ORIGINAL ARTICLE

Antifungal Efficacy of Thymol Powder Addition on Candida Albicans Adhesion to Room Temperature Vulcanized Maxillofacial Silicone: An In Vitro Study

Manar Hussein Ramadhan* , **Faiza Abdul-Ameer**

Department of Prosthodontics, College of Dentistry, University of Baghdad, Iraq

*Corresponding Author: Manar Hussein Ramadhan
Email: hussainmanar23@gmail.com

Received: 29 July 2023 / Accepted: 12 October2023

Abstract

Purpose: Microorganism colonization, namely Candida albicans (C. albicans), on silicone facial prostheses, with subsequent dermatitis and prosthesis material degradation, is another problem added to the list for maxillofacial defect patients who have already suffered a lot of physical and psychological pain during their injury and treatment journey. This study aimed to investigate the most effective percentage of thymol powder for retarding Candida albicans adhesion and colonization on the thymol-modified silicone specimens.

Materials and Methods: Study specimens were made from room-temperature vulcanized VerSilTal (VST-50) maxillofacial silicone, which is impregnated with thymol powder in percentages of 0.75 wt.% and 1 wt.%, depending on the pilot study outcomes. Forty silicone specimens were fabricated for the main study and then dispensed among four groups: group A (the negative control with no additive), groups B and C (0.75 and 1 wt.% thymol additive, respectively), and group D (the positive control with 1.4 wt.% nystatin additive). Candida adherence testing estimated the antifungal properties of thymol-modified maxillofacial silicone specimens through microscopic counting of adherent C. albicans cells on the silicone specimens' surface. ANOVA and post-hoc multiple comparison tests were used to compare groups (significance level at $P < 0.05$).

Results: Statistically, group B exhibited the maximum significant reduction in candida adherence mean value of 52.211 yeast cells/mm^2 ($P = 0.000$), compared to the rest of the study groups, including the positive and the negative controls.

Conclusion: The outcomes revealed that thymol powder could be a powerful antifungal agent when impregnated with maxillofacial silicone to produce material with inherent sanitation against *C. albicans* fungi.

Keywords: Candida Adherence; *Candida Albicans*; Maxillofacial Silicone; Thymol.

DOI:

1. Introduction

Prosthodontists and laboratory technicians adopt maxillofacial prosthetic materials to fabricate artificial replacements for patients who have lost some parts of their faces because of trauma, congenital disabilities, or surgical tumor excision [1, 2].

Any maxillofacial prosthetic material should possess a high level of biological, physical, mechanical, and aesthetic characteristics to be as durable and comfortable for the patient as possible [3].

Nowadays, maxillofacial silicone is considered the premium material developed for producing most maxillofacial prostheses. This consideration is because of its relatively good physical and mechanical characteristics, low cost, easy management, biocompatibility, and acceptable coloring and characterization techniques [4, 5].

The long period of wearing the maxillofacial prosthesis, however, places it close to facial tissue and secretions, creating a good opportunity for opportunistic microorganisms such as Candida albicans (C. albicans) to colonize both the prosthesis and the underlying facial tissue, leading to skin infection as well as silicone material degradation. Therefore, maintaining the integrity of the maxillofacial prosthesis and the health of the underlying facial tissue depends heavily on prosthesis hygiene [6].

Candida infections could range from simple mucocutaneous candidiasis to serious candidemia through the diffusion of fungal cells from their biofilm into the bloodstream; their drug-resistance capability could aggravate this [7, 8].

Furthermore, candida colonization within the fabric of a silicone prosthesis is harmful to its integrity as it causes the silicone to soften, deteriorate, and develop a fixed black stain, ultimately leading to prosthesis failure [8].

The pharmaceutical properties of medicinal herbs and their active components for human health against various ailments have been suggested by extensive epidemiological and experimental studies; they offer easier handling, fewer side effects, and lower toxicity than chemical substitutes [4, 9].

Plants can synthesize a wide variety of chemical compounds that are used to perform critical biological functions. For example, eucalyptol is the main active constituent of eucalyptus oil and has antibacterial and anti-inflammatory properties [10].

Menthol is another herbal compound that can be isolated from essential oils extracted from some species of the Mentha genus [11]. The biological properties of menthol include analgesic, anesthetic, antibacterial, and antifungal properties [12].

Also, essential oils from Laportea aestuans, highly dominated by methyl salicylate (54.50%), have in vitro antioxidant and antimicrobial activities [13].

Thymol is a natural monoterpenoid phenol, chemically termed 5-methyl-2-isopropylphenol, a colorless crystalline material with a distinctive scent. It is the main active constituent of natural thymol oil extracted from the plant Thymus vulgaris (T. vulgaris) [14].

Thymol exhibits various pharmacological properties, for example, antioxidant, antimicrobial, anti-inflammatory, and anticancer properties [15].

In this study, thymol is utilized as the antifungal agent to be added into the Room Temperature Vulcanized (RTV) VST-50 maxillofacial silicone material in an attempt to inhibit the adherence of C. albicans units on the maxillofacial silicone surface owing to its antimicrobial and anti-inflammatory properties and its safety [15].

There are no published research studies investigating the effects of thymol herbal extract as an additive to the maxillofacial silicone elastomer that inhibits candida biofilm formation on the surface of maxillofacial silicone.

This study aims to evaluate the efficacy of thymol powder incorporation into the VST-50 maxillofacial silicone matrix to inhibit C. albicans adherence.

2. Materials and Methods

All methods employed for this in vitro experimental study adhered to the Helsinki Declaration and any later amendments it received regarding human research. The institutional ethics committee approved the protocol (project No. 667222 and Ref No. 667) after fully explaining the nature and objectives of the study.

A pilot study was accomplished to investigate the more effective two percentages of thymol powder (purity of 99.5%, particle size 2 microns, BDH Limited Pool England, CAS 89-83-8) that should be incorporated into VST-50 maxillofacial silicone

matrix (Factor II Inc., USA) in the main study to act as an antifungal agent while improving or at least not affecting the other mechanical properties of the tested silicone, namely tensile strength and percentage of elongation. Concerning the candida adherence test, three silicone specimens were made for each of the six percentages (0.25, 0.50, 0.75, 1, 1.25, and 1.50 wt.%) of thymol powder additives. Another three silicone specimens were also made for the negative (-ve) control.

Tensile strength and elongation percentage tests were accomplished using dumbbell-shaped (type 2) specimens according to the International Organization for Standardization (ISO 37, 2017) [16]. The mechanical tests were examined synchronically using the same specimen through an Instron universal testing machine, the WDW-20. Again, three specimens were allocated for each weight percentage group.

According to the pilot study outcomes, 0.75 wt.% and 1 wt.% of thymol powder were nominated to be used in the main study as they exhibited a more significant reduction in candida adherence to the silicone surface as well as an improvement in the tested mechanical properties.

The main study was accomplished according to the outcomes of the pilot study. In this study, ten silicone specimens were prepared for each of the four study groups, which are group A (the -ve control without additive), group B (0.75 wt.% thymol additive), group C (1 wt.% thymol additive), and group D (the positive (+ve) control, in which 1.4 wt.% nystatin powder was incorporated, Nystatin powder was supplied by Avonchem Limited with a purity of 99% (CAS 1400-61-9). The total number of prepared and tested specimens in the main study was 40.

An inverted light microscope (D-63303, KARLKOLB, DREIEICH, GERMANY) was used to visualize and count the adherent candida cells in four fields of view on the surface of every specimen, and the mean reading was calculated.

The negative and positive control groups were used as criteria to explore the impact of thymol powder on candida adherence. Thymol powder was nominated for this study due to its antifungal characteristics.

2.1. Mold Fabrication and Specimen Preparation

The mold was fabricated from transparent acrylic sheets (Perspex cell-cast acrylic, Clairvaux Les Lacsrance, France). The shape and dimensions of the specimens' mold were designated by AutoCAD 2013. The fabrication process of these molds was accomplished by the Computer Numerical Control machine (CNC) JL-1612 [17].

Maxillofacial silicone specimens were fabricated as discs of 2 mm thickness and 10 mm diameter [18, 19].

The control silicone specimens were prepared as stated by the instructions of the manufacturer, which indicate adding the catalyst to the base of the silicone in a weight ratio of 1:10, then mixed by a Myxyvac T vacuum mixer at 360 rpm and -10 bar for five minutes [4, 20].

Concerning the experimental groups' specimens, thymol powder was incorporated into the silicone base according to its weight percentage. For example, in 10 g of the silicone base to be modified with 0.75% thymol powder, 0.75 g of thymol powder was mixed with 9.925 g of the silicone base to form 10 g of the modified base [20].

Then, the thymol and the silicone base were mixed initially without a vacuum for three minutes to prevent powder suction and then remixed for five minutes with the vacuum [4].

The silicone catalyst was then incorporated, depending on the weight of the modified base, in the previously mentioned ratio (1:10 catalyst to base) and mixed with it for five minutes under vacuum to obtain a bubble-free mixture [21].

Nystatin-modified silicone specimens (+ ve control) were prepared by the same method as thymol-modified silicone specimens and according to weight percentage, which is 1.4%.

All mold parts (cover, matrix, and bottom) were coated with a thin film of separating medium (BMS Dendal, Italy). Then, the mixture was poured into the mold [17, 22]. This was done "at a Relative Humidity (RH) of 50±10% and a controlled temperature of 23±2°C" [23]. It requires about 4-6 hours of setting time, as recommended in the manufacturer's information.

Following the setting, all specimens were retrieved from the molds, finished, and kept in a lightproof storage box under the formerly mentioned standard conditions until testing. The minimum time between vulcanization and testing should be 16 hours [23].

2.2. Preparation of Sabouraud Dextrose Agar (SDA) Medium

SDA (Oxoid Ltd Wade Road Basingstoke, Hants, UK) medium preparation was accomplished as directed by the manufacturer's instructions, sterilized by an autoclave (Portable Pressure steam sterilizer YX-2808, China) at 121 °C and 15 psi for 15 minutes, and allowed to cool down for about 30 minutes, and chloramphenicol (Oxoid Ltd Wade Road Basingstoke, Hants, UK, Cas 56-75-7) (0.05 gram per liter of medium) was added to inhibit the bacterial development in the medium. After that, the SDA medium was dispensed into Petri dishes and left to harden, then kept in the refrigerator at four °C until it needed to be used [24].

The SDA dishes were used for culturing candida isolates and then incubated aerobically for 48 hours at 37°C [25, 26].

2.3. Identification of Candida Albicans

C. albicans was identified by the following methods:

1- Colony morphology

After incubation and colonization in the SDA medium, C. albicans colonies appear dome-shaped, pearly, smooth, buttery, and pale [24, 27, 28].

2- Microscopic examination

A small part of a single, distinct C. albicans colony was picked up and blended with a normal saline droplet to produce candida suspension, which was then spread on a glass slide, dried, and fixed by the Bunsen burner flame. After that, it was stained according to Gram's method [29]. This method was the next: First, the slide was stained with crystal violet (APCO, Cas 548-62-9) for one minute and washed away by rinsing with distilled water. Second, the slide was stained with Gram's iodine stain (APCO, Cas 12298-68-9) for one minute and then washed. Third, acetone alcohol (APCO, Cas 67-64-1) was used to decolorize the slide until the solution became colorless, then rinsed with distilled water. Finally, the slide was counterstained with safranin (APCO, Cas 477-73-6) for one minute, rinsed, and dried.

The slide was examined by a light microscope (A. KRUSS OPTRONIC, Germany) at a magnification of x400.

3- Germ tube formation

A small amount of inoculum was picked up from a single distinct candida colony and mixed with 0.5 ml of serum. This mixture was then incubated for two hours at 37 °C. Then, this suspension was dispensed over a glass slide and shielded with a coverslip to be inspected under a light microscope [30, 31].

4- Biochemical identification

The VITEK-2 compact™ system (BIOMERIEUX, France) was used to accurately determine the exact species of fungi to confirm that it is C. albicans. This system compares the biochemical profile of the tested isolate with a vast database to determine the exact fungal species [24].

Candida suspension was prepared at an optical density of 2.0 McFarland standards. VITEK-2 cards were automatically filled with candida suspension, closed, and incubated in the device for 18 hours at 37 °C. The results were matched with the database to identify the microorganism [32].

2.4. Preparation of Candida Albicans Suspension

First, the Sabouraud Dextrose Broth (SDB) was prepared following the manufacturer's directions, then sterilized in an autoclave for 15 minutes at 121°C/15 psi and left to cool for about 30 minutes. Then, chloramphenicol (0.05 grams per liter of medium) was added. Then, for preparing the C. albicans suspension, this broth was mixed with C. albicans inoculum in sterile glass tubes at a concentration of 0.5 McFarland standard [24].

2.5. Candida Adherence Test

Test specimens of VST-50 RTV maxillofacial silicone material were prepared as discs of 2 mm thickness and 10 mm diameter for each study group.

Then, they soaked in sterile tubes containing about 20 ml of C. albicans suspension [18, 19].

These specimens were put in the incubator for one hour at room temperature, then rinsed for one minute by gentle agitation in normal saline and dried. Methanol (Carlo Erba, Iraq, Cas 67-56-1) was used to fix the adherent candida cells; this was followed by staining the specimens with crystal violet for one minute, washing them in normal saline for 30 seconds, and finally drying them on filter paper [18].

The prepared specimens were ready to be examined under an inverted light microscope (at a magnification power of x40) connected to the computer. First, the diameter of the Field Of View (FOV) of the microscope was calculated and found to be 0.575 mm through Equation 1 according to the manufacturer's instructions:

$$dFOV = FN/MP \quad (1)$$

where:

$dFOV$: Diameter of the field of view (mm).

FN: The field number (engraved on the objective lens).

MP: The magnification power of the objective lens.

As the FOV is circular, the area of the field of view was calculated through the area equation of circles and found to be 0.26 mm^2. Then, the mean of adherent yeast cells in four fields of view for every specimen was counted, and the result was calculated mathematically as yeast cells per one square millimeter of the FOV.

The Energy Dispersive X-Ray (EDX) device (MIRA III, TESCAN, Czech) was utilized to investigate the purity of thymol through the elemental analysis associated with electron microscopy.

3. Results

The Statistical Package for Social Sciences (SPSS) program, version 26, was used to analyze the data through descriptive and inferential statistics.

3.1. Pilot Study Results

The pilot study included a Candida albicans adherence test, a tensile strength test, and an elongation percentage test.

Table 1 shows the results of pilot study tests. Generally, the 0.25, 0.5, 0.75, and 1 wt.% thymol-modified experimental groups showed a reduction in the mean candida adherence to the silicone specimens' surfaces and an increase in the tensile strength and elongation percentage than those of the control groups. Regarding the candida adherence test, the 0.5 and 0.75 wt.% groups exhibited the lowest mean candida adherence values among the other groups. In contrast, the 1.25 and 1.5 wt.% groups showed increasing mean candida adherence values than those of the control group, which is why they were excluded from the rest of the pilot study tests.

Regarding the tensile strength test, the 0.75 and 1 wt.% groups exhibited the highest mean values among all groups. Finally, the elongation percentage test result showed the maximum increase in the mean elongation values of the test specimens belonging to the 0.25 and 0.75 wt.% groups compared to all other groups.

3.2. Main Study Results

Table 2 shows the descriptive statistics, one-way Analysis of Variances (ANOVA), homogeneity of variances, and the post-hoc multiple comparisons of the candida adherence test groups in the main study. According to the descriptive statistical analysis, experimental group B exhibited the minimum mean value of adherent candida cells (52.211 yeast cells/mm^2), followed by group C (63.653 yeast cells/mm^2), which showed a mean value lesser than that of both the +ve control group (88.653 yeast cells/mm^2) and the -ve control group (162.499 yeast cells/mm^2), with the latter having the maximum number of adherent candida cells as shown in Figure 1.

The ANOVA test showed significant differences among groups at $P = 0.000$, which means that thymol addition to the silicone matrix significantly affected the candida adherence to the silicone surfaces. In addition, the post-hoc multiple comparisons test compared the mean values of the test results between

Table 1. Statistical analysis for candida adherence (yeast cells/mm^2), tensile strength (MPa), and elongation percentage (%) tests in the pilot study

	Descriptive statistics			Post-hoc multiple comparisons		
Pilot tests	Groups	No.	Mean	Compared groups	Mean difference	*P*-value
Candida adherence	0 wt.%	3	166.346	0 wt.% - 0.25 wt.%	87.179	0.000
	0.25 wt.%	3	79.166	0 wt.% - 0.5 wt.%	104.166	0.000
	0.5 wt.%	3	62.179	0 wt.% - 0.75 wt.%	117.948	0.000
	0.75 wt.%	3	48.397	0 wt.% - 1 wt.%	98.717	0.000
	1 wt.%	3	67.628	0 wt.% - 1.25 wt.%	-26.602	0.176
	1.25 wt.%	3	192.948	0 wt.% - 1.5 wt.%	-31.730	0.055
	1.5 wt.%	3	198.076			
Tensile Strength	0 wt.%	3	6.436	0 wt.% - 0.25 wt.%	-1.006	1.000
	0.25 wt.%	3	7.443	0 wt.% - 0.5 wt.%	-0.606	1.000
	0.5 wt.%	3	7.043	0 wt.% - 0.75 wt.%	-2.326	0.230
	0.75 wt.%	3	8.763	0 wt.% - 1 wt.%	-1.446	1.000
	1 wt.%	3	7.883			
Elongation %	0 wt.%	3	734.441	0 wt.% - 0.25 wt.%	-184.334	0.087
	0.25 wt.%	3	918.776	0 wt.% - 0.5 wt.%	-0.953	1.000
	0.5 wt.%	3	735.394	0 wt.% - 0.75 wt.%	-183.604	0.089
	0.75 wt.%	3	918.045	0 wt.% - 1 wt.%	-99.733	1.000
	1 wt.%	3	834.174			

Table 2. Statistical analysis of the candida adherence result (yeast cells/mm^2) in the main study

Descriptive statistics						Post-hoc multiple comparisons		
Groups	**No.**	**Mean**	**Std. deviation**	**ANOVA *P*-value**	**Homogeneity of variances**	**Compared groups**	**Mean difference**	***P*-value**
A	10	162.499	4.73232672	0.000*	0.002	A – B	110.288	0.000*
B	10	52.211	4.41912740			A – C	98.846	0.000*
C	10	63.653	2.16435138			A – D	73.846	0.000*
D	10	88.653	2.01694035			B – C	-11.442	0.000*
						B – D	-36.442	0.000*
						C- D	-25.000	0.000*

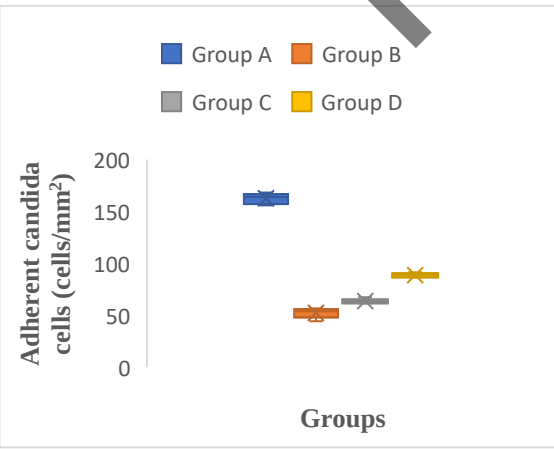

Figure 1. Box plot presentation of mean candida adherence values for all study groups

every two groups. It showed that the (-ve) control exhibited statistically significant differences as compared to experimental groups B, C, and D (*P* = 0.000); likewise, there were statistically significant differences between groups B and C, B and D, in addition to C and D (*P* = 0.000).

3.3. Energy-Dispersive X-Ray (EDX) Result

The energy-dispersive X-ray analysis demonstrated thymol purity through the elemental analysis associated with electron microscopy (Figure 2).

4. Discussion

Generally, VST-50 maxillofacial silicone possesses relatively good physical and mechanical properties, as evident in the manufacturer's information. However, patients with maxillofacial defects are prone to develop candida infections due to wearing the maxillofacial silicone prosthesis, which is in close contact with skin

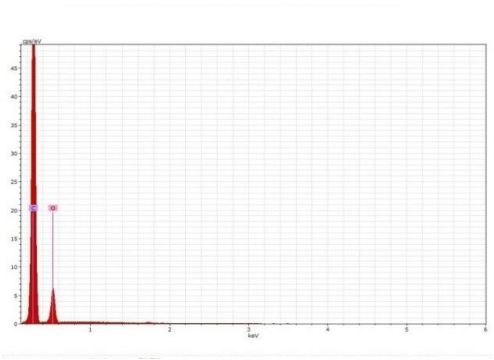

Figure 2. Energy dispersive X-ray analysis for thymol powder showing carbon (C) and oxygen (O) elements

secretions for long hours during the daytime, creating a favorable environment for candida colonization, skin infection, and silicone material degradation.

A preliminary pilot study was employed to investigate the more suitable two-weight percentages of thymol powder to be incorporated into VST-50 maxillofacial silicone, mainly in order to inhibit candida adherence and improve its mechanical performance. Accordingly, 0.75 and 1 wt.% thymol-modified groups were nominated for the main study.

According to the results of the main study, thymol powder seemed practical in inhibiting C. albicans adherence to the surface of thymol-impregnated silicone specimens, as was the aim of this study. This inhibition is essential in preventing C. albicans colonization on silicone facial prostheses and underlying tissue, with subsequent dermatitis and prosthesis material degradation.

Statistically, the antifungal efficacy of the two experimental groups, B and C, was significantly higher than the (+ve) and (-ve) control groups. This antifungal efficacy is because thymol hinders the ergosterol biosynthesis. Ergosterol is a unique fatty acid that is present only in the cell wall of fungi, and it is mandatory for their proper growth and survival. Therefore, the materials that disturb ergosterol in the fungal cell membrane render it dramatically permeable, which allows leakage of cellular constituents necessary for fungal cell survival [33] and an increase in reactive oxygen species concentration and oxidative stress, which, in turn, decreases the extracellular polymer matrix and capsular polysaccharide leading ultimately to fungal cell failure and death [34].

The adherent candida cell mean values of experimental group C specimens were higher than those of experimental group B specimens. This observation may be attributed to the leaching out of excessive thymol loading from the silicone specimens to achieve a more stable state; this could lead to a lower thymol content of the silicone specimen and, consequently, lesser antifungal efficiency [35].

The results of this test agreed with the study conducted to investigate the effect of incorporating pure natural thyme and nigella sativa oils in different concentrations (0.5%, 1%, 1.5%, and 2%) into the heat-cured acrylic resin denture base material. Their outcomes indicated a significant difference (P = 0.05), as both nigella sativa and thyme oil additives act as antimicrobial agents in the cured acrylic denture base [36].

In agreement with this work results, an earlier study had found a robust fungicidal effect of thymol and carvacrol against candida isolates [37].

This finding was also proven by an in vitro study conducted to evaluate the antifungal efficiency of thymol, carvacrol, and eugenol. The results showed that these herbal extracts inhibited fungal growth, with thymol demonstrating the maximum efficiency [38].

No previous work in the literature has studied the antifungal efficiency of thymol-modified maxillofacial silicone matrix. Therefore, in this study, we looked for the efficacy of thymol in inhibiting C. albicans adherence to the surface of maxillofacial silicone. This property is particularly promising in opening the gates towards improving patients' quality of life with maxillofacial defects.

5. Conclusion

In conclusion, this in vitro experimental study investigated the efficacy of thymol-modified maxillofacial silicone material in inhibiting the adhesion of C. albicans fungi to the surface of the silicone prostheses. Within the limitations of this study, thymol-modified silicone material exhibited significant potential against C. albicans adhesion. Silicone specimens of 0.75 wt.% thymol addition exhibited a more considerable inhibition in C. albicans adhesion than the other groups' specimens. However, further studies are required focusing on the thymol impact on other properties of the thymol-modified silicone material while considering the

patient-related factors before other recommendations can be made on using this modified silicone in standard clinical practice.

References

1- Yagthan Mohammed Haider, Zainab Salih Abdullah, Ghasak H Jani, and Norehan Mokhtar, "Evaluation of some mechanical properties of a maxillofacial silicon elastomer reinforced with polyester powder." *International Journal of Dentistry*, Vol. 2019pp. 1-6, (2019).

2- Ghassan Abdul-Hamid Naji, Mohammed Moudhaffer Ali, and Firas Abdulameer Farhan, "Physico-Mechanical Behavior of Room Temperature Vulcanized Maxillofacial Silicon after Addition of Glass Flakes." *Indian Journal of Forensic Medicine & Toxicology*, Vol. 14 (No. 1), pp. 1304-10, (2020).

3- Ahmed Aj AbdulKareem and Thekra I Hamad, "The effects of nano alumina on mechanical properties of room temperature vulcanized maxillofacial silicone (pilot study)." *Journal of Baghdad College of Dentistry*, Vol. 31 (No. 4), (2019).

4- Faiza Mohammed Hussain Abdul-Ameer, "Effect of plant-extract disinfectant solutions on the specific properties of reinforced maxillofacial silicone elastomers with nanofiller and intrinsic pigment." *European Journal of General Dentistry*, Vol. 9 (No. 02), pp. 55-61, (2020).

5- Mustafa S Tukmachi, Ihab N Safi, and Mohammed Moudhaffer M Ali, "Evaluation of mechanical properties and cytotoxicity of maxillofacial silicone material after incorporation of zirconia nanopowder." *Materials Today: Proceedings*, Vol. 42pp. 2209-17, (2021).

6- HADEEL I Ibrahim and FAIZA M Abdul-Ameer, "Influence of kappa-carrageenan powder addition on staphylococcus epidermidis adhesion on the room temperature vulcanized maxillofacial silicone." *Pak. J. Med. Health Sci*, Vol. 15p. 359, (2021).

7- Ding Li, Rui Xia, Qing Zhang, Changsen Bai, Zheng Li, and Peng Zhang, "Evaluation of candidemia in epidemiology and risk factors among cancer patients in a cancer center of China: an 8-year case-control study." *BMC infectious diseases*, Vol. 17pp. 1-8, (2017).

8- Shamsiahwati Mat-Rani, Natdhanai Chotprasert, Natchalee Srimaneekarn, and Suwan Choonharuangdej, "Fungicidal effect of lemongrass essential oil on Candida albicans biofilm pre-established on maxillofacial silicone specimens." *Journal of International Society of Preventive & Community Dentistry*, Vol. 11 (No. 5), p. 525, (2021).

9- Abayomi Sofowora, Eyitope Ogunbodede, and Adedeji Onayade, "The role and place of medicinal plants in the strategies for disease prevention." *African journal of traditional, complementary and alternative medicines*, Vol. 10 (No. 5), pp. 210-29, (2013).

10- Ruchika Agarwal and T Lakshmi, "Eucalyptus oil in dentistry: A mini Review." *Int. J. Drug Dev. Res*, Vol. 5pp. 58-61, (2013).

11- María Cecilia Martínez-Pabón and Mailen Ortega-Cuadros, "Thymol, menthol and eucalyptol as agents for microbiological control in the oral cavity: A scoping review." *Revista Colombiana de Ciencias Químico-Farmacéuticas*, Vol. 49 (No. 1), pp. 44-69, (2020).

12- Nor Azrini Nadiha Azmi, Amal Elgharbawy, Hamzah Mohd Salleh, and Adeeb Hayyan, "Evaluation of the antimicrobial performance of menthol and menthol-based deep eutectic solvents as potential future antibiotic." in *E3S Web of Conferences*, (2021), Vol. 287: *EDP Sciences*, p. 02010.

13- Ganiyat K Oloyede, "Toxicity, antimicrobial and antioxidant activities of methyl salicylate dominated essential oils of Laportea aestuans (Gaud)." *Arabian Journal of Chemistry*, Vol. 9pp. S840-S45, (2016).

14- Victor Kuete, Medicinal spices and vegetables from Africa: therapeutic potential against metabolic, inflammatory, infectious and systemic diseases. *Academic Press*, (2017).

15- Mohamed Fizur Nagoor Meeran, Hayate Javed, Hasan Al Taee, Sheikh Azimullah, and Shreesh K Ojha, "Pharmacological properties and molecular mechanisms of thymol: prospects for its therapeutic potential and pharmaceutical development." *Frontiers in Pharmacology*, Vol. 8p. 380, (2017).

16- Bahaa F Alanssari and Bayan S Khalaf, "Effect of Addition of Composite Polyamide Micro Particles and Silicone Dioxide NanoParticle on Some Mechanical Properties of Room Temperature Vulcanized Maxillofacial Silicone Elastomer Before and after Artificial Aging." *Indian Journal of Forensic Medicine & Toxicology*, Vol. 14 (No. 1), (2020).

17- Noor M Shihab and Faiza M Abdul-Ameer, "Studying some mechanical properties of maxillofacial silicone elastomer before and after incorporation of intrinsic pigments and artificial aging." *Future Dental Journal*, Vol. 4 (No. 2), pp. 244-52, (2018).

18- D Govindswamy, S Rodrigues, VK Shenoy, S Shenoy, R Shenoy, and T Yadav, "The influence of surface roughness on the retention of Candida albicans to denture base acrylic resins–An in vitro study." *Journal of Nepal Dentists Association-JNDA*, Vol. 14 (No. 1), (2014).

19- Aseel Riyadh Abdulwahhab and Raghdaa Kareem Jassim, "The effect of aloe vera extract on adherence of candida albicans and other properties of heat cure denture soft lining material." *International Journal of Medical Research & Health Sciences*, Vol. 7 (No. 3), pp. 94-103, (2018).

20- J Atta Allah and M Muddhaffer, "Influence of artificial weathering on some properties of nano silicon dioxide incorporated into maxillofacial silicone." *Indian Journal of Scientific Research*, Vol. 6pp. 423-28, (2017).

21- Abdalbasit A Fatihallah and Manar E Alsamaraay, "Effect of polyamide (Nylon 6) micro-particles incorporation into RTV maxillofacial silicone elastomer on tear and tensile strength." *Journal of Baghdad College of Dentistry*, Vol. 29 (No. 4), pp. 7-12, (2017).

22- Marcela Filié Haddad, Marcelo Coelho Goiato, Daniela Micheline dos Santos, Amália Moreno, Nuno Filipe D'almeida, and Aldiéris Alves Pesqueira, "Color stability of maxillofacial silicone with nanoparticle pigment and opacifier submitted to disinfection and artificial aging." *Journal of Biomedical Optics*, Vol. 16 (No. 9), pp. 095004-04-6, (2011).

23- ASTM Standard, "D624-00 (2012),"Standard Test Method for Tear Strength of Conventional Vulcanized Rubber and Thermoplastic Elastomers", ASTM International, West Conshohocken, PA, 2003, DOI: 10.1520/D0624-00R12." ed, (2012).

24- Huda Jaafar Naser and Faiza M Abdul-Ameer, "Evaluation of the Effect of Lemongrass Essential Oil on Candida Albicans Adhesion on Heat Cured Acrylic Based Soft Lining Material." (2023).

25- Moamin I Issa and Nabeel Abdul-Fattah, "Evaluating the effect of silver nanoparticles incorporation on antifungal activity and some properties of soft denture lining material." *Journal of Baghdad College of Dentistry*, Vol. 325 (No. 2219), pp. 1-15, (2015).

26- Jacqueline de Oliveira Zoccolotti *et al.*, "Properties of an acrylic resin after immersion in antiseptic soaps: Low-cost, easy-access procedure for the prevention of denture stomatitis." *PLoS ONE*, Vol. 13 (No. 8), p. e0203187, (2018).

27- Dalia Saad ElFeky, Noha Mahmoud Gohar, Eman Ahmad El-Seidi, Mona Mahmoud Ezzat, and Somaia Hassan AboElew, "Species identification and antifungal susceptibility pattern of Candida isolates in cases of vulvovaginal candidiasis." *Alexandria Journal of Medicine*, Vol. 52 (No. 3), pp. 269–77-69–77, (2016).

28- Alaa D Yasser and Nabeel Abdul Fatah, "The effect of addition of zirconium nano particles on antifungal activity and some properties of soft denture lining material." *Journal of Baghdad College of Dentistry*, Vol. 29 (No. 4), pp. 27-33, (2017).

29- Linda M Marler, Jean A Siders, and Stephen D Allen, Direct smear atlas: a monograph of gram-stained preparations of clinical specimens. *Lippincott Williams & Wilkins*, (2001).

30- GF Brooks, KC Carroll, J Butel, S Morse, T Mietzner, and M Jawetz, "Adelberg's medical microbiology. Sultan Qaboos Univ." *Med. J*, Vol. 7p. 273, (2007).

31- Melissa Alves *et al.*, "Unveiling the antifungal potential of two Iberian thyme essential oils: effect on C. albicans germ tube and preformed biofilms." *Frontiers in Pharmacology*, Vol. 10p. 446, (2019).

32- Ravinder Kaur, Megh Singh Dhakad, Ritu Goyal, Absarul Haque, and Gauranga Mukhopadhyay, "Identification and antifungal susceptibility testing of Candida species: a comparison of Vitek-2 system with conventional and molecular methods." *Journal of global infectious diseases*, Vol. 8 (No. 4), p. 139, (2016).

33- Ricardo Dias De Castro, Trícia Murielly Pereira Andrade de Souza, Louise Morais Dornelas Bezerra, Gabriela Lacet Silva Ferreira, Edja Maria Melo de Brito Costa, and Alessandro Leite Cavalcanti, "Antifungal activity and mode of action of thymol and its synergism with nystatin against Candida species involved with infections in the oral cavity: an in vitro study." *BMC complementary and alternative medicine*, Vol. 15 (No. 1), pp. 1-7, (2015).

34- Adam Kowalczyk, Martyna Przychodna, Sylwia Sopata, Agnieszka Bodalska, and Izabela Fecka, "Thymol and thyme essential oil—new insights into selected therapeutic applications." *Molecules*, Vol. 25 (No. 18), p. 4125, (2020).

35- Zinah Salah Mawlood, "The effect of adding citrus bergamia essential oil on Candida albicans Activity and some properties of a heat cured acrylic resin material." *University of Baghdad*, (2019).

36- Nadira A Hatim, Amer A Taqa, Wafa Abbas, and Arjwan M Shuker, "The Effect of thyme and nigella oil on some properties of acrylic resin denture base." *Al-Rafidain Dental Journal*, Vol. 10 (No. 2), pp. 205-13, (2010).

37- A Ahmad *et al.*, "Fungicidal activity of thymol and carvacrol by disrupting ergosterol biosynthesis and membrane integrity against Candida." *European journal of clinical microbiology & infectious diseases*, Vol. 30pp. 41-50, (2011).

38- Hakima Moussa *et al.*, "Action of thymol, carvacrol and eugenol on Penicillium and Geotrichum isolates resistant to commercial fungicides and causing postharvest citrus decay." *Canadian Journal of Plant Pathology*, Vol. 43 (No. 1), pp. 26-34, (2021).