016)**.

This useful oil has antipyretic, anti-hypercholesterolemic, antioxidant, analgesic and anti-inflammatory effect. In addition, VCO is not loaded with cholesterol as other oils so it plays a role in decreasing the risk of arterial blockage and hypertension development **(Rajagopal and Rajeev, 2017)**.

Alzheimer patients who receive daily coconut oil in their diet show an improvement in orientation, cognition and ability of speech **(De *et al*., 2017).**

Later in **2017 Kamalaldin *et al*.,** revealed that the VCO inhalation is considered as successful method for curing or treating asthma by reducing the allergic symptoms in asthmatic patient and reliving the inflammation of the respiratory tract.

Antibacterial, antifungal and antiviral all are important properties present in VCO **(Ogbolu *et al*., 2007; Rajagopal and Rajeev, 2017)**. A researcher found that the medium chain fatty acids are the key components of VCO which are responsible for the wide range antimicrobial activity **(Thormar *et al*., 1987)**. The exact mechanism by which these fats kill microbes is still vague; but some researches investigate the process by scanning electron microscope declared that these lipids play a role in cell membrane destruction **(Ogbolu *et al*., 2007)**.

Other studies states that the presence of Lauric acid, Capric acid and Caprylic acid is responsible for the antifungal activity of VCO, since all of these lipids are approved to act against fungi specially *Candida albicans* **(Isaacs *et al*., 1995; Ogbolu *et al*., 2007)**.

Noteworthy, *Candida albicans* is discovered to be more susceptible to VCO than to fluconazole. Moreover, fluconazole; even when used in high concentrations; it only works as fungistatic agent; i.e. it only prevents the

candida from proliferation and multiplication ; while Lauric acid and Capric acid are considered as fungicidal agents that reduce the candidal counts **(Ogbolu *et al.*, 2007)**.

Chapter Two:

Materials & Method

2.1. Materials

Materials used in this study are listed in Table (2-1).

Table (2-1): Materials used in the study

No.	Materials	Manufacturer	Source	Exp. Date
1	Virgin coconut oil	VIVA naturals	Philippine	8/2019
2	Vertex-soft (heat cured acrylic-based soft liner material)	Vertex	Netherlands	7/2019
3	Heat-curing acrylic resin	New static S.A	Antioquia, Colombia	1/2021
4	Deionized water	---	Iraq	---
5	Distilled water	---	Iraq	---
6	Polyethylene separating sheets	Amalgamated dental T.D.	England	---
7	Separating medium	BMS dental	Italy	5/2019
8	Petroleum jelly	Unilever	South Africa	2020
9	Extra hard type IV dental stone	Zhermack	Italy	9/2019
10	Wax sheets	BMS dental	Italy	---
11	Dental pumice	Silky rock	England	5/2019
12	Addition silicone impression material	Zhermack	Italy	3/2020
13	Sabouraud dextrose agar	Oxoid	England	12/2019

14	Sabouraud dextrose broth	Oxoid	England	12/2019
15	Normal saline	Almottahedon	UAE	6/2018
16	Gram stain kit	Hardy diagnostics	USA	2/2019
17	Ethyl alcohol 70%, 96%		Iraq	---
18	Vitek 2 cassettes YST	Biomerieux	France	11/2018
19	Sodium chloride, calcium chloride, potassium chloride, ammonia, monopottasium phosphate, sodium thiocyanate, disodium phosphate, urea, ascorbic acid.	Fluka	Swiss	---
20	Glucose, Mucin.	Labtech chemicals	Doha	---

2.2. Equipment and instruments

Equipment and instruments used in this study are listed in Table (2-2):

Table (2-2): Equipment and instruments used in the study

No.	Equipment's and instruments	Company	Source
1	Universal testing machine	Instron 1195	England
2	Shore A	HT	China
3	Dino-lite	AnMo company	Taiwan

4	Infrared spectrophotometer	IR Prestige-21 Shimadzu	Japan
5	Incubator	Memmert	Germany
6	Electronic balance	Sartorius BP 30155	Germany
7	Centrifuge	Hettich	Germany
8	Autoclave	Raypa	Spain
9	Densitometer	BioMerieux	Italy
10	Thermostatically controlled water bath	Memmert	Germany
11	Lathe polishing machine	Bego	Germany
12	Hydraulic press	BegoHydrofix	Germany
13	Light Microscope	Olympus	Japan
14	Hood	Azbil group	Japan
15	Refrigerator	Samsung	Korea
16	Probe sonication apparatus	Soniprep-150	England
17	Dental vibrator	Bego	Germany
18	Vortex mixer	Stuart	UK
19	Electronic digital caliper	Fujian	China
20	Dental flasks	Broden	Sweden
21	Clamps	HANUA	U.S.A
22	Sterilized petri dishes	JRZ plastilab	Lebanon
23	Transport swabs (Amies transport medium)	AFCO (Al Hanoof)	Jordan

24	Inoculation loops	Citotest	China
25	Glass spreader		China
26	Autoclavable sterilized tubes		China
27	Bunsen burner		China
28	Tweezers	Zamberg	Germany
29	Disposable syringes	Al Hanoof	Jordan
30	Finishing burs (stone, acrylic, fissure, sand paper)		Germany
31	Rubber bowl, spatula, wax knife		China
32	Micropipette	Dragon lab	China
33	Disposable pipette		China
34	Prosthetic hand piece	Marathon	Korea
35	Blades, Scalpel		Germany
36	Beakers, graduated tubes		China
37	Glass flasks		China
38	Ph meter	Jenway	Cyprus

2.3. Methods

2.3.1. Pilot study

To determine the best concentration of coconut oil to be added to the heat cured acrylic-based soft liner material (Figure 2-1) a pilot study was performed. Three concentrations of coconut oil were selected (2.5%, 5% and 7.5%) by volume of the monomer and compared with the control group (0% coconut oil).

The pilot study aimed to assess the effect of coconut oil on *Candida albicans* activity and shear bond strength of heat cured acrylic-based soft denture liner material, five samples were used for each test. Moreover, pilot study aimed to determine the best dilution of *Candida* suspension that showed a countable range of colonies.

Figure (2-1): A) Heat cured acrylic soft liner material (Vertex); B) Virgin coconut oil

2.3.1.1. Viable counts test for *Candida albicans*

Table 2-3 represents the result of viable count test for the control compared with three concentrations of coconut oil (2.5%, 5% and 7.5%) that were used in the pilot study.

Table (2-3): The results of *Candida albicans* viable counts test (CFU/mL) in the pilot study

N	Control (CFU/mL)	2.5% (CFU/mL)	5% (CFU/mL)	7.5% (CFU/mL)
1	176	76	83	77
2	140	48	65	97
3	163	45	72	89
4	144	64	67	83
5	132	60	88	90
Mean	151	58.6	75	87.2

2.3.1.2. Shear bond strength test

Table 2-4 shows the results of shear bond strength of the control compared to three concentrations of coconut oil (2.5%, 5% and 7.5%) that were used in the pilot study.

Table (2-4): The results of shear bond strength test (N/mm^2) at different concentrations in the pilot study

N	Control	2.5% (N/mm^2)	5% (N/mm^2)	7.5% (N/mm^2)
1	0.456	0.4208	0.4368	0.4576
2	0.4992	0.4544	0.384	0.408
3	0.4464	0.4112	0.496	0.3936
4	0.4928	0.4848	0.4624	0.464
5	0.5136	0.4736	0.4192	0.4272
Mean	0.4816	0.44896	0.43968	0.43008

The result of the pilot study showed that as the concentration of virgin coconut oil is increased the antifungal efficiency of the soft liner samples along with the shear bond strength between the soft liner sample and the underlying denture base is decreased. So according to this result; the 5% and 7.5% groups were excluded and the 2.5% group was included and a new concentration was added which is 1.5% VCO.

2.3.2. Samples grouping

At the end of sample preparation, consequently, three hundred sixty samples were obtained. Four different tests were performed in this study, for each test three experimental groups were investigated according to the VCO concentration used in the pilot study (control 0%, 1.5% and 2.5%). The tests were performed at different periods of time (24 hours in distilled water, 2 weeks and 4weeks in artificial saliva), ten samples were used for each time interval, as shown in Figure (2-2).

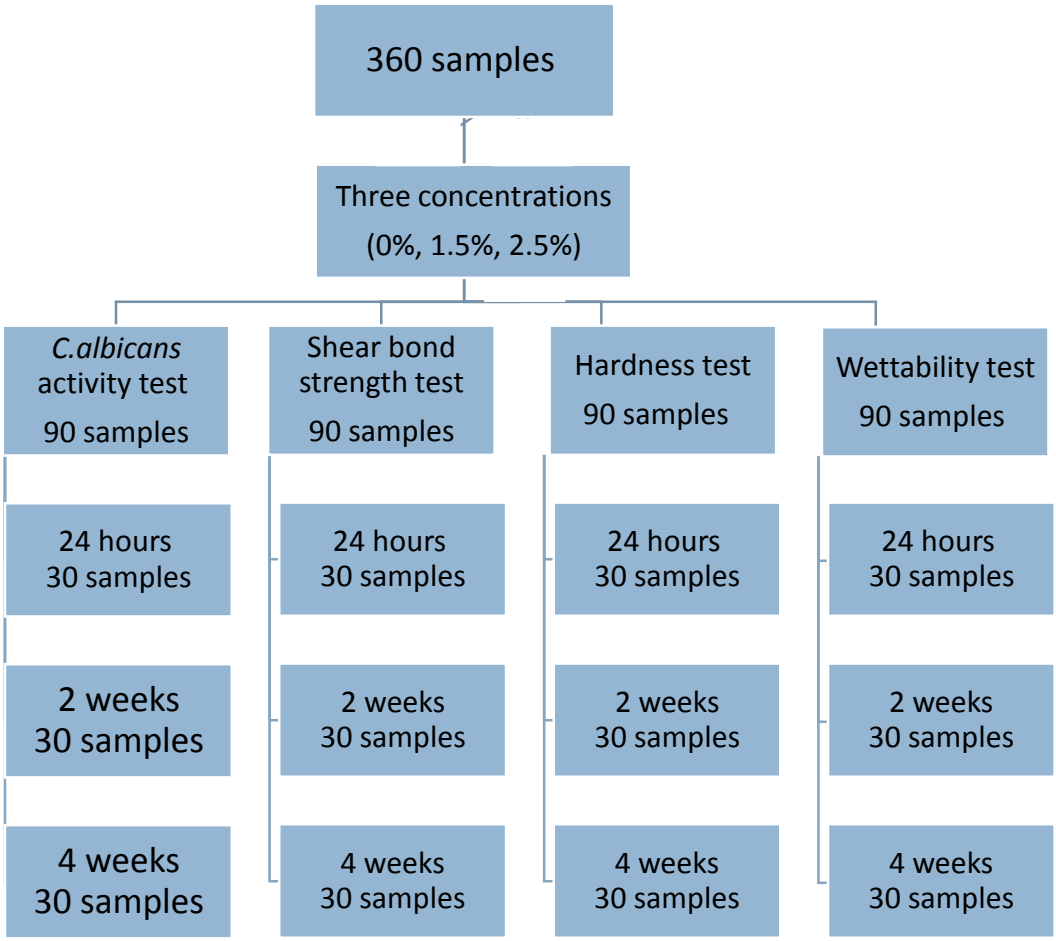


Figure (2-2): Flow chart for the samples grouping with detailed description.

2.3.3. Fourier Transform Infrared spectroscopy (FTIR)

A small number of control and experimental samples were scratched and tested using the FTIR spectrophotometer analysis to inspect the presence of any sort of chemical reaction between the VCO and soft liner materials (Figure 2-3).

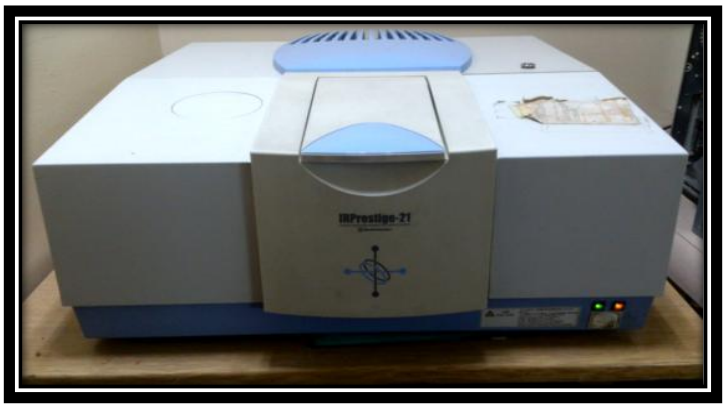


Figure (2-3): FTIR spectrophotometer

2.3.4. Preparation of artificial saliva

One liter of artificial saliva was made by adding 0.002g of ascorbic acid, 0.160g of NH_4Cl , 2.700g of mucin , 0.330g of KH_2PO_4, 0.170g of $CaCl_2$, 1.270g of KCl, 0.160g of NaSCN, 0.030g of glucose, 0.200g urea, 0.340g of Na_2HPO_4 and 0.580 of NaCl (Bacto-Mucin-Bacteriological) in 1000 mL of distilled water. Then, the solution titration was done by adding phosphate buffer of 26.4 mL, 0.06 M $Na_2HPO_42H_2O$ and 7.36 mL 0.06 M KH_2PO_4. The pH value of neutral artificial saliva (pH=7) was measured using pH meter **(Klimek *et al*., 1982).** The artificial saliva was changed every day.

Figure (2-4): a) Artificial saliva salts; b) pH meter device

2.3.5. Microbiological aspect of the study

2.3.5.1. Fabrication of control and experimental soft liner samples used to asses *C.albican*s viable counts

A) Mold preparation

Ninety samples measuring 10×10×2.3 mm, length, width and thickness respectively (30 from each group) were prepared using special design plastic molds to make the final shape of the soft liner samples **(Chladek *et al*., 2011)** (Figure 2-5). Dental flasks were prepared and coated with a layer of petroleum jelly as a separating medium, the lower part of the flask was loaded to its half with a freshly mixed dental plaster (W/P ratio: 50mL/100g) and then completed with a freshly mixed extra hard dental stone (W/P ratio: 25mL/100g) according to the manufacturer instructions. Before the stone set, half the thickness of the mold was immersed in the stone and the other was left above the stone level to prevent the molds from getting submerged inside the stone which may make it difficult to remove later. Eighteen molds were placed randomly inside each flask. After setting, the stone with invested plastic molds were coated with a layer of separating medium. The upper part of the flask is then placed over the lower one and loaded with a layer of extra hard dental stone followed by a layer of dental plaster and cover tightly with the flask cover. The flask was left one hour to make sure the stone was completely set and then opened to extract the plastic molds.

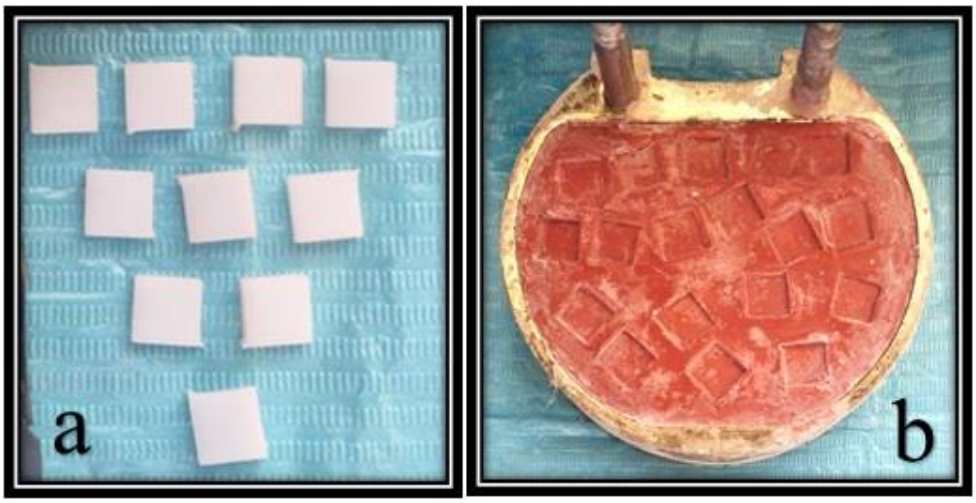


Figure (2-5): a) Plastic molds; b) Mold spaces

B) Heat cured acrylic based soft liner material mixing

Heat cured acrylic soft liner is available as powder and liquid; according to the manufacturer instructions mixing ratio by volume/parts by weight is 1mL liquid to 1.2 g of powder. A glass beaker was cleaned and dried well, powder and liquid were added and mixed for 30 seconds; the container was covered tightly and left for about 15 minutes to reach dough stage. The two halves of the flask were painted with separating medium and left to dry. As the dough time has ended the material was ready for packing.

C) Adding virgin coconut oil to soft liner material

Experimental samples were prepared by adding two different concentrations of VCO (1.5%, 2.5% by volume) to soft liner liquid. The volume of VCO was subtracted from the volume of soft liner liquid to obtain accurate P/L ratio (Table 2-5) **(S. Abed Karkosh *et al*., 2018)**. In dry clean glass beaker the required amount of VCO was added and completed with soft liner liquid; this mixture was mixed for 20 seconds using probe sonication apparatus at 120 W

and 60 KHz for complete homogeneity (Figure 2-6) **(Muttagi and Subramanya, 2017)**. Then, soft liner powder was added immediately to the previous mixture and mixed as previously mentioned.

Table (2-5): proportioning and mixing of soft liner samples

Sample	Soft liner Powder (g)	Soft liner Liquid (mL)	VCO (mL)
control	12 g	10 mL	0 mL
1.5% VCO	12 g	9.85mL	0.15 mL
2.5% VCO	12 g	9.75mL	0.25 mL

Figure (2-6): Probe sonication apparatus

D) Packing

After soft liner mixture reached dough stage, the dough was packed into mold spaces and a polyethylene sheet was placed over it; the two parts of the flask were placed over each other and placed under continuous pressure using the hydraulic press to guarantee an equal distribution of the material to all mold spaces; then, the pressure was gradually decreased and the flask was opened to expel all excess material using sharp knife. A layer of separating medium was

applied over the stone after the removal of polyethylene sheet and left to dry; the flasks parts were re-approximated under pressure until edge to edge contact was achieved and re-placed under the press (100Kg/cm^2) for five minutes. The flasks were removed from the press and clamped using the clamps and placed inside the water bath to be cured.

E) Curing, deflasking and finishing

According to the manufacturer instructions; the curing cycle was performed by heating the water up to 70 °C for 90 minutes; then the temperature was elevated up to 100 °C for 30 minutes. Digital thermostatic control water bath was used to complete the curing process (Figure 2-7a). After curing, the flasks were removed from the water bath and left to cool down gradually at room temperature for about thirty minutes followed by 15 minutes of cooling under tap water to insure complete cooling of flask metal.

The flasks were opened and the samples were extracted and finished with sharp blade to cut away all excess materials, then a fine grit polishing burs (Vertex) and sand papers were used to remove flashes all around samples (Figure 2-7b, c).

Samples to be used after 24 hours were stored in distilled water, while others to be used after 14 days and 30 days were stored in artificial saliva at 37 °C using incubator. (Figure 2-7d) **(Yasser, 2017).**

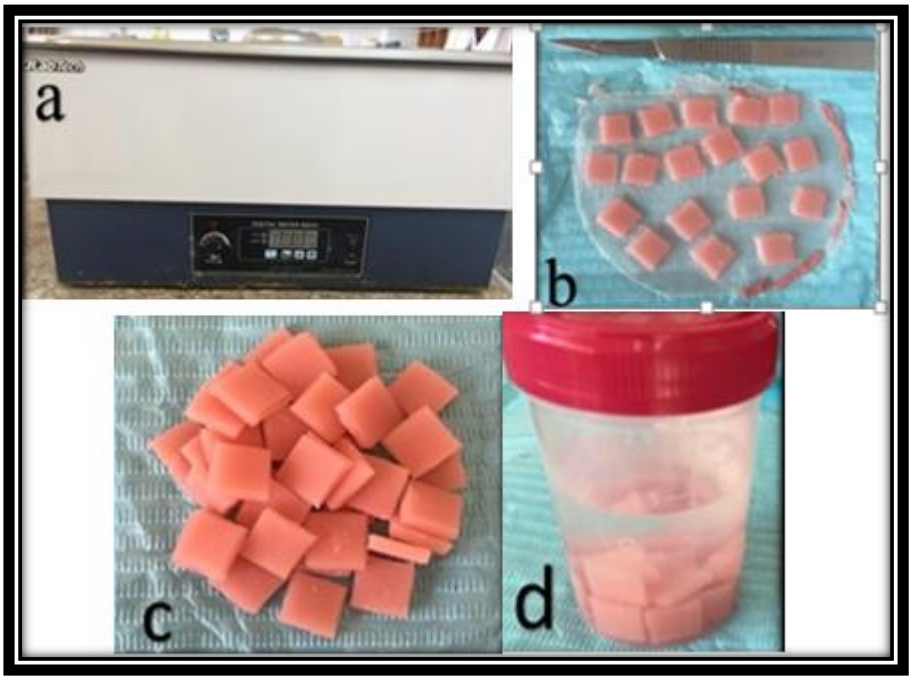


Figure (2-7): a) Digital water bath; b) Samples finishing using blade; c) Finished samples; d) Sample storage in artificial saliva

2.3.5.2. *Candida albicans* isolation

Candida albicans isolates were collected from the oral cavity of 15 patients attended Baghdad university/college of dentistry/prosthodontics clinics, all patients were medically fit denture wearers of 50-65 years old with an indication of denture stomatitis. Sterile cotton swab was used to collect the isolate by rubbing the palatal mucosa gently, swab was inoculated immediately in sabouraud dextrose agar (SDA) as a primary isolation media **(Manikandan and Amsath, 2013)**.

The cotton swabs were then cultured on sabouraud dextrose agar plates and incubated inside an incubator at 37 °C for 48 hours, for further investigations the cultured plates were preserved in refrigerator at 4 °C **(de Sousa *et al*., 2016**) (Figure 2-8).

Figure (2-8): some materials and instruments used in the microbiological aspect of the study

2.3.5.3. Sabouraud dextrose agar preparation

According to manufacturer instructions (Oxoid company), clean dry beaker was filled with 1000 ml of distilled water, 62 g of sabouraud dextrose agar is weighed using precise electronic balance and added to the distilled water and the beaker was sealed using a sterile cotton, culture media was then sterilized using an autoclave for 15 minutes at 121 °C/15 psi, and then media was left over the counter to cool down gradually up to 47 °C.

To prevent the growth of a wide range of gram-negative and gram-positive bacteria in the SDA media, 0.05 g of Chloramphenicol antibiotic was added for each 1000 ml **(Brooks *et al*., 2012)**.

After the media had cooled down, it was poured inside petridishes, covered and left until the agar got harden, then it was kept in refrigerator at 4 °C **(Issa and Abdul-Fattah, 2015)**.

2.3.5.4. *Candida albicans* identification

a) Macroscopic examination

Candida albicans colonies had a pearl-shape appearance which was pasty, creamy, smooth and slightly convex as a dome on SDA **(ElFeky *et al.*, 2016)**.

b) Microscopic examination

Wood stick was used to take small amount of inoculums from single isolated colony and emulsified in normal saline drop placed over glass slide to make spreaded *Candidal* suspension that left to dry at room temperature. Then the glass slide was passed above Bunsen burner flame many times for few seconds for fixing. After that the slide was stained using a gram stained kit as the following procedure; Crystal violet was used first to overflow the glass slide for 1 minute then washed with tap water, second, gram's iodine was placed over the slide for another 1 minutes and also washed with tap water, third, the slide was decolorized using acetone-alcohol by directing the glass slide at angle so all the solution was dissolved and the slide turned from blue to colorless, after that slide had placed under water for quick washing. Finally, safranin stain was used as counter stained for 1 minute then washed and left over filter paper to dry. Slide was then examined under light microscope (Figure 2-9a) **(Marler *et al.*, 2001)**.

c) Germ tube formation

Suspension was prepared from small amount of inoculums which obtained from single isolated colony, then added to 0.5 mL of serum. The suspension was transferred to the incubator and kept at 37 °C for 2 hours. By using

disposable pipet, a small amount of suspension was spreaded over the slide and covered to be examined under low power magnification using light microscope (Figure 2-9a, b) **(Brooks *et al.*, 2012)**.

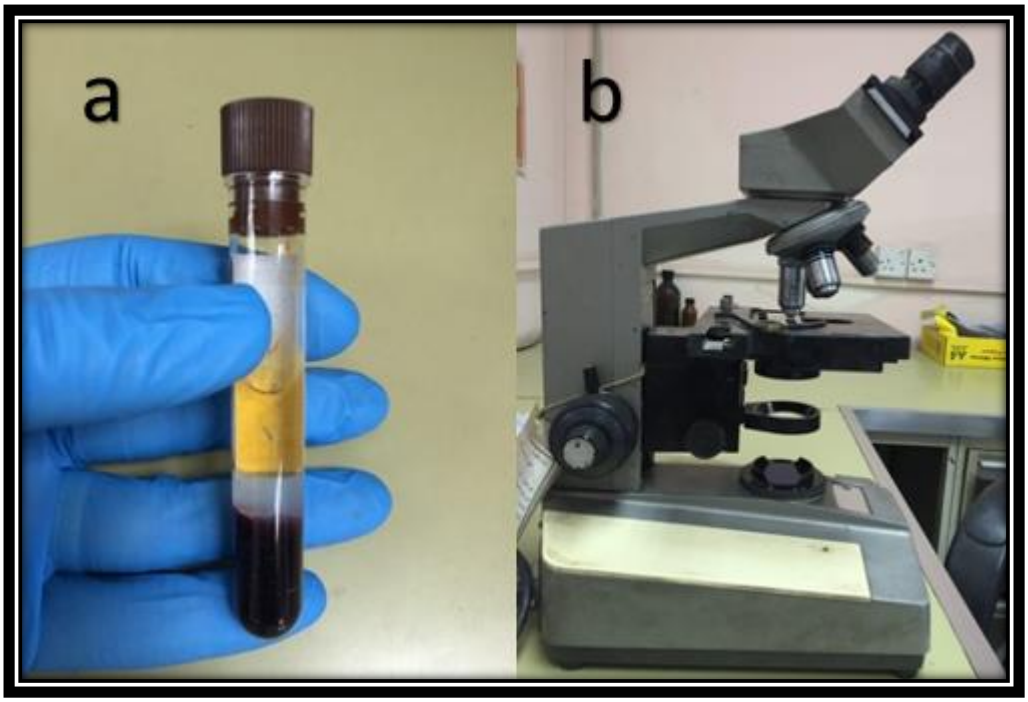


Figure (2-9): a) Light microscope, b) Blood serum

d) Biochemical identification

For more accurate determination of fungal isolates and to determine the exact species of fungi; Vitek-2 compact system with its Vitek-2 cards were used to confirm that it is *Candida albicans* (the test was conducted in a private lab). Vitek-2 system works by comparing the biochemical profile using a huge database (Figure 2-10a) **(Cejudo *et al*., 2010)**.

A small amount of *Candida* inoculums was diluted with normal saline in a test tube, the solution was measured with McFarland densitometer until 2.0 McFarland standards was obtained, Vitek-2 cards were filled automatically, closed and incubated using special cards with *Candida* suspension (Figure 2-10b). Cards were incubated inside the device at 37 °C for 18 h and the optical

density reading was repeated automatically every fifteen minutes; according to the reading obtained by the device the biochemical profile was determined and explained using a special algorithm **(Kaur *et al*., 2016)**.

Figure (2-10): a) Vitek-2 compact system; b) Vitek yeast cards

2.3.5.5. Sabouraud dextrose broth preparation

As manufacturer (Oxoid Company) directed; using electronic balance, 30 g of sabouraud dextrose broth was weighed and added to 1000 mL of distilled water in a glass beaker, broth was sterilized in autoclave at 121 °C/15 psi for fifteen minutes and left over the counter to gradually cool down up to 47 °C.

For bacterial growth prevention, 0.05 g Chloramphenicol antibiotic was added for each 1000 mL of culture media **(Brooks *et al*., 2012)**.

2.3.5.6. Assessment of VCO loaded samples effect on *Candida albicans* viable counts

To assess the antifungal effectiveness of VCO loaded samples, viable count test was done by making a *Candida albicans* suspension of about 10^7CFU/mL which equal to 0.5 McFarland standards. This suspension was prepared by

diluting a small amount of inoculums in a test tube containing normal saline and measuring this solution with McFarland densitometer device. This process was repeated many times either by adding more normal saline for more dilution or by adding an additional amount of inoculum to increase solution density until a 0.5 MaCfarland was obtained (Figure 2-11a). Then, 0.1% of this suspension was taken using micropipette and added to a test tube containing 0.9% sabouraud dextrose broth, after that, the sample was immersed in this tube and incubated at 37 °C for 24 hours (Figure 2-11b). 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 (Figure 2-11c). 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 (Figure 2-11d). Third dilution was chosen because it demonstrated a countable range of 30-300 CFU **(Sutton, 2011).**

All viable counts presented over SDA surface were counted by the naked eye and a statistical analysis was performed. Antifungal efficiency (AFE) was calculated using following formula:

$$AFE\ [\%] = \frac{Vc - Vt}{Vc} \times 100\%$$

In which number of viable colonies of control samples was represented by Vc and number of viable colonies of experimental samples was represented by Vt **(Chladek *et al*., 2011)**.

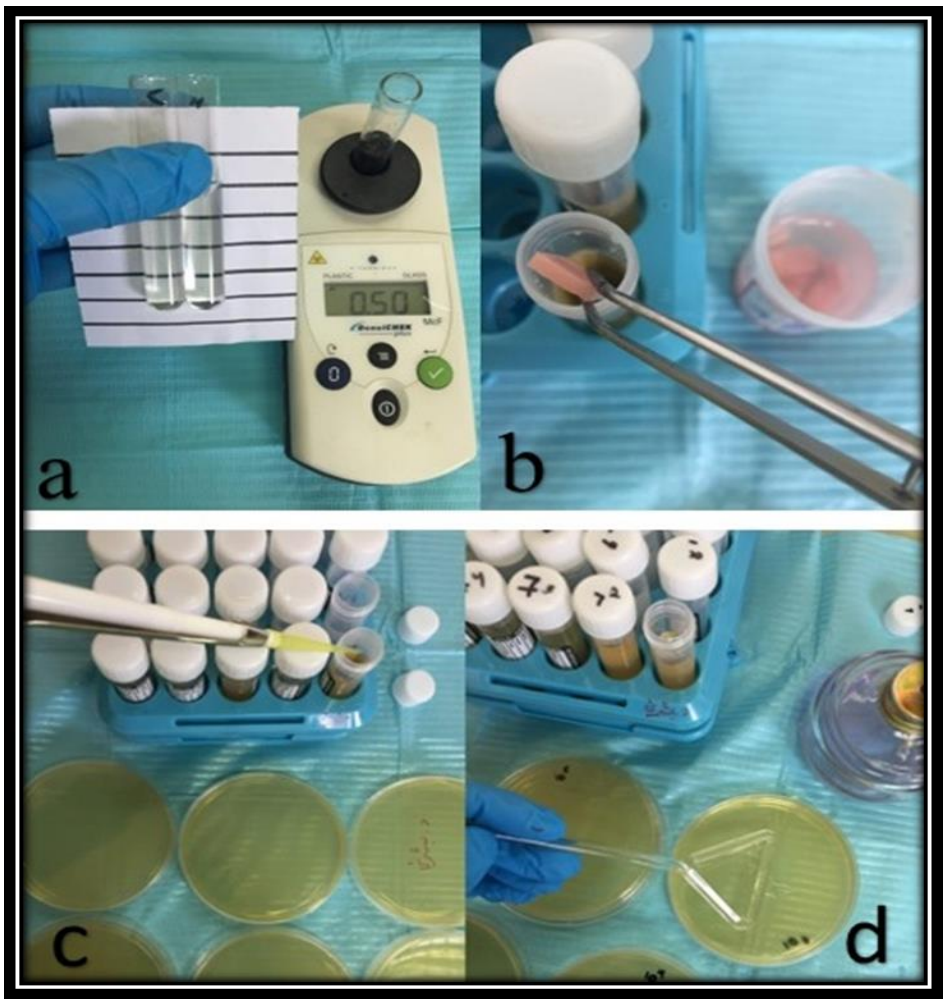


Figure (2-11): a) Preparation of *Candida* suspension equal to 0.5 McFarland standard using McFarland densitometer ; b) Sample placement inside test tube; c) Serial dilution; d) Spreading 0.1% of solution over SDA surface.

2.3.6. Shore A hardness test

A disk-shape plastic molds measuring 30mm in diameter and 3mm thickness **(Abraham and Abdul-Fattah, 2017)** were used to create mold spaces inside the dental stone. The soft liner control and experimental samples were prepared in the same manner as previously mentioned in (2.3.5.1)(Figure 2-12a); 90 samples were prepared. Samples to be used after 24 hours were stored in

distilled water while others to be used after 14 days and 30 days were stored in artificial saliva at 37 °C.

Hardness of soft liner samples was measured using Shore A durometer (Figure 2-12b), readings were obtained from different five points that were pointed on each sample (one point on the center of the sample and the other 4 points were marked 6mm away from the center **(Tukmachi and Moudhaffer, 2017)** and the average of these readings was taken automatically from the durometer. Distance between the indenter and samples was 20 mm and the penetration time was 5 seconds **(Issa and Abdul-Fattah, 2015)**.

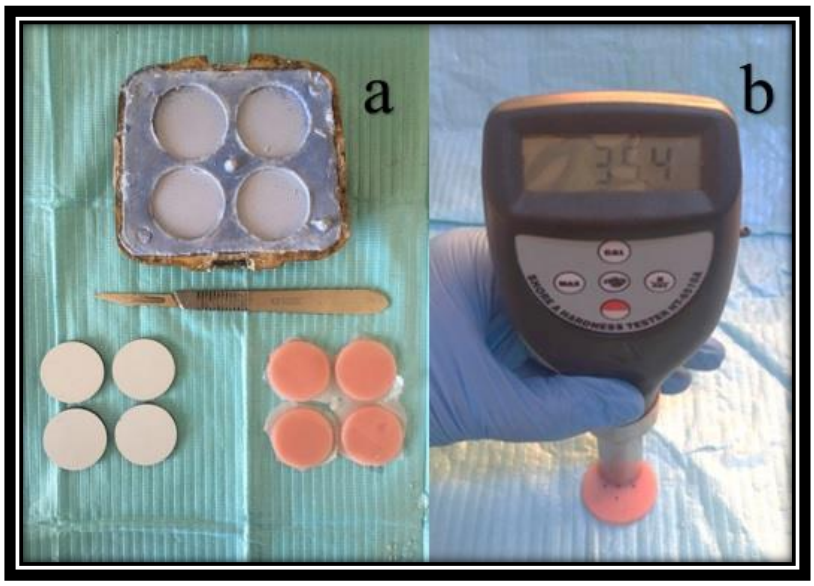


Figure (2-12): a) Plastic molds and soft liner samples for hardness test; b) Shore A durometer and sample testing

2.3.7. Shear bond strength test

2.3.7.1. Sample design

In order to evaluate the bond between the acrylic denture base and soft lining material; 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 were 5 mm depth, 25 mm width and 75 mm length with 3 mm depth stopper and 13 mm handle thickness **(Yasser, 2017)** (Figure 2-13).

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 of 3 mm depth, 25 mm length and 25 mm width **(Abdulwahhab and Jassim, 2018)**.

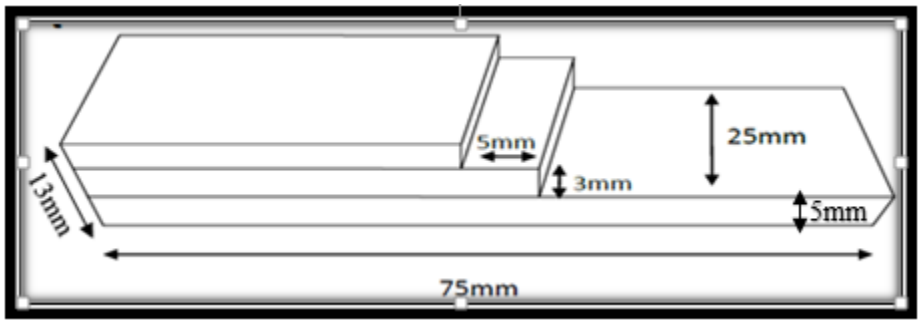


Figure (2-13): Dimension of shear bond acrylic block used for shear bond strength test.

2.3.7.2. Heat cured acrylic blocks fabrication

A) Mold fabrication

A plastic block measuring 75 mm length, 25 mm width, 5 mm depth and 3 mm stopper depth was constructed. This plastic block was invested completely in addition silicone impression material and left until silicone had set, the plastic block with the surrounding silicone were submerged into the lower part of a metal flask containing freshly mixed extra hard dental stone and left until stone had set, after that, separating medium was applied all over the plastic and silicone surfaces and stone and the upper part of the flask was placed over the lower one, filled with dental stone and covered. After stone set, the flask was opened and the plastic mold was extracted lifting a space for the heat cured acrylic material to be placed **(Issa and Abdul-Fattah, 2015)** (Figure 2-14).

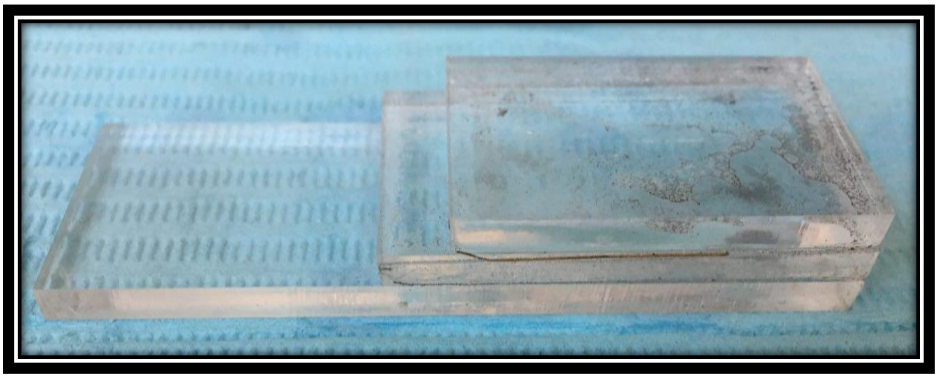


Figure (2-14): plastic pattern of shear bond test block

B) heat cured acrylic proportioning and mixing

In a clean dry beaker, 2.2 g of heat cured acrylic resin powder were mixed with 1 mL of liquid according to manufacturer's directions. The beaker was sealed to prevent monomer evaporation and left to reach to the dough stage so it can be packed.

C) Packing

After acrylic resin material achieved dough stage, the material was packed inside the mold space and a layer of polyethylene sheet was placed, upper and lower parts of the flasks were re-approximated until edge to edge contact was achieved and placed under pressure using the hydraulic press to insure equal distribution of the material. After that, the pressure was gradually decreased, the flask was opened, polyethylene sheet was removed and all excess material were cut using sharp blade. Layer of separating medium was applied over stone surface, and the flask was reclosed under pressure and left for five minutes under 100 kg/cm^2. For curing, the clamped flask was then placed inside the water bath.

D) Curing, deflasking, finishing and polishing

Using digital water bath with a thermostatic control, curing process was performed. As directed by manufacturers, the flasks were heated for 30 minutes at 70 °C, then the temperature was elevated up to 100 °C for another 30 minutes. After that, the flasks were taken out and left to cool gradually for 30 minutes at room temperature followed by 15 minutes tap water cooling, after complete cooling, the flasks were opened and acrylic samples were extracted.

Acrylic blocks were finished to remove all excess materials all around the sample using acrylic bur, stone bur, and sand paper respectively. Polishing was done for all samples faces except the one that will bond with soft liner material; rag wheel followed by lathe polishing machine with pumice were used to polish the samples (Figure 2-15). Acrylic blocks were stored at 37 °C in distilled water for 48 hours before being used as directed by ADA specification No.12 (1999).

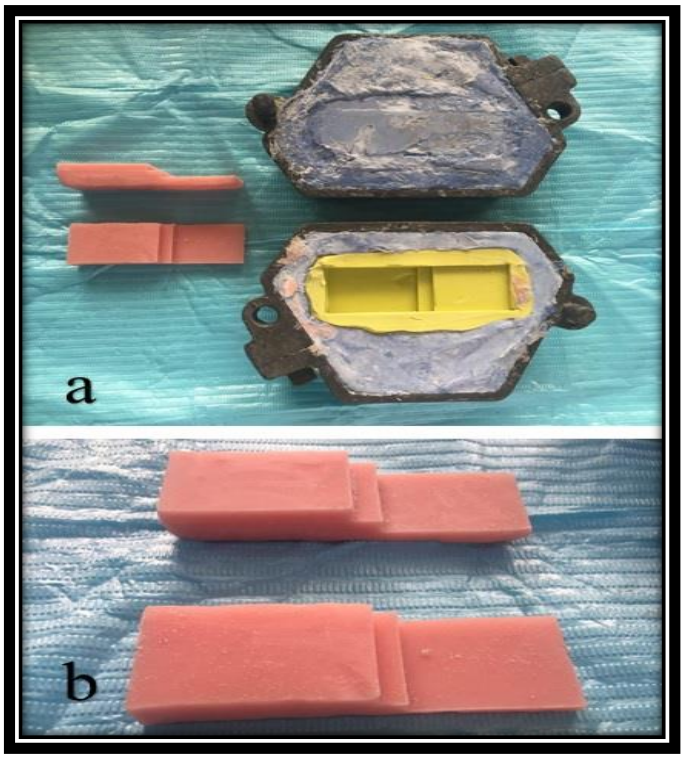


Figure (2-15): a) Deflasking of acrylic shear block; b) Finished shear test blocks.

2.3.7.3. Final sample fabrication

a) Mold preparation

Two acrylic blocks were placed facing each other for every single shear bond strength test sample, the space remained between them had dimension of 3 mm depth, 25 mm length and 25 mm width and was filled with wax in order to fix the two blocks together. Fixed sample was completely immersed in addition silicone material and left until complete setting of silicone was achieved. Custom-made flask was filled with freshly mixed hard dental stone and the silicone with the immersed samples were invested inside the stone and covered with a petroleum gel coated flask cover. De-waxing procedure was carried out and all wax remnants were cleaned using running hot water and soaps, then the flask and samples were left for complete drying **(Issa and Abdul-Fattah, 2015)** (Figure 2-16).

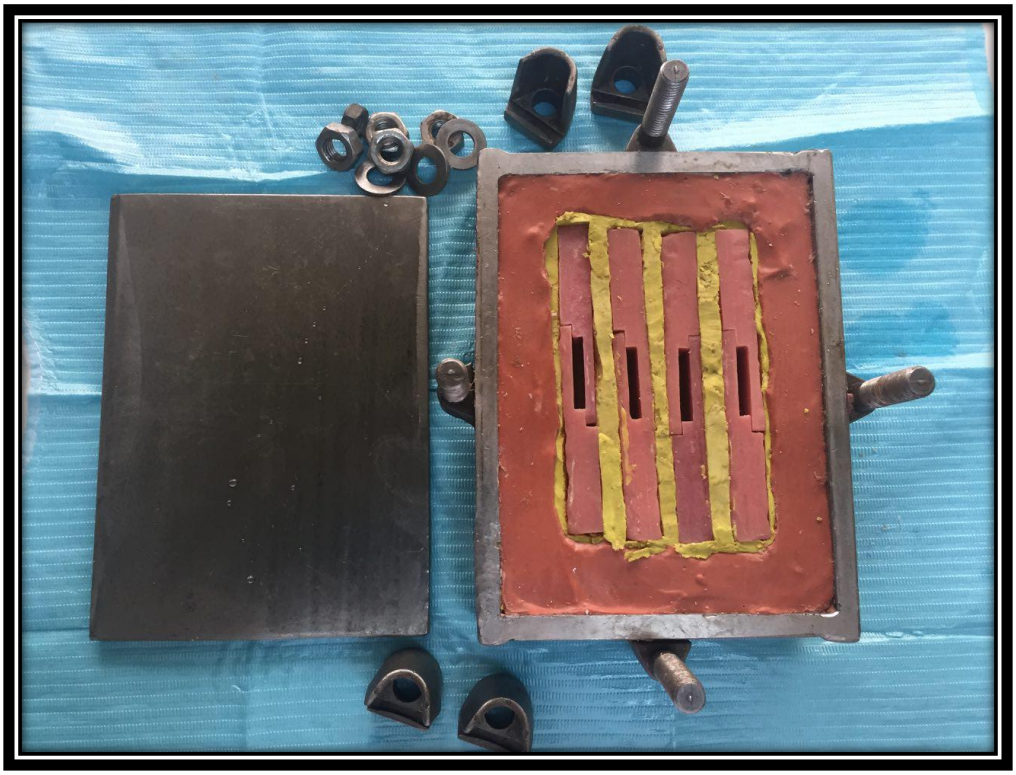


Figure (2-16): custom made flask for preparation of the final shape of shear strength test sample

b) Soft lining material placement

Soft lining material was prepared for control and experimental samples as previously explained in (2.3.5.1). Soft lining material was incrementally inserted and condensed in the space to prevent air bubbles formation within the material, the space was over filled, the flask was covered under pressure (1 Kg) and tightly screwed until edge to edge contact was achieved. The flask was then placed in digital water bath to be cured as previously mentioned in (2.3.5.1). All samples were finished by cutting excess soft lining material using sharp blade; 90 samples were prepared, 30 for each group. Samples to be used after 24 hours were stored in distilled water while others to be used after 14 days and 30 days were stored in artificial saliva at 37 °C **(Abraham and Abdul-Fattah, 2017)** (Figure 2-17).

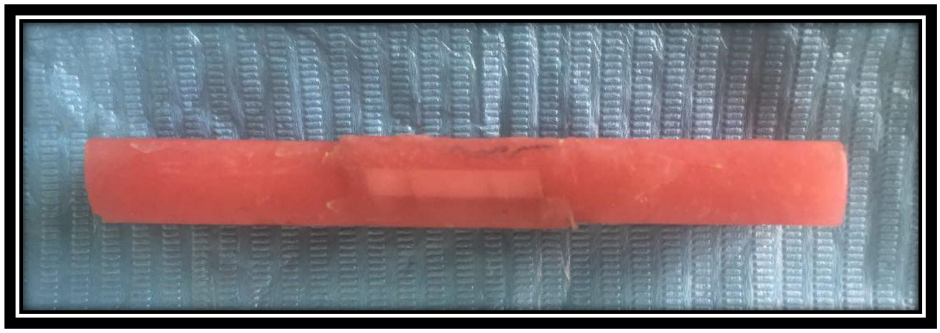


Figure (2-17): Final shape of shear bond sample.

2.3.7.4. Shear bond strength test

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 represent 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) (Figure 2-18).

$$\text{Bond strength (N/mm}^2\text{)} = \frac{Maximum\ load}{cross\ sectional\ area} = \frac{F}{A}$$

Figure (2-18): a) Instron machine; b) Testing of shear bond strength of soft lining material.

2.3.8. Wettability test:

Ninety samples were prepared (30 from each group) to evaluate the wettability of the experimental soft lining samples and compare it with that of control samples. The static sessile drop method was used in this study, a side view of a liquid drop on a solid substrate placed on horizontal flat base was captured and analyzed using optical subsystem **(Zgura *et al*., 2010)**.

The contact angle which is the angle developed between the liquid (distilled water), solid (sample surface) and air was captured using Dino-lite digital microscope that take a magnified picture (45x magnification) of the sample profile with a drop of distilled water over its surface. In order to standardize the drop size, a micropipette was used to give 40 µL distilled water drop and held vertically over the surface of a horizontally placed soft liner sample (Figure 2-19). Dino-lite digital microscope was placed parallel to the sample surface and a magnified picture was captured and analyzed using the special software of the

microscope (Dino-Capture). This software automatically drew a tangent; the angle formed between the baseline of the distilled water drop and the tangent located at the three-phase-lines air/solid/liquid was measured to give the contact angle value. As the contact angle increased the wettability decreased and vies versa **(Al-Shaikhli and Khamas, 2012; Al-Azawi and Al-Nakkash, 2016)**.

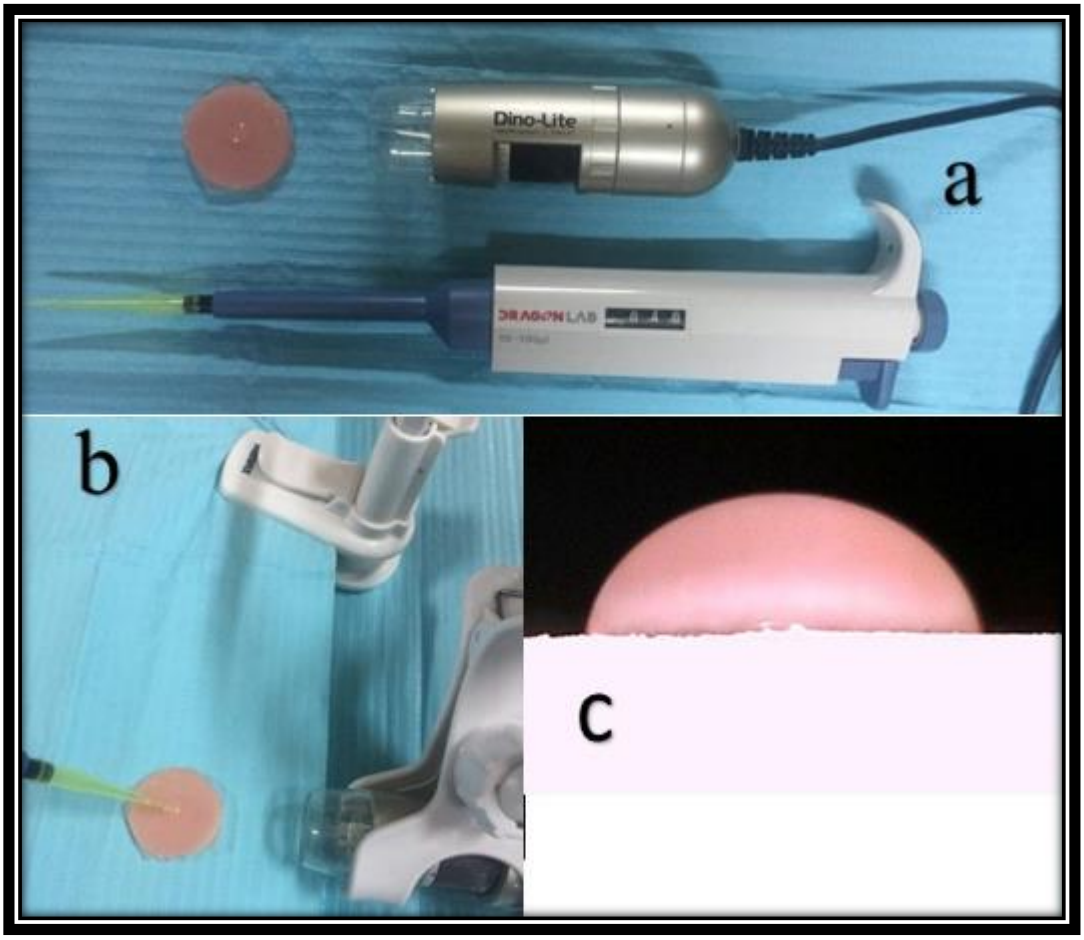


Figure (2-19): a) Dino-lite digital microscope, micropipette; b) Microscope placed parallel to the sample; c) Magnified picture of distilled water drop.

2.3.9 Statistical analysis:

The results of this research were analyzed using SPSS (statistical package for social science – version 24) computer software.

The following statistical methods were employed:

1- Descriptive statistics which include:
   - Means.
   - Standard deviation.
   - Graphical presentation by bar-chart.

2- Inferential statistics:

   Two way ANOVA (analysis of variance) was used to compare means among all groups.

   Tukey's multiple comparisons test was used to show the significance among different groups.

   A "P" value of >0.05 was considered as statically non-significant, ≤ 0.05

   Was considered as significant and < 0.01 was considered as highly significant.

Chapter Three:

Results

In this study, the following tests and investigations were performed. The results will be presented in this chapter:

1- FTIR spectroscopy
2- Identification of *Candida albicans*
3- Evaluating viable counts of *Candid albicans*
4- Shore A hardness test
5- Shear bond strength test
6- Wettability test

3.1. FTIR (Fourier Transform Infrared) Spectroscopy

The results of Fourier Transform Infrared Spectroscopy analysis demonstrate no difference in the spectra between control sample and experimental sample since the pattern and the alignment of the absorption peaks didn't show any change. This indicates that there is no chemical interaction between soft lining material and virgin coconut oil (Figure 3-1). The slight change seen in the pattern of the spectra range of 3200 to 3600 cm^{-1} is related only to the moisture content; i.e. the amount of water absorbed by the sample.

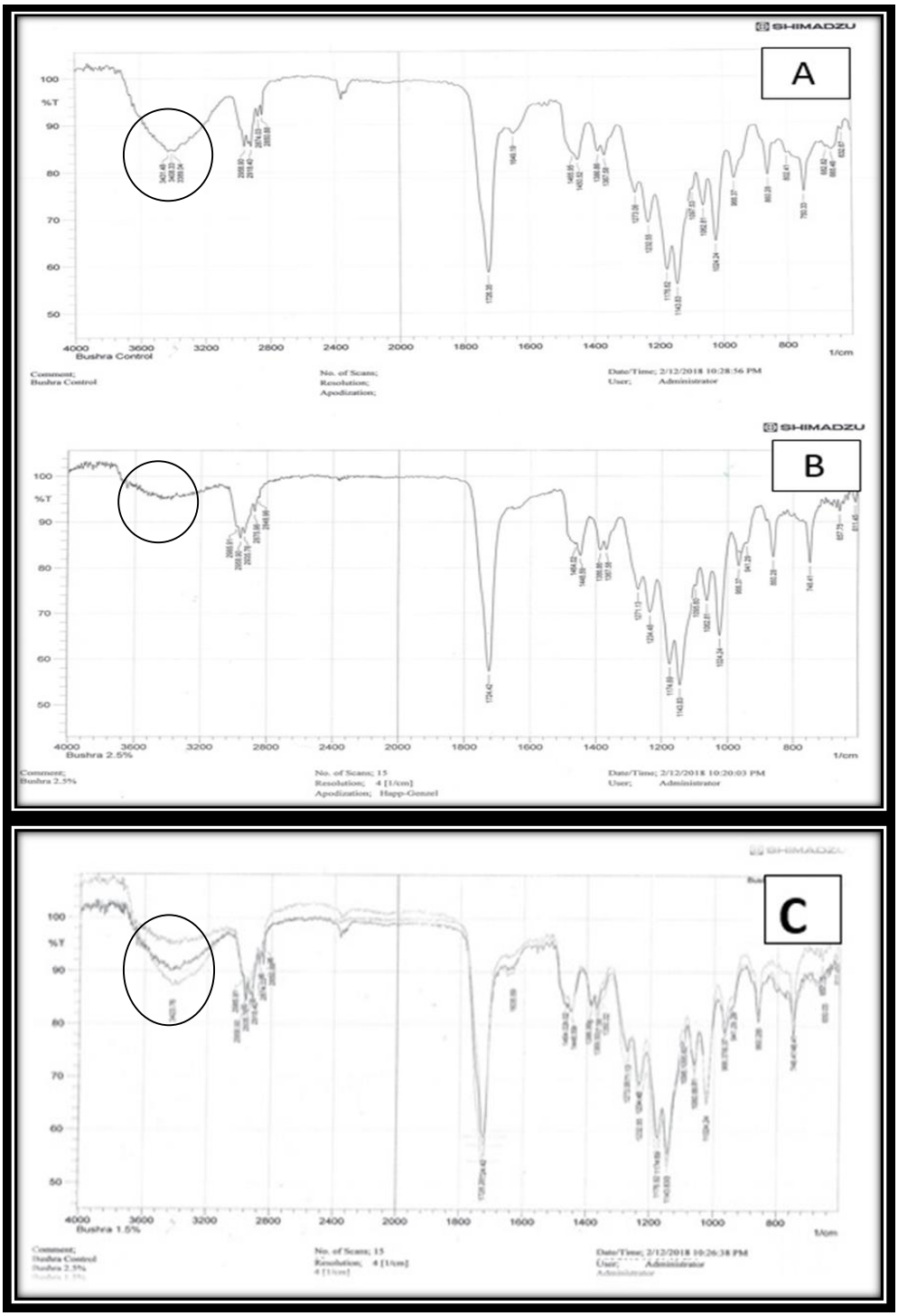


Figure (3-1): A) FTIR analysis of control sample; B) FTIR analysis of experimental sample; C) FTIR analysis of control and experimental sample

3.2. Identification of *candida albicans*

a) Colony morphology:

Candida albicans colonies had a pearl-shape, pasty and creamy appearance on SDA (Figure 3-2).

Figure (3-2): *Candida albicans* colonies on sabouraud dextrose agar.

b) Microscopic examination:

Under light microscopic examination, *candida albicans* appear as a small gram positive oval or budding cells as seen in Figure 3-3.

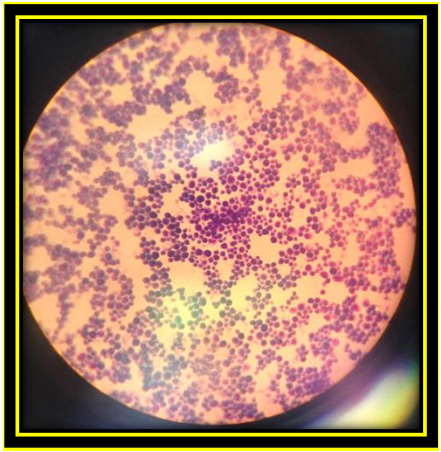


Figure (3-3): *Candida albicans* showed as gram positive cells under light microscope

c) Germ tube formation:

Germ tube formation appears as an outgrowth extending tube like structure, as shown in Figure 3-4.

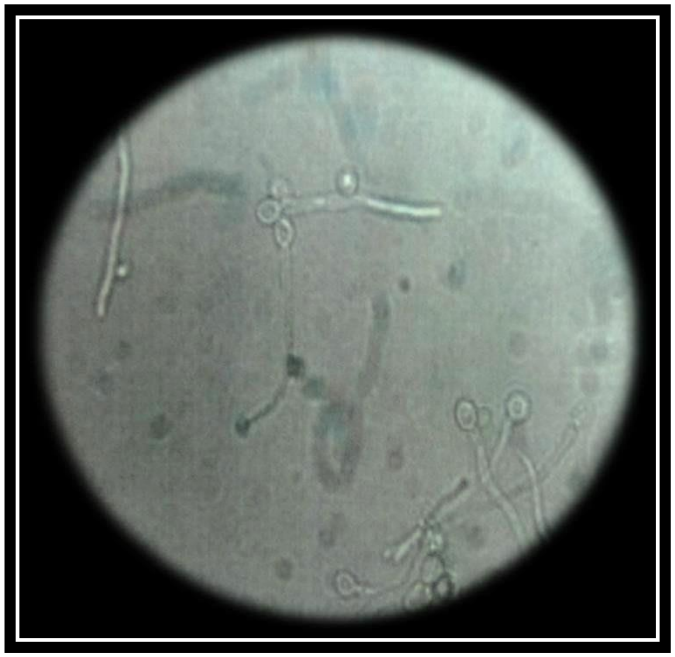
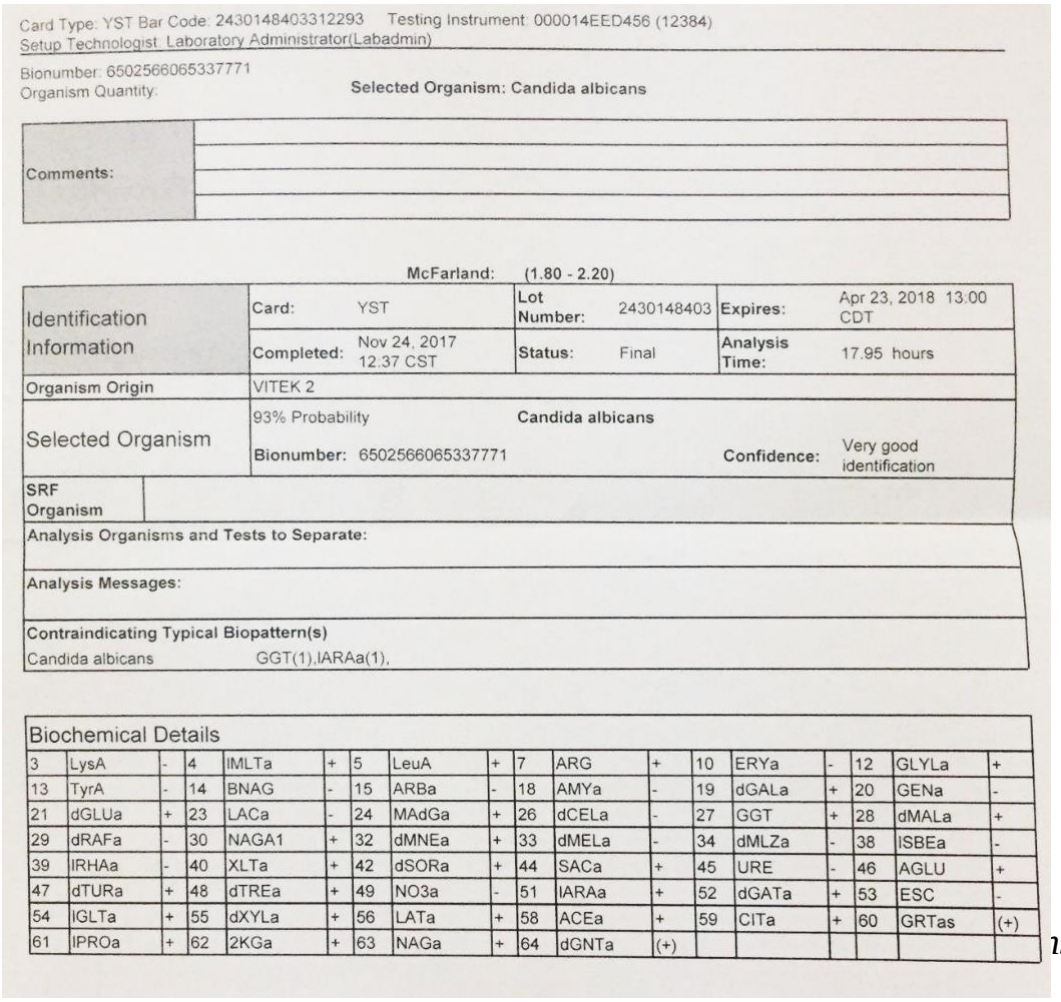


Figure (3-4): Germ tube formation under light microscope

d) Biochemical identification:

The biochemical profile of the *Candida* isolates using Vitek-2 YST system based on the detection of different enzymes in each yeast species. The resultant colors at the end of the incubation period were coded and compared with the differential Chart to identify the species. The end result revealed the probability of 93% *candida albicans* which was a very good identification (Figure 3-5).



Card Type: YST Bar Code: 2430148403312293 Testing Instrument: 000014EED456 (12384)
Setup Technologist: Laboratory Administrator(Labadmin)

Bionumber: 6502566065337771
Organism Quantity:

Selected Organism: Candida albicans

Comments:	

McFarland: (1.80 - 2.20)

Identification Information	Card:	YST	Lot Number: 2430148403	Expires:	Apr 23, 2018 13:00 CDT
	Completed:	Nov 24, 2017 12:37 CST	Status: Final	Analysis Time:	17.95 hours
Organism Origin	VITEK 2				
Selected Organism	93% Probability		Candida albicans		
	Bionumber: 6502566065337771			Confidence:	Very good identification
SRF Organism					
Analysis Organisms and Tests to Separate:					
Analysis Messages:					
Contraindicating Typical Biopattern(s)					
Candida albicans	GGT(1),IARAa(1),				

Biochemical Details

3	LysA	-	4	IMLTa	+	5	LeuA	+	7	ARG	+	10	ERYa	-	12	GLYLa	+
13	TyrA	-	14	BNAG	-	15	ARBa	-	18	AMYa	-	19	dGALa	+	20	GENa	-
21	dGLUa	+	23	LACa	-	24	MAdGa	+	26	dCELa	-	27	GGT	+	28	dMALa	+
29	dRAFa	-	30	NAGA1	+	32	dMNEa	+	33	dMELa	-	34	dMLZa	-	38	ISBEa	-
39	IRHAa	-	40	XLTa	+	42	dSORa	+	44	SACa	+	45	URE	-	46	AGLU	+
47	dTURa	+	48	dTREa	+	49	NO3a	-	51	IARAa	+	52	dGATa	+	53	ESC	-
54	IGLTa	+	55	dXYLa	+	56	LATa	+	58	ACEa	+	59	CITa	+	60	GRTas	(+)
61	IPROa	+	62	2KGa	+	63	NAGa	+	64	dGNTa	(+)						

ns.

3.3. 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* for 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 3-6, Figure 3-7 and Table 3-1). AFE for the experimental groups in the first incubation period was 81.1% and 60.3%, ordinarily.

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 the mean values of the control group, also the lowest values were noticed in 1.5% coconut oil group of 33.1 CFU/mL and 35.2 CFU/mL, respectively, while the mean values of the control group were 156.5 CFU/mL and 160 CFU/mL ordinarily, as shown in Figure 3-6. 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.

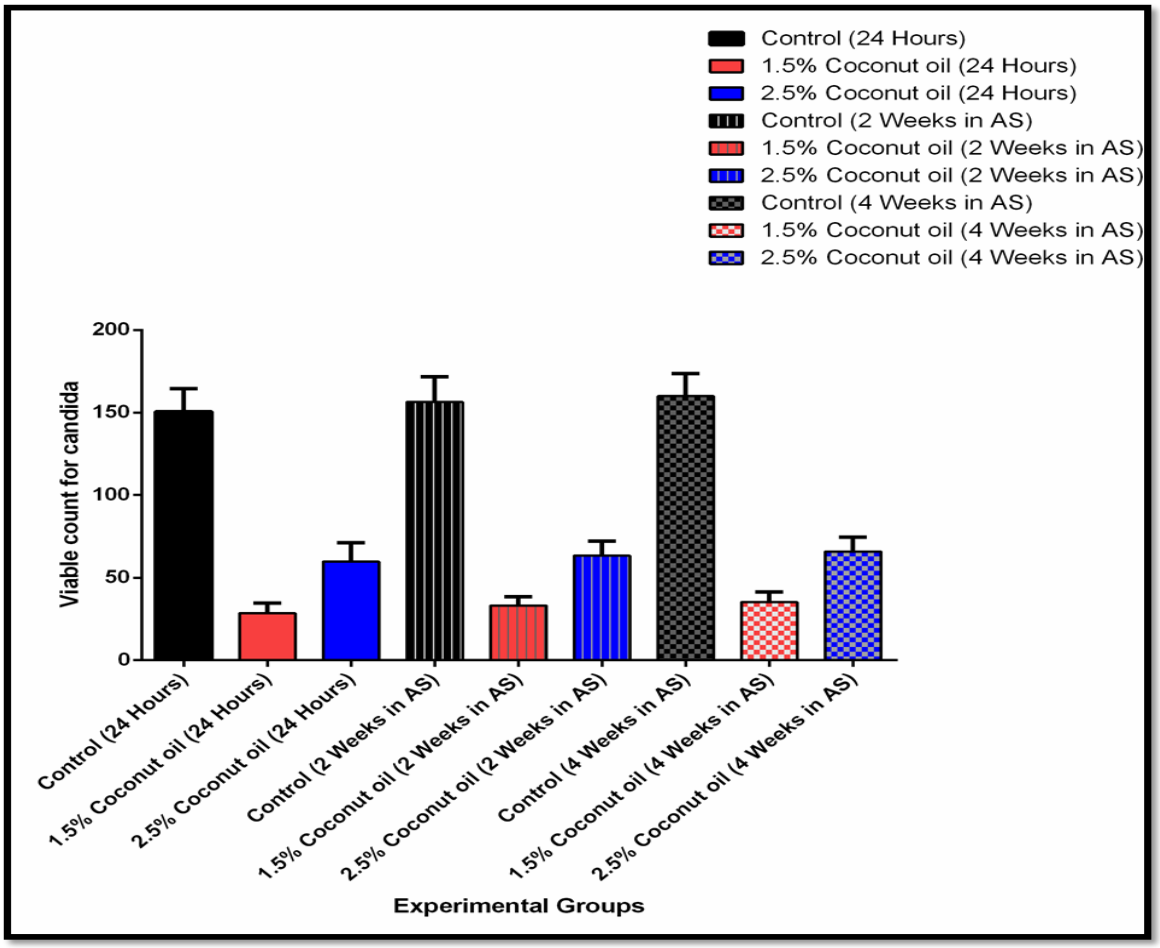


Figure (3-6): Bar chart showing mean values and standard deviation of viable counts of *Candida albicans* for control and experimental groups at different periods of incubation

The descriptive statistics of viable counts for control and experimental groups in different periods of incubation are listed in Table 3-1.

Table (3-1): Descriptive statistics of viable counts of *Candida albicans*

Incubation period	Group	N	Mean (CFU/mL)	S.D.	Min.	Max.
After 24 hours of incubation	Control	10	150.5	14.02	132	176
	1.5% of coconut	10	28.4	6.204	19	39
	2.5% of coconut	10	59.8	11.26	45	76
After 2 weeks of incubation	Control	10	156.5	15.13	135	178
	1.5% of coconut	10	33.1	5.363	25	41
	2.5% of coconut	10	63.3	8.845	51	75
After 4 weeks of incubation	Control	10	160	13.74	139	179
	1.5% of coconut	10	35.2	6.25	25	44
	2.5% of coconut	10	65.9	8.621	55	79

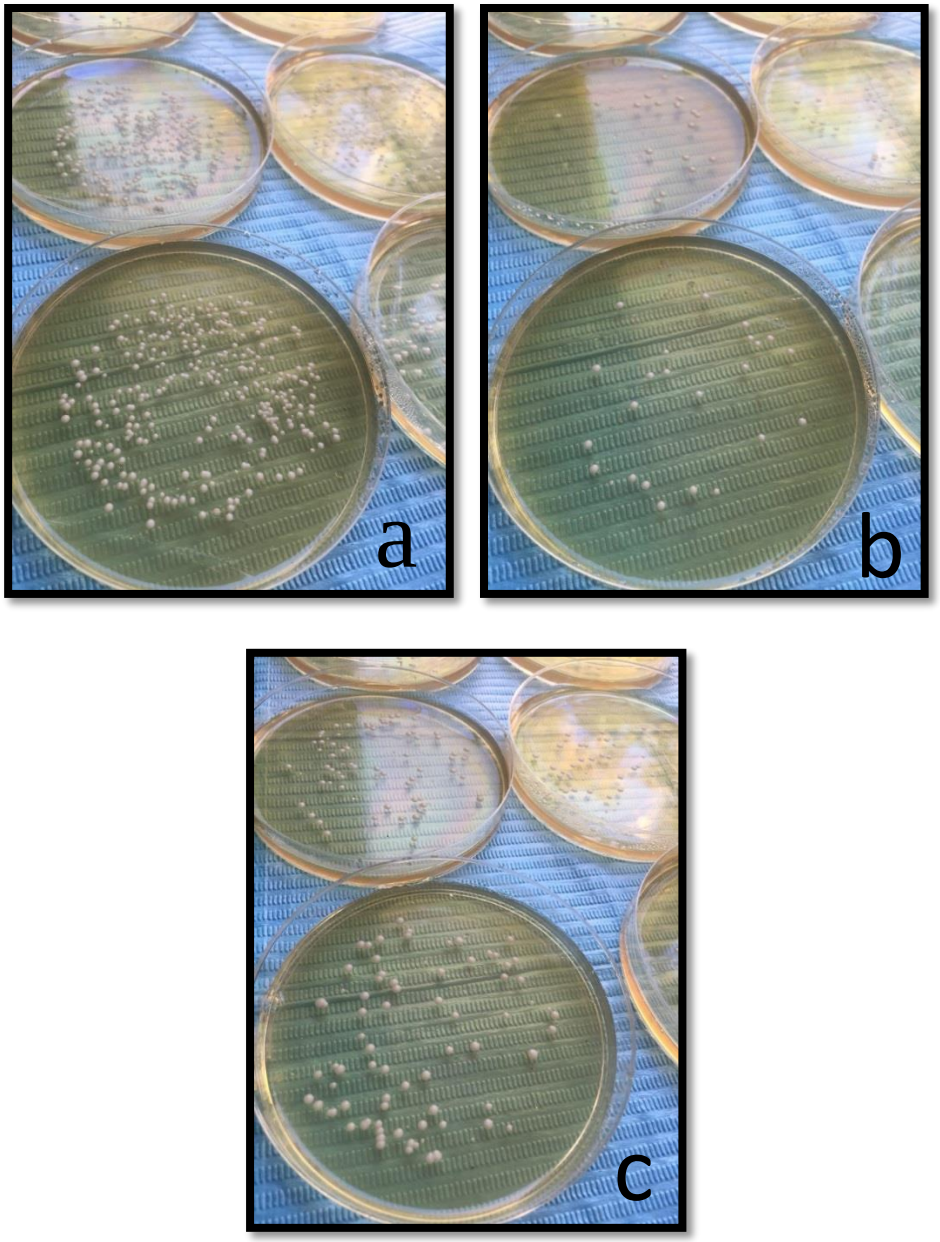


Figure (3-7): Viable counts of *Candida albicans* after 24 hours incubation of: a) Control samples; b) Experimental samples (1.5% coconut oil); c) Experimental samples (2.5% coconut oil)

In Table 3-2, 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 ($p=0.0252$). A non-significant interaction was seen between concentrations and incubation periods ($p=0.9884$).

Table (3-2): Comparison of average values of viable counts test using two way ANOVA

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Concentration	247694.867	2	123847.433	1114.565	0.0000	.965
Incubation period	856.267	2	428.133	3.853	0.0252	.087
Concentration * Incubation period	35.267	4	8.817	.079	0.9884	.004

Tukey's multiple comparisons test was used to compare mean values of different groups. In the 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 to 1.5% group 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 from 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 1.5% (4 weeks), 2.5% samples also showed a non-significant difference 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 3-3.

Table (3-3): 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 of incubation	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
	1.5%	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
	2.5%	2.5% (2 weeks)	-3.5	0.9982	NS
		2.5% (4 weeks)	-6.1	0.9372	NS
After 2 weeks of incubation	Control	1.5% (2 weeks)	123.4	< 0.0001	HS
		2.5% (2 weeks)	93.2	< 0.0001	HS
		Control (4 weeks)	-3.5	0.9982	NS
	1.5%	2.5% (2 weeks)	-30.2	< 0.0001	HS
		1.5% (4 weeks)	-2.1	> 0.9999	NS
	2.5%	2.5% (4 weeks)	-2.6	0.9998	NS
After 4 weeks of incubation	Control	1.5% (4 weeks)	124.8	< 0.0001	HS
		2.5% (4 weeks)	94.1	< 0.0001	HS
	1.5%	2.5% (4 weeks)	-30.7	< 0.0001	HS

3.4. Shore A hardness test

Results of shore A hardness test after 24 hours of incubation in distilled water showed that both experimental groups (1.5% and 2.5% coconut oil) had a lower mean values than control group, the experimental group with 1.5% of coconut oil showed the lowest value of 39.63 in this period as shown in (Figure 3-8, Table 3-4).

At the second and third periods of evaluation (2 and 4 weeks incubation in artificial saliva), mean values of experimental groups (1.5% and 2.5% coconut oil) were less than mean value of control group, also the lowest values were noticed in 1.5% coconut oil group (45.41 and 42.99) respectively, while mean values of control group were 56.01 and 53.57, respectively as shown in (Figure 3-8, Table 3-4).

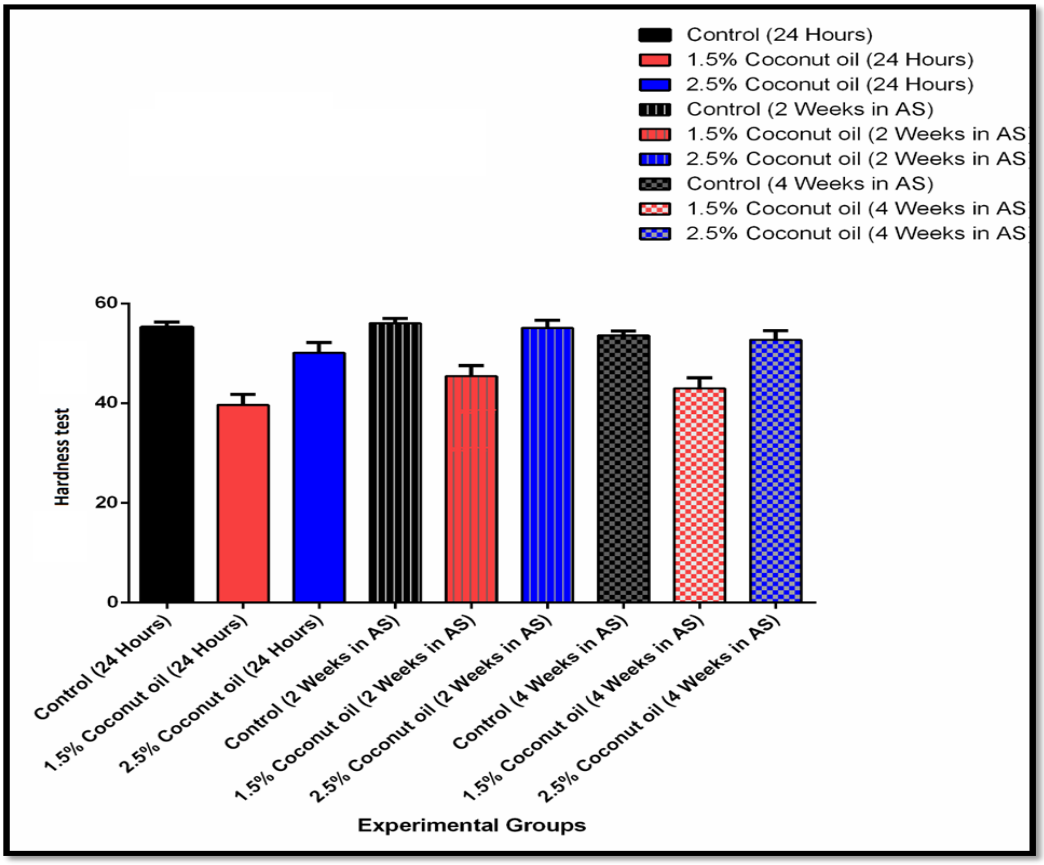


Figure (3-8): Bar chart showing mean values and standard deviation of shore A hardness for control and experimental groups at different periods of incubation

The descriptive statistics of shore A hardness test for control and experimental groups at different periods of incubation are listed in Table 3-4.

Table (3-4): Descriptive statistics of shore A hardness test

Incubation period	Group	N	Mean (shore A unit)	S.D.	Min.	Max.
After 24 hours of incubation	Control	10	55.24	1.01	53.6	56.8
	1.5% of coconut	10	39.63	2.167	36.1	43.4
	2.5% of coconut	10	50.13	2.034	47.2	53.7
After 2 weeks of incubation	Control	10	56.01	0.9814	54.5	57.2
	1.5% of coconut	10	45.41	2.168	41.9	48.1
	2.5% of coconut	10	55.11	1.501	53	57.6
After 4 weeks of incubation	Control	10	53.57	0.9068	52.4	55.1
	1.5% of coconut	10	42.99	2.079	39.6	46.2
	2.5% of coconut	10	52.74	1.776	50.6	56.3

Two way ANOVA (Table 3-5) indicated a highly significant difference among concentrations of coconut oil addition ($p< 0.01$), and among incubation periods ($p< 0.01$). A highly significant interaction was seen between concentrations and incubation periods ($p< 0.01$).

Table (3-5): Comparison of average values of hardness test using two way ANOVA

	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Concentration	2552.547	2	1276.273	440.606	.0000	.916
Incubation period	226.338	2	113.169	39.069	.0000	.491
Concentration * Incubation period	97.393	4	24.348	8.406	.0000	.293

Tukey's multiple comparisons test was used to compare mean values of different groups. In the first incubation period control group showed a highly significant difference compared to both experimental groups in the same incubation period, while it was non-significant difference compared to both control groups in different incubation periods, 1.5% samples showed a highly significant difference with 2.5% samples in the same incubation period, and it was a highly significant difference also when compared to 1.5% samples in 2 and 4 weeks incubation period. Regarding 2.5% samples, the difference was highly significant with 2.5% samples after 2 weeks incubation period while it was significant when compared with the same concentration after the third incubation period.

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

showed a non-significant difference with the same concentration in the third incubation period.

The third control group in the 4 weeks incubation period showed a highly significant difference when compared to 1.5% (4 weeks), while the difference was non-significant with 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 periodic group, as shown in (Table 3-6).

Table (3-6): Tukey's multiple comparisons test of different groups for shore A hardness test results

Period	Groups		Mean difference	P value	Sig.
After 24 Hours of incubation	Control	1.5% (24 hours)	15.61	< 0.0001	HS
		2.5% (24 hours)	5.11	< 0.0001	HS
		Control (2 weeks)	-0.77	0.985	NS
		Control (4 weeks)	1.67	0.4402	NS
	1.5%	2.5 % (24 hours)	-10.5	< 0.0001	HS
		1.5% (2 weeks)	-5.78	< 0.0001	HS
		1.5% (4 weeks)	-3.36	0.0014	HS
	2.5%	2.5% (2 weeks)	-4.98	< 0.0001	HS
		2.5% (4 weeks)	-2.61	0.0303	S
After 2 weeks of incubation	Control	1.5% (2 weeks)	10.6	< 0.0001	HS
		2.5% (2 weeks)	0.9	0.961	NS
		Control (4 weeks)	2.44	0.0552	NS
	1.5%	2.5% (2 weeks)	-9.7	< 0.0001	HS
		1.5% (4 weeks)	2.42	0.0591	NS
	2.5%	2.5% (4 weeks)	2.37	0.0698	NS
After 4 weeks of incubation	Control	1.5% (4 weeks)	10.58	< 0.0001	HS
		2.5% (4 weeks)	0.83	0.976	NS
	1.5%	2.5% (4 weeks)	-9.75	< 0.0001	HS

3.5. Shear bond strength test

Results of the 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/mm^2 in this period, followed by 1.5% samples of 0.4637 N/mm^2, as shown in (Figure 3-9, Table 3-7).

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 value 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 seen in (Figure 3-9, Table 3-7).

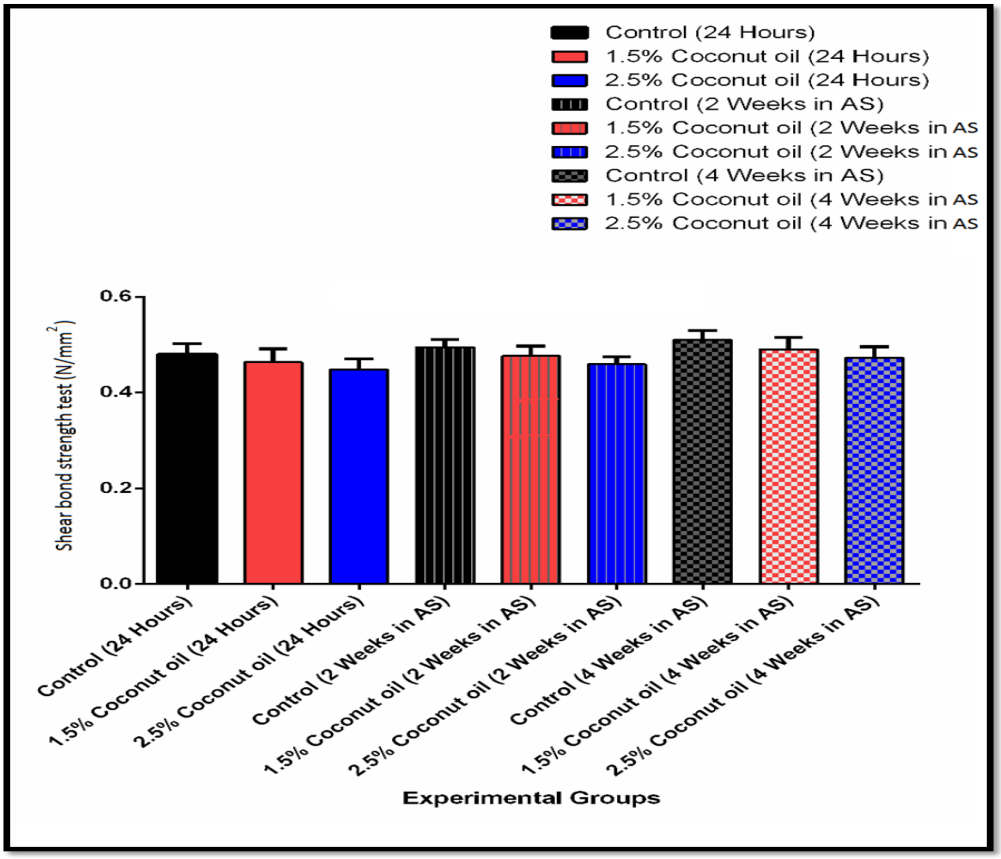


Figure (3-9): Bar chart showing mean values and standard deviation of shear bond strength for control and experimental groups at different periods of incubation

The descriptive statistics of shear bond strength test for control and experimental groups in different time periods of incubation are listed in Table 3-7.

Table (3-7): Descriptive statistics of shear bond strength test

Incubation Period	Group	N	Mean (N/mm^2)	S.D.	Min.	Max.
After 24 hours of incubation	Control	10	0.4806	0.02184	0.4464	0.5136
	1.5%	10	0.4637	0.02767	0.408	0.496
	2.5%	10	0.4483	0.02236	0.4112	0.4848
After 2 weeks of incubation	Control	10	0.4942	0.0167	0.4608	0.5168
	1.5%	10	0.4765	0.02089	0.4464	0.5088
	2.5%	10	0.4589	0.01573	0.4384	0.4832
After 4 weeks of incubation	Control	10	0.5102	0.01909	0.4768	0.536
	1.5%	10	0.4904	0.02459	0.464	0.536
	2.5%	10	0.4725	0.02296	0.4336	0.5008

Two way ANOVA (Table 3-8) indicated a highly significant difference among concentrations of coconut oil addition ($p< 0.01$), and among incubation periods ($p< 0.01$). A non-significant interaction was seen between concentrations and incubation periods ($p=0.9967$).

Table (3-8): Comparison of average values of shear bond strength test using two way ANOVA

	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**	**Partial Eta Squared**
Concentration	.019	2	.009	19.859	0.0000	.329
Incubation period	.011	2	.005	11.591	0.0000	.223
Concentration * Incubation period	7.726E-5	4	1.931E-5	.041	0.9967	.002

Tukey's multiple comparisons test was used to compare mean values of different groups. In the 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-significant difference compared to the control group after 2 weeks incubation periods but significant when compared to the control group after 4 weeks incubation periods. The 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 1.5% samples after 2 and 4 weeks incubation periods. Regarding 2.5% samples, the difference was also non-significant with 2.5% samples 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 3-9.

Table (3-9): Tukey's multiple comparisons test of different groups for the 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
	1.5%	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
	2.5%	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
	1.5%	2.5% (2 weeks)	0.0176	0.6081	NS
		1.5% (4 weeks)	-0.01392	0.8463	NS
	2.5%	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
	1.5%	2.5% (4 weeks)	0.01792	0.5846	NS

3.6. Wettability test (by measuring static contact angle)

Results of wettability test (by measuring static contact angle) 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 of contact angle than the control group, the experimental group with 1.5% of coconut oil showed the lowest value in this

period (71.74°), followed by 2.5% samples (74.44°), as shown in (Figure 3-10, Figure 3-11 and Table 3-10).

At the second period of evaluation (2 weeks of incubation in artificial saliva), the mean values of experimental groups (1.5% and 2.5% coconut oil) were less than the mean value of the control group, also the lowest mean value was noticed in 1.5% coconut oil group (65.31°), as shown in (Figure 3-10, Table 3-10).

At the third evaluation period (4 weeks of incubation), the mean value of 1.5 % samples (74.44°) were higher than that of the control group (71.74°), while the mean value of 2.5 % samples (71.36°) were lower than that of control group (71.74°) (Figure 3-10, Table 3-10).

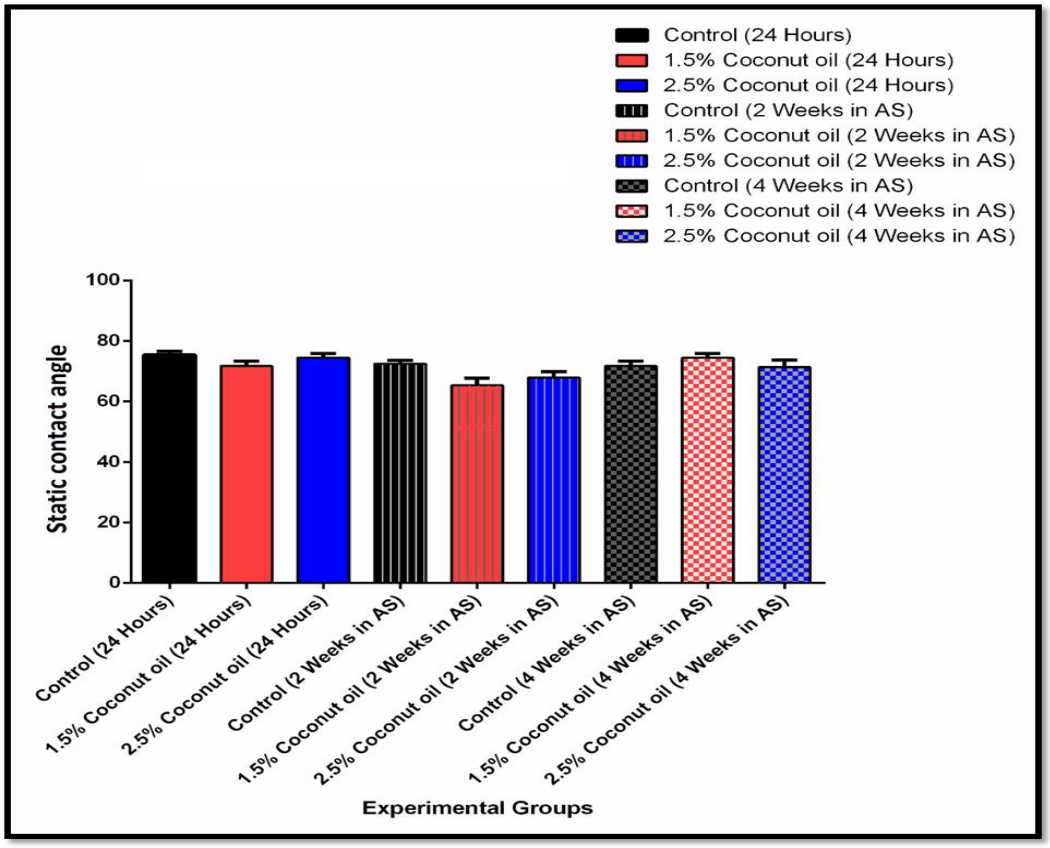


Figure (3-10): Bar chart showing mean values and standard deviation of static contact angles for control and experimental groups at different periods of incubation

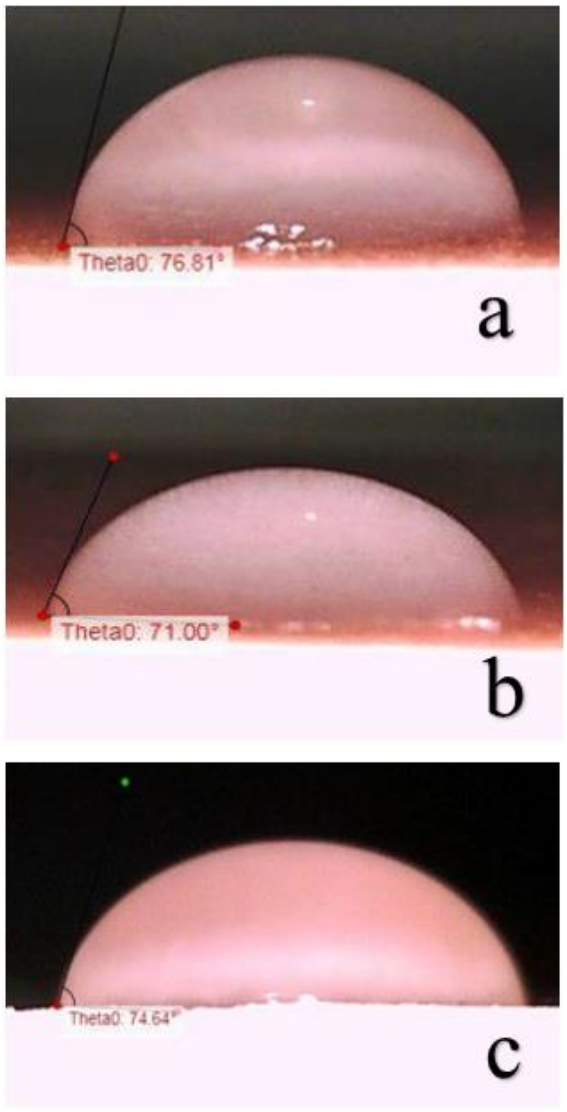


Figure (3-11): Static contact angle of: a) Control samples; b) Samples contain 1.5% VCO; c) Samples contain 2.5% VCO

The descriptive statistics of static contact angle test for control and experimental groups in different periods of incubation are listed in Table 3-10.

Table (3-10): Descriptive statistics of static contact angle test

Incubation period	Group	N	Mean (°)	S.D.	Min.	Max.
After 24 hours of incubation	Control	10	75.38	1.257	73.7	77.1
	1.5% of coconut	10	71.74	1.623	69.4	74.4
	2.5% of coconut	10	74.44	1.425	72.4	76.2
After 2 weeks of incubation	Control	10	72.44	1.126	70.9	74.2
	1.5% of coconut	10	65.31	2.327	62.6	69.3
	2.5% of coconut	10	67.92	1.928	64.6	70.4
After 4 weeks of incubation	Control	10	71.74	1.623	69.4	74.4
	1.5% of coconut	10	74.44	1.425	72.4	76.2
	2.5% of coconut	10	71.36	2.312	68.9	75.2

Two way ANOVA (Table 3-11) indicated a highly significant difference among concentrations of coconut addition ($p< 0.01$), and among incubation periods ($p< 0.01$). A highly significant interaction also was seen between concentrations and incubation periods ($p< 0.01$).

Table (3-11): Comparison of average values of static contact test using two way ANOVA

	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Concentration	200.934	2	100.467	33.516	.0000	.453
Incubation period	539.496	2	269.748	89.990	.0000	.690
Concentration * Incubation period	235.930	4	58.983	19.677	.0000	.493

Tukey's multiple comparisons test was used to compare mean values of different groups. In the first incubation period, the control group showed a high significant difference compared to 1.5% samples and non-significant difference with 2.5% samples in the same incubation period, while it was highly significant difference compared to both control groups in different incubation periods, 1.5% samples showed a significant difference with 2.5% samples in the same incubation period, and it was a highly significant difference also when compared to 1.5% samples in 2 weeks and significant when compared to 1.5% samples in 4 weeks incubation period, regarding 2.5% samples the difference was highly significant with 2.5% samples after 2 weeks incubation period while it was non-significant when compared with the same concentration after 4 weeks incubation period.

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

In the 4 weeks incubation period, the control group showed a significant difference when compared to 1.5% samples and a non-significant difference with 2.5% samples, While 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 3-12).

Table (3-12): Tukey's multiple comparisons test of different groups for static contact angle

Period	Groups		Mean difference	P value	Sig.
After 24 Hours of incubation	Control	1.5% (24 hours)	3.64	0.0076	HS
		2.5% (24 hours)	0.94	0.7726	NS
		Control (2 weeks)	2.94	0.0068	HS
		Control (4 weeks)	3.64	0.0076	HS
	1.5%	2.5 % (24 hours)	-2.7	0.0191	S
		1.5% (2 weeks)	6.43	0.0075	HS
		1.5% (4 weeks)	-2.7	0.0191	S
	2.5%	2.5% (2 weeks)	6.52	0.0007	HS
		2.5% (4 weeks)	3.08	0.0554	NS
After 2 weeks of incubation	Control	1.5% (2 weeks)	7.13	< 0.0001	HS
		2.5% (2 weeks)	4.52	0.0102	S
		Control (4 weeks)	0.7	0.9857	NS
	1.5%	2.5% (2 weeks)	-2.61	0.3408	NS
		1.5% (4 weeks)	-9.13	< 0.0001	HS
	2.5%	2.5% (4 weeks)	-3.44	0.0793	NS
After 4 weeks of incubation	Control	1.5% (4 weeks)	-2.7	0.0191	S
		2.5% (4 weeks)	0.38	0.9999	NS
	1.5%	2.5% (4 weeks)	3.08	0.0554	NS

Chapter Four:

Discussion

Soft denture lining materials are widely used nowadays to improve patient's acceptance and compliance specially for those who cannot bear denture related stresses. Although the addition of the soft lining material to the denture base has several advantages; but the prolonged use of the soft lined removable prosthesis has direct adverse effects on the oral environment. The most important one being the accumulation and colonization of *Candida albicans* represented orally as a soft tissue condition named denture induced stomatitis **(Perchyonok, 2017).**

An important line of the treatment of denture induced stomatitis is prescribing antifungal drugs. Unfortunately, the increased resistance of *Candida albican* to these antifungal drugs makes it necessary to develop an alternative line of management using a naturally derived products such as plants extracts **(Pachava *et al* ., 2015; Atai *et al* ., 2017)**. Medicinal Plants extracts are considered as excellent alternative to antimicrobial drugs with less or no side effects; this makes a worldwide tendency towards herbal-based medicines **(Bakhshi *et al* ., 2012; Atai *et al* ., 2017)**. Among the naturally derived herbal medicaments are the oils which are considered as a promising therapeutic line for oral microbial infections **(Petrović *et al* ., 2014; Perchyonok, 2017**).

Virgin coconut oil is a medicinal plant extract obtained from a fresh coconut meat and known with its antipyretic, anti-inflammatory, antimicrobial and antioxidant properties **(Rajagopal and Rajeev, 2017).** VCO mainly consists of medium chain fatty acids such as Lauric acid, Capric and Caprylic acids which are approved to act against fungi particularly *Candida albicans* **(Ogbolu *et al* ., 2007)**.

This study aimed to produce soft denture lining material with potent antifungal activity particularly against *Candida albican*s, which consider as the main cause of denture induced stomatitis, via incorporating VCO to the acrylic-

based heat cured soft denture lining material with minimal effect on the material properties.

Relatively, large numbers of studies have focused on the assessment of antifungal activity of removable dental prostheses using various kinds of organic oils. In spite of having wide applications, to the best of our knowledge, work regarding the antifungal activity of virgin coconut oil incorporation to the dental prostheses has little been attempted.

4.1. Antifungal activity

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 (control). This antifungal effect of VCO on *Candida albicans* can be explained by the ability of virgin coconut oil to disintegrate the cell membrane of fungi as stated by **Kannan and Mohammed (2014)** who declared that 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. The Lauric acid, which is found in a high percentage in VCO, is 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 exhibits potent antifungal effect. This fact was also in agreement with **Ogbolu *et al* . (2007)** who demonstrated that VCO is considered as a natural fighter for fungi which has the ability to destruct their cells membrane. The most recent was **Rawat *et al* . (2017**) who studied the antifungal effect of incorporating 25% VCO into tissue conditioner and found that specimens containing VCO interfered with *Candida*

*albican*s growth and showed an inhibition zone on the surface of sabouraud dextrose agar when compared to the control group.

The result also exhibited a highly significant decrease of the mean values of the viable counts of *Candida albican* for 1.5% VCO when compared to that of 2.5% for all time intervals; this could be explained by the dynamic process that takes 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 events: leaching out of plasticizer with other soluble contents, and fluid absorption such as water or saliva uptake. On the other hand, adding oil to the sample will result in reduction of porosity, producing a more compact sample with less water micro bockets inside **(Aziz, 2015)**; While **Gutiérrez *et al* . (2014)** stated that this fact is a concentration dependent, for example the 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 albican*s as the time interval increased ($p>0.05$). After 4 weeks incubation period, the mean of viable counts of *Candida albican*s 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 long period of time result in a rougher and more porous sample creating a suitable environment for *Candida albican*s colonization and proliferation, as suggested by **(Chladek *et al* ., 2012).**

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 fact is in a good agreement with the result obtained by **(Carandang, 2008)**; 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.

4.2. Shore A hardness

Hardness is a physical property that represent the surface resistance to permanent indentation **(Rajaee *et al* ., 2014)**, and it is a simple way to measure the modulus of elasticity of a material **(Pavan *et al* ., 2007)**.

One of the most important advantages of soft lining material is impact absorption during masticatory cycles, this requires a soft non-rigid viscoelastic material to act as a cushion between denture base and residual ridge. So lower hardness value is considered a desirable feature for soft lining materials. Nevertheless, no limitation was found for the Shore A hardness values to be considered as clinically acceptable. However, a scale ranging from 13 to 49 Shore A hardness units during 24 hours was considered as acceptable range for the material to be used clinically **(Sakaguchi and Powers, 2012)**.

After 24 hours, the incorporation of different concentrations of VCO (1.5% and 2.5%) to the heat cured acrylic-based soft liner caused a highly significant reduction in the mean values of the material hardness ($p<0.01$) when compared to the control group (0% VCO). While after two and four week's incubation periods, this reduction in the mean values in comparison with the control group was also

highly significant for 1.5% VCO group but non-significant for 2.5% group. This could be related to the fact that coconut oil is considered as a potential alternative choice of plasticizers in polymer industry, since this oil is mainly composed of fatty acids which can work as a potent plasticizer, even in low concentrations, by increasing polymer chain mobility and decreasing material viscosity as explained in the previous studies by **(Bhasney *et al* ., 2017).** Moreover, as approved by the FTIR test in this study; no chemical reaction was shown between VCO and lining material, this means that the bond presents between lining material and VCO is physical rather than a chemical. This physical bond explains the low hardness values and high material resiliency **(Hussein *et al* ., 2009)**.

The present study showed a highly significant decrease in the hardness values for 1.5% VCO when compared to the 2.5% group for all time intervals. This could be attributed to the higher amount of VCO which makes a state of instability inside the sample as more VCO will migrate to the sample surface result in more rapid loss of VCO this in turn decreases the amount of plasticizer presents inside the 2.5% samples in a faster rate than 1.5% sample. This phenomenon also may be responsible for the non-significant reduction in the mean value of 2.5% VCO samples after 2 and 4 weeks incubation periods in artificial saliva in comparison with control groups of the same incubation periods; since as time interval increased more VCO will be lost from the sample surface making the hardness values closer to that of the control groups.

For each concentration, the results also demonstrated an increase in the hardness values after 2 weeks incubation period in artificial saliva in comparison to that of 24 hours storage in distilled water, then the values start to decrease again after 4 weeks incubation in artificial saliva. A non-significant difference was obtained for the control group, whilst a highly significant for 1.5% group ($p<0.01$)

and significant for 2.5% group ($p<0.05$). This could be attributed to the dynamic process which takes place during long term storage of soft liner samples in water or any aqueous media in which the reported increase of hardness values after 2 weeks could be explained by leaching out of the plastizcer molecules along with VCO, this in turn can reduce the polymer chains movements and decrease the resiliency of the material **(Hussein *et al* ., 2009)**.

From another standpoint, the leaching out-uptake is a time dependent procedure and diffusion monitored; so as the time interval of sample storage is increased, more soluble contents will be released and the smaller size of water molecules will start to diffuse and fill the micro pockets created inside the samples acting as alternative plasticizer and facilitating the polymer chains mobility as explained by **(Rajaee *et al* ., 2014),** mostly leading to re-decrease of hardness values after 4 weeks of storage. The difference in the significance for each concentration, could be related to the amount of water absorbed via each sample group.

4.3. Shear bond strength

Debonding between soft lining material and the underlying denture base is considered one of the most common failure noticed in clinical practice. Debonding does not only affect the quality of material surface; it also creates a potential environment for microbial colonization **(Goiato *et al* ., 2015)**.

In this study, shear bond strength test was chosen to evaluate the bond strength between soft lining material and the underlying acrylic denture base; since most of the forces applied over the soft lining material in the oral cavity are represented by shear and tear.

For all time intervals; the incorporation of 1.5% and 2.5% VCO caused a decrease in the shear bond strength mean values compared with those in the 0% VCO group (control). 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 **(Rajaganesh *et al* ., 2016)**. 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 interfere with the bond between the two materials. This was stated by **Mutluay and Ruyter (2005)** who declared that a weak bond can be caused by any material presents in the bonding layer before or during the processing. 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 might be regarded to the higher concentration of oil which results 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 demonstrates 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 increase in the stiffness of the samples. This result was consistent with those reported by **Rawat *et al* . (2017)**, who 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.

4.4. Wettability

Wettability is a fundamental requirement for a denture base, it allows the saliva to easily spreaded over the denture surface increasing its retention. Moreover, wettability plays a role in minimizing accumulation of *Candida* on the denture surface **(Muttagi and Subramanya, 2017)**.

Contact angle is an essential parameter in the measurement of wettability of denture lining materials. Contact angle is the angle formed by a tangent to the drop of liquid and the solid surface (**GPT9, 2017)**. This angle is a unique feature for each substance because it is related to the surface energy of the solid substances and surface tensions of liquid substances. The highest is the contact angle the lowest is the wettability value **(Jin *et al* ., 2009)**.

After 24 hours and 2 weeks incubation periods, the results revealed that the incorporation of 1.5% and 2.5% VCO caused a decrease in the mean values of static contact angle (increase wettability), when compared to 0% VCO group (control). This difference was highly significant for 1.5% group in both incubation periods ($p<0.01$), while it was non-significant for 2.5% in the first period and significant in the second one ($p>0.05$).

This may be attributed to the higher surface energy acquired by the soft liner samples after VCO addition, since VCO known by its high surface activity, high flow rate, high saturation and less viscosity compared with other oils **(Siddiqui and Ahmad, 2013),** it is possible that adding VCO to soft lining material could

result in higher surface energy of the samples, this consequently reduce the contact angle of the water droplet with the sample surface result in overall increase in the material wettability **(Kasraei and Azarsina, 2012)**.This was in agreement with **Muttagi and Subramanya (2017)** who reported that seed oil incorporated soft liner samples demonstrated a better wettability than control group in which a significant reduction of the mean values of the contact angle was noticed after oil addition in comparison to the control group. Moreover, a previous study was conducted by **(Dankovich and Hsieh, 2007)** trying to fabricate a hydrophobic material, by coating it with different plants oils states that all utilized oils were able to produce a hydrophobic material with less water sorption except coconut oil and relates this to the fact that coconut oil contains mostly saturated fatty acids with very low percent of unsaturated fatty acids, on the contrary to other oils used in the previous study which contain mostly unsaturated fatty acids that have the ability to form a cross-linked network to increase the hydrophobicity of the material.

For the third incubation period (After 4 weeks), the results demonstrated a significant increase in the mean values of static contact angles for 1.5% VCO group in comparison to the control group. This unexpected increase need to undergo further investigations and may be related to the long period of incubation in which some components of artificial saliva was adsorbed or accumulated over the sample surfaces reducing its surface energy and adversely affecting its wettability by increasing the contact angle with water droplet. In addition, this adsorption of salivary components is not constant for all groups and varies with the difference in chemical composition of the outmost layer of the sample surface at the time of measurement as stated by **Sipahi *et al* . (2001)** who declared that one cannot make a clear conclusion about the interaction between the solid and liquid materials since the wettability of a solid surface covered by organic layer mainly

depend on the surface chemical properties of the most external atoms presents on the solid surface which require a very complicated and extensive investigations to be determined. This explanation can also be applied for the release and accumulation of free VCO over the sample surface decreasing its surface energy and impairing its wettability by creating a large contact angle with water droplet. Although incorporation of VCO into soft denture lining material can improve its wettability as mentioned previously, but knowing that the behavior of liquid differs in accordance to its state whether it is pure liquid or it is mixed with other components because the composition present in the surface of a mixture is not necessarily similar to that presents in the bulk **(Thangaraja *et al* ., 2016).**

Comparing 1.5% VCO group to 2.5% VCO group, the results showed that the mean value of 1.5% was lower than that of 2.5% and this is also supports the previously mentioned explanation in which the more VCO incorporated in the sample leads to more instability in the sample and faster release rate of the oil from the sample. This result explain the lesser beneficial effect of VCO in the wettability of 2.5% group than 1.5% group. This is why 1.5% group demonstrated a lower contact angles with improved wettability. For each concentration of experimental samples, statistical results also demonstrate a highly significant decrease in mean values of the static contact angle after 2 weeks incubation period in artificial saliva in comparison to that of 24 hours storage in distilled water, and this reduction had continued for the control group only. Researches studying the effect of water storage on the wettability of soft lining material for a very long period of time were not conducted yet, but **Jin *et al* . (2009)** stated that the wettability of heat cured acrylic based soft denture lining material was increased after 24 hour of water storage due to leaching out of plasticizer and water imbibition.

While after 4 weeks of incubation, the mean values of contact angle of the experimental groups were increased, this might be related to the accumulation of free VCO or saliva components over the samples surfaces impairing its wettability.

Chapter Five:

Conclusions and Suggestions

5.1 Conclusions

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 decreased (non-significantly) as the time interval of incubation period is increased.
3) The addition of VCO into soft lining material resulted in decreased the hardness and shear bond strength of the material for both experimental groups compared to the control group; the1.5% group showed the lowest hardness values, whilst the 2.5% groups showed the lowest shear bond strength values for all incubation periods.
4) Adding VCO into soft denture lining material result in a more hydrophilic material with no adverse effect on the material wettability.

5.2 Suggestions

1) Evaluating the effect of coconut oil incorporation into soft lining material on other mechanical properties such as roughness, tear strength, tensile strength, resiliency and water sorption and solubility.
2) Studying the thermocycling effect on the release of coconut oil from soft lining material at different time intervals.
3) Investigating the effect of adding coconut oil on other bacterial and fungal activity and some properties of a heat-cured acrylic denture soft lining material.
4) Evaluating the effect of coconut oil incorporation on some properties of silicon maxillofacial material.

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Appendices

Appendix I: Data representing values of viable count of candida albicans (CFU/mL).

Viable count

	Control			1.5% Coconut Oil			2.5% Coconut Oil		
	24 Hours	2 Weeks	4 Weeks	24 Hours	2 Weeks	4 Weeks	24 Hours	2 Weeks	4 Weeks
1	143	146	175	29	34	37	76	57	77
2	138	162	163	25	37	32	48	69	74
3	166	173	147	27	30	39	45	73	68
4	154	140	155	39	29	31	64	53	79
5	149	168	161	33	25	44	60	75	62
6	176	151	139	22	41	25	71	51	58
7	140	178	172	19	37	27	45	71	55
8	163	135	166	31	32	37	63	67	63
9	144	168	179	24	39	42	71	62	56
10	132	144	143	35	27	38	55	55	67
Avg	150.5	156.5	159.9	28.4	33.1	35.2	58	63.3	65.9

Appendix II: Data representing value of shore A hardness test.

Hardness

	Control			1.5% Coconut Oil			2.5% Coconut Oil		
	24 Hours	2 Weeks	4 Weeks	24 Hours	2 Weeks	4 Weeks	24 Hours	2 Weeks	4 Weeks
1	55.4	56.8	53.6	37.9	47.8	46.2	51.3	54.6	52.3
2	55.3	57.2	54.2	39.4	44.6	41.2	50.5	55.7	50.7
3	56.7	56.4	55.1	36.1	45.9	42.3	50.2	54.3	51.7
4	55.1	56.3	53.8	41.4	43.4	40.9	49.0	53.1	52.4
5	54.2	56.6	53.5	38.6	46.7	44.5	47.7	55.9	53.9
6	53.6	54.8	52.5	38.0	45.6	42.6	53.7	57.6	56.3
7	54.8	54.8	52.8	41.7	42.8	43.1	47.2	54.4	53.9
8	56.8	56.9	54.7	39.1	41.9	45.2	52.2	53.0	54.0
9	54.7	55.8	52.4	40.7	47.3	39.6	48.6	55.7	51.6
10	55.8	54.5	53.1	43.4	48.1	44.3	50.9	56.8	50.6
Avg	55.24	56.01	53.57	39.63	45.41	42.99	50.13	55.11	52.74

Appendix III: Data representing value of shear bond with acrylic resin (N/mm^2).

Shear bond

	Control			1.5% Coconut Oil			2.5% Coconut Oil		
	24 Hours	2 Weeks	4 Weeks	24 Hours	2 Weeks	4 Weeks	24 Hours	2 Weeks	4 Weeks
1	0.456	0.4992	0.52	0.4432	0.4736	0.4928	0.4208	0.4752	0.488
2	0.4992	0.5168	0.5312	0.496	0.5088	0.5008	0.4544	0.4608	0.4704
3	0.4464	0.4896	0.5024	0.4592	0.4976	0.536	0.4112	0.4416	0.4528
4	0.4928	0.4608	0.4944	0.4752	0.4896	0.5248	0.4848	0.4384	0.4576
5	0.5136	0.4752	0.536	0.4944	0.4624	0.4656	0.4736	0.4752	0.496
6	0.4848	0.4976	0.4768	0.4608	0.4464	0.4768	0.4528	0.4832	0.4912
7	0.4976	0.5104	0.4912	0.472	0.4816	0.4848	0.4352	0.448	0.4336
8	0.4672	0.5072	0.5088	0.44	0.4528	0.464	0.4496	0.4528	0.5008
9	0.4608	0.4896	0.5152	0.408	0.4592	0.4672	0.4592	0.4464	0.4496
10	0.488	0.496	0.5264	0.488	0.4928	0.4912	0.4416	0.4672	0.4848
Avg	0.48064	.49424	.51024	.46368	.47648	.4904	.44832	.45888	0.47248

Appendix IV: Data representing wettability test by measuring contact angle (°)

Wettability

	Control			1.5% Coconut Oil			2.5% Coconut Oil		
	24 Hours	2 Weeks	4 Weeks	24 Hours	2 Weeks	4 Weeks	24 Hours	2 Weeks	4 Weeks
1	76.37	73.14	67.16	72.93	62.83	69.22	74.37	67.49	71.22
2	74.86	71.33	68.25	70.38	68.19	65.39	72.93	69.84	73.95
3	73.69	70.87	66.89	74.38	64.81	68.47	76.11	70.42	69.20
4	75.44	73.63	68.93	71.48	65.30	67.11	73.25	68.83	70.46
5	76.22	72.26	65.48	72.59	63.73	64.57	75.66	64.56	74.31
6	75.51	73.61	67.30	69.36	66.91	66.83	73.44	66.73	68.91
7	77.12	71.45	64.57	70.8	66.28	68.90	74.16	67.81	70.52
8	73.67	74.21	67.82	69.77	69.33	70.13	75.78	65.34	69.06
9	76.80	72.19	66.92	72.38	63.18	69.54	76.19	68.72	75.16
10	74.12	71.69	67.33	73.15	62.55	67.95	72.39	69.60	70.74
Avg	75.380	72.438	67.065	71.722	65.311	67.811	74.428	67.934	71.353

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جامعة بغداد

كلية طب الأسنان

تأثير إضافة زيت جوز الهند على نشاط المبيضات البيض وبعض خصائص مادة تبطين الطقم اللينة الاكريليكية

رسالة

مقدمة إلى مجلس كلية طب الأسنان جامعة بغداد كجزء من متطلبات نيل شهادة الماجستير في التعويضات الاصطناعية

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دكتوراه في التعويضات الصناعية

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الخلاصة

المقدمة: إن تراكم المتعضيات المجهرية تعتبر احدى اهم المشاكل الرئيسية التي تواجه مادة تبطين طقم الأسنان اللينة خلال الاستعمال. وتظهر هذه المشكلة على شكل التهاب ناجم عن طقم الاسنان، والذي تسببه الفطريات وخاصة المبيضات البيض. لذلك فإن تطوير مادة مبطنة لينة تحتوي على نظام لايصال العلاج يصبح أمرا ضروريا.

الهدف من الدراسة: كان الهدف من الدراسة هو بحث فاعلية تراكيز مختلفة من زيت جوز الهند النقي بعد ادخالها في تركيب مادة تبطين الاسنان الطرية المتصلبة بالحرارة كمضاد للفطريات ضد المبيضات البيض. وبالاضافة إلى ذلك تقييم الصلابة والقابلية للابتلال وقوة الالتصاق القصية بمادة الطقم بعد الاضافة. تم تقييم الفحوص جميعها بفترات زمنية مختلفة.

المواد والطرق: تم تحضير ثلاث مئة وستين عينة عن طريق اضافة ١,٥% و ٢,٥% (بالحجم) من زيت جوز الهند النقي إلى مادة تبطين طقم الاسنان اللينة الأكريليكية المتصلبة بالحرارة. تم تقسيم عينات الدراسة إلى أربع مجموعات (٩٠ عينة لكل مجموعة) اعتمادا على نوع الفحص؛ و تشمل فحص نشاط المبيضات البيض وفحص الصلابة وفحص قوة الالتصاق القصية وفحص قابلية الابتلال. ثم تم اعادة تقسيم كل مجموعة إلى ثلاث مجموعات فرعية (المجموعة الضابطة ٠% و١,٥% و٢,٥%) اعتمادا على تركيز الاضافة لزيت جوز الهند النقي (عشر عينات لكل مجموعة فرعية). كل مجموعة تقييمها في فترات مختلفة (٢٤ ساعة في الماء المقطر، أسبوعان وأربعة أسابيع في اللعاب الصناعة)، لكل فترة من الفترات تم استخدام عشر عينات. تم إجراء فحص (FTIR) لتحديد إذا ماكان هناك تفاعل كيميائي بين زيت جوز الهند ومادة التبطين الطرية.

النتائج: بخصوص فحص نشاط المبيضات البيض فإن إضافة ١,٥% و ٢,٥% من زيت جوز الهند النقي سبب انخفاضا عالي التأثير على متوسط القيم لعدد الخلايا القابلة للحياة من المبيضات البيض عند مقارنتها بالمجموعة الضابطة. وعلى النقيض من ذلك فإن زيادة تدريجية (غير مهمة إحصائيا) لعدد الخلايا القابلة للحياة تم الحصول عليها كلما زادت الفترة الزمنية. أظهر اختبار الصلابة نقصانا في معدل صلابة العينات بعد اضافة ١,٥% و ٢,٥% من زيت جوز الهند مقارنة بالمجموعة الضابطة، كان هذا النقصان عالى الأهمية إحصائيا بعد ٢٤ ساعة من التخزين في الماء المقطر. أظهرت النتائج سلوكا متذبذبا في أوقات تخزين مختلفة فكانت النتائج تزداد ثم يتبع الزيادة نقصانا في قيمة الصلابة بعد تخزينها لمدة اسبوعين واربعة اسابيع في اللعاب الصناعي. وكان هناك نقصان غير مهم احصائيا في قيم قوة الالتصاق القصية لمجموعة الـ ١,٥% بينما كان هناك نقصان مهم احصائيا لمجموعة الـ ٢,٥% مقارنة بالمجموعة الضابطة. وعلى خلاف ذلك فإن كلا من المجموعات الاختبارية اظهرت زيادة غير مهمة احصائيا في قوة الالتصاق بازدياد وقت التخزين للعينات. وبخصوص فحص قابلية الابتلال، أظهرت النتائج نقصانا في قيمة زاوية التماس بعد ٢٤ ساعة

واسبوعين من وقت التخزين، كان هذا النقصان عالي الأهمية إحصائيا لمجموعة الـ ١,٥% . بينما ازداد متوسط القيم لزاوية التماس في المجموعات الاختبارية بعد اربعة أسابيع من التخزين .

الخلاصة: تم وبنجاح اضافة زيت جوز الهند النقي إلى مادة تبطين طقم الأسنان اللينة وعمل زيت جوز الهند كمضاد دوائي محتمل للفطريات مع نظام إيصال دواء مستمر ضد المبيضات البيض. ويظهر أن إضافة ١,٥% من زيت جوز الهند كان صاحب التأثير الإيجابي الأكبر ضد الفطريات، مع قيم أفضل للصلابة وقابلية الابتلال، ومع تأثير سلبي أقل أيضا على قوة الالتصاق القصية.