Effects of Oregano-Extract on the Surface Hardness and Roughness of VST-30 Silicone

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Abstract

Background Disinfecting solutions might cause physical and chemical deterioration of silicone elastomers. Phytotherapy may be a viable disinfection option. However, its impact on the silicone material›s characteristics is uncertain. **Objectives** The present study aims to examine the effect of oregano essential oil as a disinfection solution on the surface hardness and surface roughness of VST-30 silicone. **Materials and Methods** Forty specimens were prepared and divided into four main groups (n=10), one control group and three experimental groups depend on immersion period of (1 day, 1 month, and 6 months. The experimental specimens were immersed in oregano solution. The surface hardness was measured using a (Shore A) durometer. While the surface roughness was measured with a digital portable surface roughness tester device and the surface characteristics were examined using Scanning Electron Microscopy (SEM). The data were statistically analyzed using one-way ANOVA and the post-hoc Tukey test ($p < 0.05$). **Results** A significant reduction in the surface hardness of the specimens was noticed after the immersion in an Oregano solution compared to the control group. While a non-significant difference in the surface roughness was reported after each immersion period. SEM images showed that the silicone after immersion shows a smooth surface with no evident cracks. **Conclusions** Oregano oil solution can be used as disinfectant material for VST-30 maxillofacial silicone with the least damage to the surface of the prosthesis.

Keywords: Disinfection; hardness; maxillofacial silicone;mregano oil; surface roughness.

Introduction

Unfortunately, ablative surgery, trauma, and congenital abnormality cause a substantial proportion of people to have facial abnormalities, (Manjula, 2017). Maxillofacial prostheses are commonly used to conceal facial abnormalities or deformities in individuals when surgery is not an option, (Guiotti et al, 2016a). Barnhart presented the first use of maxillofacial silicone for facial prostheses in 1960, the silicone is one of the materials that can be used for facial defects restoring due to its texture, strength, and durability, as well as the ease in which it can be handled and colored, and also for the patient comfort, (Montgomery and Kiat & Amnuay, 2010). Maxillofacial silicone elastomers should also possess tear resistance, tensile strength, and elongation at the break that are excellent, a hardness that is comparable to skin on the defect side, and enough bonding to the underlying retaining substrate, (Hatamleh and Watts, 2010). The loss of static and dynamic physical properties of elastomers, as well as discoloration of the prostheses in a service environment, are two important problems with maxillofacial prostheses used to rehabilitate patients with extra-oral-facial defects, (Goiato et al, 2009a). However Environmental exposure, such as ultraviolet (UV) light, air pollution, and changes in humidity and temperature, as well as handling, washing, and removal of the prosthesis, are the most common causes of prosthesis degradation, (Karakoca et al, 2010). The prosthesis should be disinfected daily for 3 to 5min by having the patients brushing their prosthesis gently. Disinfection is necessary not just to preserve the prostheses from microorganisms, but also to protect the surrounding tissues, (Goiato et al, 2010). To clean the prosthesis and avoid bacteria accumulation, disinfecting solutions containing alkaline peroxides, chlorhexidine solutions, and neutral soap are usually utilized, (Babu et al, 2018). Some researchers believe that digital friction, even when applied gently, causes the compounds incorporated into the elastomer matrix to detach, also cleaning silicone prostheses with chemical disinfection via immersion that has been proposed as an alternative but they all seem to influence the properties of an elastomer content, (Goiato et al, 2009a; Pesqueira et al, 2011; Eleni et al, 2013b). Since deterioration is directly linked to prosthesis aesthetics, leading to its replacement, the mechanical properties of maxillofacial elastomers must be similar to the facial part or tissue that is replaced and stay stable in performance, (Goiato et al, 2012). Disinfection may damage the material›s surface rather than its bulk. Thus, it could be interesting to look into not just an elastomer›s mechanical behavior but its surface alterations, (Hatamleh et al, 2011). The resistance and durability of the facial prosthesis are dependent on the hardness of the used material. Simultaneously, the material should be soft and flexible enough to allow the patient›s facial musculature to move freely, (Goiato et al, 2009a). Disinfection with Phytotherapy solutions may be suitable for maxillofacial silicone prostheses because the elastomers' properties can be preserved, (Guiotti et al, 2016b; Abdul-Ameer, 2020). Essential oils and plant extracts have been evaluated on a global scale as possible sources of new antimicrobial agents and alternatives to treat infectious diseases due to their antifungal, antibacterial, and antiviral effects, (Astani et al, 2010). Many studies evaluated the effect of plant extract solutions as a disinfectant solution on the hardness of maxillofacial silicone at different immersion periods, (Guiotti et al, 2016b; Tetteh et al, 2018; Jaffer, 2019). A study made by Eleni et al, in 2013 also evaluated the surface hardness of two maxillofacial silicone after disinfection, (Eleni et al, 2013a). Other studies were observed the surface roughness of the silicone material after different disinfection procedures, (Goiato et al, 2009b; Fouad and Moudhaffer, 2016; El Afandy and Fawzy, 2019). Origanum vulgare (Family- Lamiaceae) is an annual, perennial, and shrubby herb that is native to the Mediterranean, Euro-Siberian, and Irano-Siberian regions, (Vokou et al, 1993). The long-standing use of Origanum vulgare in traditional medicine has prompted interest in developing novel pharmacological formulations in a variety of fields. Despite there were many medicinal qualities that have been discovered in recent studies, including antibacterial, antiviral, antioxidant, and anti-inflammatory properties, (Oniga et al, 2018), but there was no previous search that studied the effect of this plant extract on the properties of maxillofacial silicone materials. So, the purposes of this study were to evaluate the effect of oregano essential oil as a disinfection solution on the surface hardness and surface roughness of VST-30 silicone elastomer.

Materials and Methods

A VST-30 Versiltal platinum-catalyzed, vinyl terminated RTV silicone elastomer was used to fabricate the study specimens (Factor II Inc., Lakeside, USA). The material was supplied in two parts, (A) was the base and (B) was the cross-linking agent catalyst. Forty specimens were fabricated and divided into four main groups (n=10), (I) is the control group without immersion, and the three other experimental groups divided according to immersion period (II) for one day, (III) for one month, and (IV) for six months.

Pilot study

The purpose of the pilot study was to identify the antibacterial activity of the Oregano oil and to establish the optimal concentration that prevents the growth of Staphylococcus aureus and Staphylococcus epidermidis. First, the oil was extracted by hydro-distillation using Clevenger apparatus, (Kulaksiz et al, 2018). The bacterial identification was made by the VITEK system, (Biomerieux, France), (Sulaiman et al, 2018), and the bacterial suspension was prepared in Mueller Hinton Broth, (Sutton, 2011). Agar-well diffusing test was used to determine the Minimum Inhibition Concentration (MIC) of the oil, (Kabbashi et al, 2015). Two-fold serial dilutions were made to obtain various concentrations of the essential oil to be distributed on the agar plate that was previously prepared and seeded with the bacterial inoculum. The concentrations of antimicrobial solution that inhibited the growth of the tested bacteria cultures were shown in table (1). The optimum concentration selected to be used in this study was 0.4%.

Specimens preparation

To obtain the specimens, Auto CAD 2015 computer software was used to design the mold dimensions, then by the aid of a plasma CNC (Computer Numerical Control) machine, the plastic matrices (Plexi, Korea) was made with holes that represent the square specimens› dimension of (25×25×6)mm width, length and thickness respectively, (Chi, 2014). The silicone was mixed in the proportion of 10:1 for the base and catalyst following the recommendation of the manufacturer’s instructions. The required amount of the base material was poured inside the bowl and placed in the vacuum mixer (Multivac 3 Degussa, Germany) for 5min in a clockwise direction and the vacuum was (28) inches HG according to the manufacturer’s instructions. The mixture was poured slowly and continuously until all the specimens holes were filled with silicone, then the cover was placed cautiously from one side and holding the other side away from the mold then slowly lay it over the mold. According to the manufacturer, the silicone was vulcanized after 30min. After complete vulcanization, the specimens were carefully removed from the mold, (Pinheiro et al, 2014). Flashes and excess silicone were trimmed away by using sterile scissors. All specimens were stored in a zip-lock plastic bag inside a custom-made light-proof box till testing, (Abdul-Ameer, 2020).

Conditioning of the specimens

The experimental specimens were subjected to disinfection by immersion in Oregano solution. The disinfection procedure was accomplished in three-time durations using simulating time of immersion, (Tetteh et al, 2018). 5min represents disinfection/1 day; 150min represents disinfection/1 month, and 900min represents disinfection/6 month.

Testing procedures

Hardness test

The hardness of maxillofacial silicone material was measured using the (Shore A) hardness tester durometer. The specimens were fabricated according to ISO 7619-1:2010 specifications. The measurement method is based on the penetration of the needle onto the surface of the material with a constant load of 5N, Figure (1.A). Three readings were collected for each specimen and the average value was calculated, (Çevik, 2018).

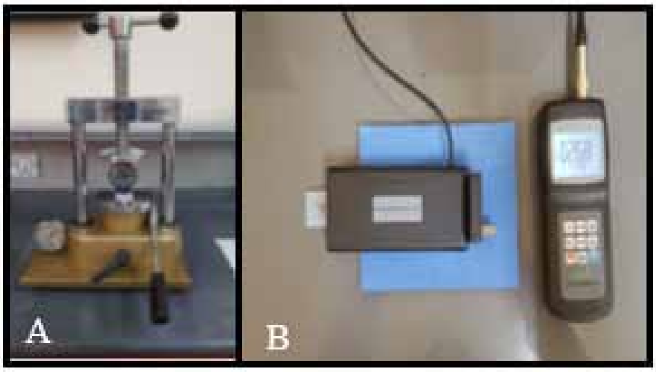

Figure (1): A, Shore A durometer surface hardness unit; B, Surface roughness Profilometer testing device.

Surface roughness test

Roughness is the measure of the finer irregularities of surface texture that are characteristic of the materials. Surface roughness average (Ra) is estimated as the arithmetic regular deviation of the surface valleys and peaks conveyed in micro-inches or micrometers, (Al-Dharrab et al, 2013). For the measurements of the surface roughness test, a portable digital roughness tester (6200S, China) was used with a 6mm measurement course, Figure (1.B), three readings were taken for each specimen then the average mean value was achieved, (Goiato et al, 2009a).

Scanning electron microscope (SEM)

SEM examination was established for one specimen from each group with ×500 magnifications with an accelerating voltage of 10kV (Inspect S50, USA), (Babu et al, 2018).

Statistical analysis

Data were analyzed by using Statistical Package for Social Science (IBM SPSS-V24) (SPSS, Chicago, Illinois, USA). One-way ANOVA and post-hoc Tukey test (HSD) was used to analyze the significant differences between the experimental and the control groups at a level of significance ($p \leq 0.05$).

Results

Figure (2) showed a decrease in the mean values for all the experimental groups with the highest mean value in the control group without immersion (I). The mean values of the experimental groups were highest in group (II) of 1 day/immersion, followed by group (III) of 1 month/immersion, while the lowest mean value was in the group (IV) of 6 months/immersion. ANOVA test shows that there was a significant difference between the non-treated and the experimental treated groups as in, Table (2). For further analysis, the post-hoc Tukey test (HSD) was used. It shows a significant difference among the non-treated and other treated groups, but there were non-significant differences between different immersion intervals, Table (3).

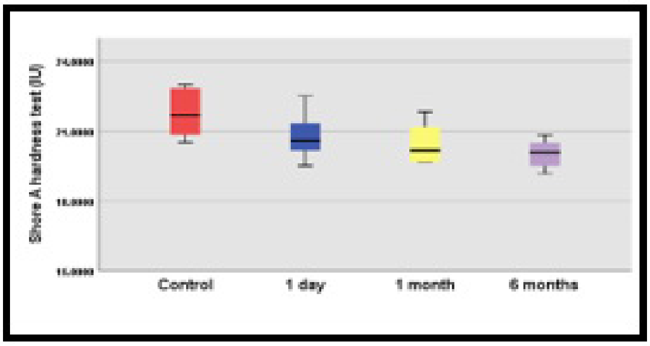

Figure (2): Box plot of Shore A hardness represents the mean values.

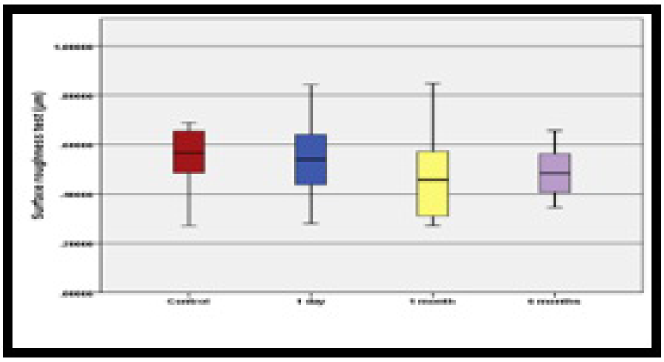

Figure (3): Box plot of surface roughness represents the mean values.

For the surface roughness, the higher mean value was in the group (II) of 1 day/immersion followed by non-treated group (I) than that of the group (IV) of 6 months/immersion, while group (III) of 1 month/immersion showed the least surface roughness mean value, Figure (3). In table (4), one-way ANOVA showed the non-significant differences among the studied groups. Finally, the SEM images showed that silicone surfaces were tended to get less roughness after immersion, Figure (4).

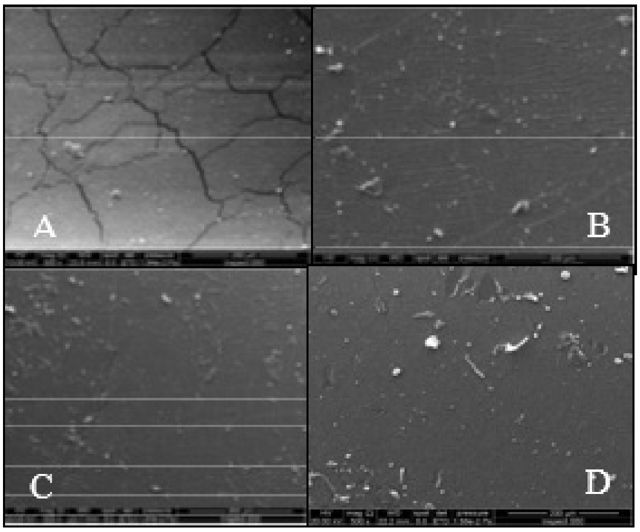

Figure (4): SEM image of VST 30 silicone elastomer, A, Control specimen; B, After immersion for 1 day; C, After immersion for 1 month; and D, After immersion for 6 months.

Table (1): Mean values of inhibition zone of Oregano at different concentrations.

Inhibition zone (mm)	Con.	100%	50%	25%	12.5%	6.25%	3.1%	1.6%	0.8%	0.4%	0.2%	0.1%	0.05%	DMSO
	S. aur.	45.33	51.33	57.66	41.66	41.00	29.00	26.66	14.33	10.00	0	0	0	0
	S. epi.	35.66	53.00	42.66	35.33	38.66	28.33	20.33	17.33	10.66	0	0	0	0

Table (2): One-way ANOVA for shore A hardness among the study groups.

	Sum of Squares	df	Mean Square	F-test	(*P*-value) Sig.
Between Groups	15.283	3	5.094	7.525	0.000 *S
Within Groups	24.372	36	0.677		
Total	41.555	39			

* S: Significantly different at $p \leq 0.05$

Table (3): Tukey HSD test of Shore A hardness test among the study groups.

Study groups		Mean Difference	Std. Error	*P*-value	Sig.
Group (I)	Group (II)	0.9999	0.3679	0.047	*S
	Group (III)	1.2499	0.3679	0.009	*S
	Group (IV)	1.6833	0.3679	0.000	*S
Group (II)	Group (III)	0.2499	0.3679	0.904	**NS
	Group (IV)	0.6833	0.3679	0.264	**NS
Group (III)	Group (IV)	0.4333	0.3679	0.645	**NS

*S: Significantly different at $p \leq 0.05$, **NS: Non-significantly different $p > 0.05$.

Table 4: One-way ANOVA for surface roughness of all study groups.

	Sum of Squares	df	Mean Square	F-test	(*p*-value) Sig.
Between Groups	0.036	3	0.012	0.493	0.689 **NS
Within Groups	0.886	36	0.025		
Total	0.923	39			

Discussion

Maxillofacial elastomers› hardness should be kept within a wide range of acceptable values. Given the varied toughness and hardness of the various facial regions, this range is about between 10 and 45, depending on the region of the face that needed to be replaced, (Eleni et al, 2013a). However, the researchers claim that this increased softness is advantageous and that the materials are suitable for facial prostheses even when the Shore A units are less than 25, (Goiato et al, 2009a). In this study, the hardness decreased significantly after immersion of the silicone specimens in the plant extract solution, this could be due to the decomposition of the cleaning solutions into carbon dioxide, carbon monoxide, and sulfur dioxide. That might cause changes in elastomers after disinfection with antimicrobial solutions, which could lead to either a harder or softer material, (Hatamleh and Watts, 2010). These findings concur with another study when they used Hydrastis Canadensis plant-extract solutions as an antimicrobial disinfectant and found that the hardness values were significantly decreased after immersion, (Guiotti et al, 2016b). Long-term storage of silicone materials, according to the literature, can enhance water absorption, resulting in reduced hardness, (Babu et al, 2018). This could explain the significant decrease in hardness values which can be due to disinfection solution absorption after the immersion period. These results may agree with Hatamleh et al, 2011, who found that after immersing silicone specimens in an antimicrobial silicone-cleaning solution for 30 hours, the hardness was reduced. Babu et al. in 2018 also found a general decrease in hardness values after (60 days) disinfection period due to absorption of the disinfection solution, (Hatamleh et al, 2011; Babu et al, 2018). Also, an agreement was found with Tetteh et al., in 2018 who found a significant decrease in hardness values of silicone after immersion in tea tree and Manuka oil solutions for different time periods, (Tetteh et al, 2018). On the other hand, a disagreement was found between the present study results and that concluded by Jaffer in 2019 who inferred a significant increase in hardness values after using three concentrations of the Misiwak extract as disinfecting material. This could be due to different silicone materials or different disinfection procedures and materials used as a disinfectant, (Jaffer, 2019). According to the findings, the values of hardness in this study are considered to be clinically acceptable. The results of surface roughness of the three experimental groups showed a decreased mean value of roughness when compared with the non-treated group except for that of 1 day/immersion that showed a slight increase in the surface roughness. It›s possible that this is attributable to the low concentration of the essential oils, as well as the fact that all of the ingredients in the natural product have a low and harmless influence. The results agree with other studies that found that surface roughness of the silicone material was decreased after different disinfection procedures, (Goiato et al, 2009b; Fouad and Moudhaffer, 2016; El Afandy and Fawzy, 2019). According to the immersion intervals, the results indicated a non-significant difference in the immersion period, this could be due to the low concentration of the essential oil. The silicone surface became less rough with time. This could be due to the completed polymeric chain that was promoted by the continuous polymerization process during immersion, (Goiato et al, 2009a). This is probably in agreement with Babu et al. in 2018 who found a significant decrease in surface roughness values after 60 days of storage in a chemical disinfectant solution, (Babu et al, 2018). These findings, on the other hand, may disagree with those of Al-Dharrab et al, in 2013, who found a significant increase in silicone surface roughness after 6 months of storage. This could be due to the long immersion time, which caused chemical structure degradation, micro-cracks, and pores on the material›s surface, (Al-Dharrab et al, 2013).

Conclusions

Within the limitations of this study, the surface roughness of VST-30 maxillofacial silicone was not significantly affected by the oregano plant-based solution as an antibacterial disinfectant, and even though the hardness was significantly decreased after the immersion, it was still within the acceptable clinical range.

Conflict of interest

We are the authors (Zainab Abid Ali Habeebullah, and Assist. Prof. Hawraa Khalid Aziz) state that the submitted manuscript for this paper is original. It has not been published previously, and it›s part of MSc. dissertation and is not under consideration for publication elsewhere, and that the final version has been seen and approved by all authors.

References

ABDUL-AMEER, F. M. H. 2020. 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, 9, 55-61.

AL-DHARRAB, A. A., TAYEL, S. B. & ABODAYA, M. H. 2013. The effect of different storage conditions on the physical properties of pigmented medical grade I silicone maxillofacial material. International Scholarly Research Notices journal, 2013. https://doi.org/10.1155/2013/582051

ASTANI, A., REICHLING, J. & SCHNITZLER, P. 2010. Comparative study on the antiviral activity of selected monoterpenes derived from essential oils. Phytotherapy Research journal, 24, 673-679. https://doi.org/10.1002/ptr.2955

BABU, A. S., MANJU, V. & GOPAL, V. K. 2018. Effect of chemical disinfectants and accelerated aging on maxillofacial silicone elastomers: An In vitro Study. Indian Journal of Dental Research, 29, 67. https://doi.org/10.4103/ijdr.ijdr_272_16

ÇEVIK, P. 2018. Evaluation of Shore A hardness of maxillofacial silicones: the effect of dark storage and nanoparticles. European oral research journal 52, 99. https://dx.doi.org/10.26650%2Feor.2018.469

CHI, Y., HSIN-. 2014. Effect of Silica Filler on the Mechanical Properties of Silicone Maxillofacial Prothesis.

EL AFANDY, H. M. & FAWZY, A. M. 2019. Evaluation of mechanical properties of maxillofacial silicone after long term exposure to different conditions. Egyptian Dental Journal, 65, 2681-2689. https://dx.doi.org/10.21608/edj.2019.72629

ELENI, P. N., KROKIDA, M. K., POLYZOIS, G. L. & GETTLEMAN, L. 2013a. Effect of different disinfecting procedures on the hardness and color stability of two maxillofacial elastomers over time. Journal of Applied Oral Science, 21, 278-283. https://dx.doi.org/10.1590%2F1679-775720130112

ELENI, P. N., PERIVOLIOTIS, D., DRAGATOGIANNIS, D. A., KROKIDA, M. K., POLYZOIS, G. L., CHARITIDIS, C. A., ZIOMAS, I. & GETTLEMAN, L. 2013b. Tensile and microindentation properties of maxillofacial elastomers after different disinfecting procedures. Journal of the mechanical behavior of biomedical materials, 28, 147-155. https://doi.org/10.1016/j.jmbbm.2013.07.013

FOUAD, M. & MOUDHAFFER, M. 2016. Effect of disinfection on some properties of heat-vulcanized maxillofacial silicone elastomer reinforced by nano silicone dioxide. Journal of Baghdad College of Dentistry, 28, 16-21 https://doi.org/10.12816/0033205

GOIATO, M. C., HADDAD, M. F., SINHORETI, M. A., DOS SANTOS, D. M., PESQUEIRA, A. A. & MORENO, A. 2010. Influence of opacifiers on dimensional stability and detail reproduction of maxillofacial silicone elastomer. Biomedical engineering online journal 9, 1-9. https://doi.org/10.1186/1475-925x-9-85

GOIATO, M. C., PESQUEIRA, A. A., MORENO, A., DOS SANTOS, D. M., HADDAD, M. F. & BANNWART, L. C. 2012. Effects of pigment, disinfection, and accelerated aging on the hardness and deterioration of a facial silicone elastomer. Polymer degradation and stability journal, 97, 1577-1580. https://doi.org/10.1016/j.polymdegradstab.2012.06.033

GOIATO, M. C., PESQUEIRA, A. A., SANTOS, D. M. D. & DEKON, S. F. D. C. 2009a. Evaluation of hardness and surface roughness of two maxillofacial silicones following disinfection. Brazilian oral research journal, 23, 49-53. https://doi.org/10.1590/s1806-83242009000100009

GOIATO, M. C., PESQUEIRA, A. A., SANTOS, D. M. D., ZAVANELLI, A. C., RIBEIRO, P. D.. 2009b. Color stability comparison of silicone facial prostheses following disinfection. Journal of Prosthodontics: Implant, Esthetic and Reconstructive Dentistry 18, 242-244. https://doi.org/10.1111/j.1532-849x.2008.00411.x

GUIOTTI, A. M., CUNHA, B. G., PAULINI, M. B., GOIATO, M. C., DOS SANTOS, D. M., DUQUE, C., CAIAFFA, K. S., BRANDINI, D. A., DE OLIVEIRA, D. T. N. & BRIZZOTTI, N. S. 2016a. Antimicrobial activity of conventional and plant-extract disinfectant solutions on microbial biofilms on a maxillofacial polymer surface. The Journal of prosthetic dentistry, 116, 136-143. https://doi.org/10.1016/j.prosdent.2015.12.014

GUIOTTI, A. M., GOIATO, M. C., DOS SANTOS, D. M., VECHIATO-FILHO, A. J., CUNHA, B. G., PAULINI, M. B., MORENO, A. & DE ALMEIDA, M. T. G. 2016b. Comparison of conventional and plant-extract disinfectant solutions on the hardness and color stability of a maxillofacial elastomer after artificial aging. The Journal of prosthetic dentistry, 115, 501-508. https://doi.org/10.1016/j.prosdent.2015.09.009

HATAMLEH, M. M., POLYZOIS, G. L., SILIKAS, N. & WATTS, D. C. 2011. Effect of extraoral aging conditions on mechanical properties of maxillofacial silicone elastomer. Journal of Prosthodontics: Implant, Esthetic and Reconstructive Dentistry, 20, 439-446. https://doi.org/10.1111/j.1532-849x.2011.00736.x

HATAMLEH, M. M. & WATTS, D. C. 2010. Mechanical properties and bonding of maxillofacial silicone elastomers. Dental Materials Journal, 26, 185-191. https://doi.org/10.1016/j.dental.2009.10.001

JAFFER, A. P. N. T. 2019. EFFECTOFSIWAK EXTRACTSON THE HARDNESSPROPERTY OF VINYL ADDITION CURED SILICONE MAXILLOFACIAL PROSTHETIC MATERIAL. Journal of Duhok University, 42-48. https://doi.org/10.26682/sjuod.2020.22.1.6

KABBASHI, A. S., MOHAMMED, S. E. A., ALMAGBOUL, A. Z. & AHMED, I. F. 2015. Antimicrobial activity and cytotoxicity of ethanolic extract of Cyperus rotundus L. American Journal of Pharmacy and Pharmaceutical Sciences, 2, 1-13.

KARAKOCA, S., AYDIN, C., YILMAZ, H. & BAL, B. T. 2010. Retrospective study of treatment outcomes with implant-retained extraoral prostheses: survival rates and prosthetic complications. The Journal of prosthetic dentistry, 103, 118-126. https://doi.org/10.1016/s0022-3913(10)60015-7

KULAKSIZ, B., SEVDA, E., ÜSTUNDAĞ-OKUR, N. & SALTAN-İŞCAN, G. 2018. Investigation of antimicrobial activities of some herbs containing essential oils and their mouthwash formulations. Turkish Journal of Pharmaceutical Sciences, 15, 370. https://dx.doi.org/10.4274%2Ftjps.37132

MANJULA, N. 2017. Effect of denture cleansers and accelerated aging on the color stability of maxillofacial silicone: An in vitro study. Indian Journal of Multidisciplinary Dentistry, 7. https://doi.org/10.4103/ijmd.ijmd_39_17

MONTGOMERY, P. C. & KIAT-AMNUAY, S. 2010. Survey of currently used materials for fabrication of extraoral maxillofacial prostheses in North America, Europe, Asia, and Australia. Journal of Prosthodontics: Implant, Esthetic and Reconstructive Dentistry, 19, 482-490. https://doi.org/10.1111/j.1532-849x.2009.00538.x

ONIGA, I., PUȘCAȘ, C., SILAGHI-DUMITRESCU, R., OLAH, N.-K., SEVASTRE, B., MARICA, R., MARCUS, I., SEVASTRE-BERGHIAN, A. C., BENEDEC, D. & POP, C. E. 2018. Origanum vulgare ssp. vulgare: Chemical composition and biological studies. Molecules journal 23, 2077. https://doi.org/10.3390/molecules23082077

PESQUEIRA, A. A., GOIATO, M. C., DOS SANTOS, D. M., HADDAD, M. F., RIBEIRO, P. D. P., COELHO SINHORETI, M. A. & SUNDEFELD, M. L. M. M. 2011. Effect of disinfection and accelerated aging on color stability of colorless and pigmented facial silicone. Journal of Prosthodontics: Implant, Esthetic and Reconstructive Dentistry, 20, 305-309. https://doi.org/10.1111/j.1532-849x.2011.00693.x

PINHEIRO, J. B., REIS, A. C., PISANI, M. X., LEITE, V. M. F., SOUZA, R. F., PARANHOS, H. F. O. & CLáUDIA HELENA, S.-L. 2014. Microstructural characterization and evaluation of the properties of polymeric materials for maxillofacial prosthetics. Journal of Medical Engineering Technology, 38, 67-75. https://doi.org/10.3109/03091902.2013.864715

SULAIMAN, I. M., BANERJEE, P., HSIEH, Y.-H., MIRANDA, N., SIMPSON, S. & KERDAHI, K. 2018. Rapid detection of Staphylococcus aureus and related species isolated from food, environment, cosmetics, a medical device, and clinical samples using the VITEK MS microbial identification system. Journal of AOAC International, 101, 1135-1143. https://doi.org/10.5740/jaoacint.17-0284

SUTTON, S. 2011. Measurement of microbial cells by optical density. Journal of Validation technology, 17, 46-49.

TETTEH, S., BIBB, R. J. & MARTIN, S. J. 2018. Mechanical and morphological effect of plant based antimicrobial solutions on maxillofacial silicone elastomer. Materials journal 11, 925. https://doi.org/10.3390/ma11060925

VOKOU, D., KOKKINI, S. & BESSIERE, J.-M. 1993. Geographic variation of Greek oregano (Origanum vulgare ssp. hirtum) essential oils. Biochemical Systematics and Ecology journal, 21, 287-295. https://doi.org/10.1016/0305-1978(93)90047-U