# "TO EVALUATE AND COMPARE THE EFFECT OF OREGANO OIL (ORIGANUM VULGARE) AND 1% CLOTRIMAZOLE ON ANTIMICROBIAL EFFICACY, AGAINST CANDIDA ALBICANS, STAPHYLOCOCCUS AUREUS AND COLOR STABILTY OF MAXILLOFACIAL SILICONE - AN IN-VITRO STUDY."


**By**

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# LIST OF ABBREVIATIONS USED IN THE STUDY

| ABBREVATIONS | FULL FORMS |
|---|---|
| COT | *Clotrimazole* |
| UV | Ultraviolet |
| ORAC | Oxygen radical absorbance capacity |
| RTV | Room temperature vulcanized |
| ASTM | American Society for Testing and Materials |
| ISO | International Organization for Standardization |
| Wt% | Percentage by weight |
| Ti | Titanium |
| Zn | Zinc |
| Ce | Cerium |
| SEM | Scanning electron microscope |
| TiO2 | Titanium dioxide |
| ZnO | Zinc oxide |
| CeO2 | Cerium oxide |
| BaSO4 | Barium sulphate |
| ANOVA | Analysis of Variance |
| N | Newton |
| MPa | Megapascal |
| Hz | Hertz |
| MIC | Minimum inhibitory concentration |
| CSNPs | Chitosan nanoparticles |
| RCT | Randomised control trial |

# ABSTRACT

## STATEMENT OF PROBLEM

Maxillofacial prosthesis is a reliable option for restoring any maxillofacial defects. A frequent problem incurred that is evident with the use of the materials is black discoloration of the inside surfaces of prosthesis after they have been worn for certain period of time. The presence of porosities, together with the modification of the anatomy of the facial tissues as a result of the lesion, may compromise the natural balance of the microbial flora, favoring microbial colonization.

## PURPOSE

To evaluate and compare the effect of Oregano oil on antimicrobial efficacy against *Candida albicans, Staphylococcus aureus* tear and colour stability of maxillofacial silicone after incorporating Oregano oil in it.

## MATERIALS AND METHODS

Sample size estimation was done using G power software. For antimicrobial properties against *Candida albicans Staphylococcus aureus,* the study group (n=66) and control group (n=66) both had 66 numbers in each group. Control group had two groups: Group 1 of Silicone+ 1%COT+ C. albicans (n=33) and Group 2 of Silicone +1% COT + S. aureus (n=33). While study groups: Group 3 of Silicone + Oregano oil + C. albicans (n=33) and Group 4 of Silicone + Oregano oil + s. aureus (n=33) . For color stability, both the study group and control group has 33 (n=33) in each group. Group 1 of Mdx4-4210 silicone elastomer+color pigments had (n=33) numbers while Group 2 under study group of silicone + oregano oil emulsion + color pigments had (n=33) numbers. The data was entered in Microsoft excel and analyzed using SPSS

(statistical product service solution / statistical package for social sciences) version 25. Descriptive characteristics of the sample was carried out in terms of mean and standard deviation. Normality of data was explored by Kolmogorov – Smirnov and Shapiro wilk test with $p$ value <0.05 as the reference for using parametric or non – parametric tests.

## RESULTS

The comparative effect of Origanum oil on antibacterial efficacy was superior to 1% Clotrimazole with a significant statistical difference. Origanum oil showed superior effect in comparison to 1% Clotrimazole.

## CONCLUSION

The present study and its findings have indicated that silicone elastomer is the material of choice for maxillofacial prosthesis. The clinical inertness, biocompatibility, mechanical properties and ease of manipulation make it a material of choice. Origanum oil has better resistance to the adhesion of *Candida albicans*, also reducing the fungal adherence and colonization without compromising its physical properties. The antimicrobial effects of the Oregano have been successfully utilized to inhibit the growth of several foodborne pathogenic bacteria. The production of aflatoxins and spores is initiated by Oregano; also an inhibitory effect against foodborne fungi thus inhibiting fungal growth.

**KEYWORDS:** Maxillofacial silicone elastomer, Origanum oil, Candida albicans, Staphylococcus aureus, colour stability.

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# INTRODUCTION

For the purpose of correcting maxillofacial deformities and enhancing quality of life, a maxillofacial prosthesis is regarded as an effective intervention option.[1] A maxillofacial prosthesis material must be simple to manufacture, resistant to manufacturing variations, and possess the physical as well as mechanical qualities similar to human tissue it is replacing.[2]

Clinical inertness, biocompatibility, mechanical qualities, and simplicity of handling are some of the inherent properties, hence the material of choice in maxillofacial prosthesis is silicone elastomer.[3] Silicone, also referred to as polydimethylsiloxane, is a polymer that is frequently utilized in the biomedical sector.[4] Barnhart made the first mention of silicone prosthetics in 1960.[5] The main objective of maxillofacial prosthesis is to restore appearance of the patient in order to boost self-esteem, confidence and support as normal a life as is feasible for the patients. Therefore, it is crucial to create prostheses only with best possible physical characteristics and aesthetics, and to maintain both their look and functionality during the service lifespan. Furthermore, silicone prosthetic care and durability remain a concern[6].

The longevity of a facial prosthesis made of silicone is 14.5 to 36 months. The main justification for replacing a facial prosthesis is a change in appearance brought on by changes in physical characteristics and color[6]. The silicone elastomers discoloration is the most typical cause for replacing facial prosthetics[7]. UV (ultraviolet) radiation has a significant impact. Fading of the color is attributed to the

external environmental conditions like weather; solar radiation, temperature, and moisture.[8,9]

A good maxillofacial prosthesis is fabricated using pigmentation and coloration. In clinics, the colorings both intrinsic and extrinsic are frequently employed to mimic the prosthesis to human oral tissues. Intrinsic coloring, which establishes the fundamental color and translucency, is less prone to damage from handling and the environment than extrinsic coloring, but it is more likely to alter the composition and characteristics of the mixture[10].

Black discoloration of the inner surfaces of prosthetics after being worn for a while has emerged as a problem with the usage of these materials. This issue affects nasal prostheses because of constant moist air, nasal secretions being constantly passing through nasal apertures, making them more prone to contamination. The major factors for replacing a facial prosthesis include color loss, attrition on the borders of the prosthesis, and staining that is caused by fungus.[11].

Additionally, the occurrence of porosities may impair the normal equilibrium of the microbial flora, favoring microbial colonization, along with the alteration of the facial tissue morphology brought on by the lesion. [12].

The frequency of major fungal infections has grown in recent years. Opportunistic mycoses, which are primarily caused by degenerative diseases like cancer and diabetes and are often treated with immunosuppressant, primarily affect those who have immunological suppression[13]. The yeasts mostly are involved in the etio-pathogenesis of mycotic illnesses includes *Candida* and the *Cryptococcus* genus[14]. Plants as natural sources for antimicrobial medications have received little

attention since the invention and extensive usage of synthetic antimicrobials in the previous century[14]. A large amount of scientific data has shown that essential oils and its constituents have a broad range of biological activity, making it significant in a number of fields[14].

The lamiaceae plant family in particular produces significant amounts of essential oil[15]. Biotypes of *Origanum* and other species are commonly utilized by the pharmaceutical industries due to their great variety of chemical properties and aroma[15]. At a range of altitudes, *Origanum* species are plentiful on stony slopes and steep mountain terrain (0-400 m) [16]. Along with more than fifty additional varieties, *O. vulgare L* (oregano) has been used in folk remedy to treat a variety of diseases[17].

Europe, Mediterranean, and southern and central Asian continents are all home to *Origanum* oil. The primary components, 4-terpineol, carvacrol, and thymol, which have the following effects, such as analgesic, antifungal, antiseptic, antitoxic, antiviral, and bactericidal, despite the presence with over 22 other chemicals. It prevents *Candida albicans* from forming new cells or expanding their mycelium. It exhibits improved resilience to *Candida albicans* adhesion and also decreases fungal adherence and colonization without significantly impairing the material's physical properties[18].

Oregano's antibacterial properties have been effectively used to stop the growth of a number of foodborne pathogenic bacteria. Aflatoxin and spore generation, in addition to fungal growth, have both been shown to be inhibited by its inhibitory action on foodborne fungus. Numerous developing parameters that influence the concentration of their essential oil and chemical make-up have an impact on the

proportion of monoterpenes, the main chemicals having biological activity, in oregano plants [19].

The volatile compounds in oregano's essential oil, particularly carvacrol and thymol, have proved to be the reason for the herb's antibacterial impact as well as individual components is what causes oregano's antimicrobial activity. In their 2012 study of the chemical makeup of *O. vulgare spp*. essential oil taken at southern Italy, Bonfanti et al. identified thymol and -terpinene as the primary constituents. The predominant antibacterial components were carvacrol (66%) and p-cymene (14%). The bacteriostatic or bactericidal effects, the survival/inactivation dynamics displayed depends on the essential oil concentration. The lag phase was significantly lengthened in subminimal inhibitory concentrations, but cell death was evident right away after exposure to the highest tested concentrations.

Oregano essential oil and extracts have been shown to be among the most potent antioxidants of all the popular herbs and spices in studies on their antioxidant activity. There are further reports on oregano antioxidants with phenolic and glucosidic structures that are distinct from thymol and carvacrol (Avila-Sosa et al., 2010a). LC-DAD-ESI/MS analysis by Lin et al. (2007) stated more than 20 flavonoids in Mexican oregano extract which used 15-5.0 L/mL) of essential oil.

Additionally, extracts from Mexican oregano have been reported to have antioxidant action seen in a study done by (Gonzalez-Güereca et al., 2007), as well as antimutagenic and antioxidant properties reported by (Martnez-Rocha et al., 2008). Olmedo et al. (2014) assessed the antioxidant activity of essential oil and molecularly distilled fractions of *Origanum vulgare L*. Due to the differences in their constituents;

the fractions distilled demonstrated better antioxidant properties than the residue fractions, due to limit lipid oxidation processes in sunflower oil, while the short-path molecular distillation fractions displayed greater antioxidant activity than whole essential oil. By measuring the oxygen radical absorbance capability, Bentayeb et al. (2014) assessed the antioxidant effects of oregano essential oil (ORAC). Thymol and carvacrol demonstrated 72-85% of oregano essential oil's antioxidant potential.[6]

Few studies have been conducted in the past, however none have examined the antibacterial effectiveness of oregano oil against different yeasts and the color stability of silicone used in the maxillofacial region. Therefore, the objective of this in vitro study is to assess Origanum oil's potential as an additive/inclusion to maxillofacial silicone prosthesis as well as its impact on mechanical properties including color stability. The null hypothesis stated that adding oregano oil had no impact on the antibacterial capabilities and color stability of maxillofacial silicone elastomer.

# NEED FOR THE STUDY

The best material for a maxillofacial prosthesis ought to be simple to process, insensitive to processing variables, and comprise all the necessary physical and mechanical qualities which mimic to those found in the human tissue it is restoring. For the purpose of correcting maxillofacial deformities and enhancing quality of life, a maxillofacial prosthesis is regarded as an effective therapeutic strategy. The clinical inertness, biocompatibility, mechanical characteristics, and simplicity of handling, silicone elastomer, these properties make this a preferred material for maxillofacial prostheses.

Polydimethylsiloxane, also called as silicone, a dental material frequently utilized in the field of biomedicine. Maxillofacial prosthesis ultimate goal is to enhance the patient's looks so that self-esteem/confidence can be elevated and the patient can lead a socially normal life. Therefore, it becomes crucial to create prostheses with the best possible physical characteristics and aesthetic, as well as to maintain their appearance and function during their lifespan.

However, there are concerns about the durability and maintenance of silicone prosthesis. Black staining of the inner surfaces of prosthesis after prolonged usage is a challenge which has emerged as a result of using these materials. This concern mainly affects nasal prostheses, particularly prone to contamination due to the continuous flow of moist air and nasal secretions through the apertures. Fungal proliferation is the cause of discoloration.

Germination and mycelial growth of Candida albicans are regulated/ inhibited by Origanum oil. It exhibits improved resilience to *Candida albicans* adhesion and also inhibits fungus attachment and colonisation without significantly impairing the material's physical qualities.

Oregano's antibacterial properties have been effectively used to stop the proliferation of a range of foodborne pathogenic bacteria. Its inhibitory impact has also been shown to be effective against fungi that are foodborne, preventing the development of aflatoxins and spores in addition to fungal growth. Particularly carvacrol and thymol, have particularly shown to be accountable for antimicrobial impact of oregano as well as individual components, are two volatile chemicals present in its essential oil that are mainly accountable for the antimicrobial property of oregano. In this study, the antibacterial effectiveness of oregano oil against *Candida albicans, Staphylococcus aureus,* and color stability of maxillofacial silicone after oregano oil incorporation were evaluated and compared.

**Operational definition**

Maxillofacial prosthetics: A discipline of prosthodontics known as maxillofacial prosthetics focuses on treating people who have congenital abnormalities or developmental problems.[3]

Silicones: Maxillofacial prosthetics employ silicones. It typically has a putty-like consistency and is a white, opaque substance. It can be supplied as putty with one component or two components[13].

Oregano oil: In addition to having antiviral and antifungal characteristics, oregano oil is well known for its antibacterial action. They are effective anti-inflammatory, anti-cancer, anti-antioxidant, and anti-diabetic substances[5].

# HYPOTHESIS

**NULL HYPOTHESIS:**

The antimicrobial properties and color stability of the maxillofacial silicone elastomer are not significantly altered by the addition of oregano oil.

**ALTERNATIVE HYPOTHESIS:**

The antimicrobial properties and color stability of silicone elastomer used in maxillofacial prosthesis are significantly impacted by the addition of oregano oil.

# AIM AND OBJECTIVES

## AIM

To evaluate and compare the effect of Oregano oil on antimicrobial efficacy against *Candida albicans*, *Staphylococcus aureus*, and assess the color stability of maxillofacial silicone after incorporating Oregano oil.

## OBECTIVES

1. To evaluate antifungal and antimicrobial efficacy of silicone impregnated with 1% Clotrimazole and different concentrations of oregano oil against *C. albicans* and *Staphylococcus aureus.*
2. To evaluate colour stability of maxillofacial silicone impregnated with different concentrations of oregano oil and colour pigments.
3. To compare antifungal and antimicrobial efficacy of silicone impregnated with 1% Clotrimazole and different concentrations of oregano oil against *C. albicans* and *Staphylococcus aureus.*
4. To compare the colour stability of maxillofacial silicone impregnated with different concentrations of oregano oil and colour pigments.

# REVIEW OF LITERATURE

1) **Andres CJ et al. (1992)**[1] carried out a comprehensive review regarding the physical property testing of elastomers used in maxillofacial prosthetics, to find an ideal material or combination of materials to treat patients with disfigured or missing facial features or body parts. The goal of this review was to identify the materials currently being used for these procedures using a global survey. The study also sought input on the benefits and drawbacks of the materials now in use, techniques for intrinsic and extrinsic colouring, and the qualities that an "ideal" material ought to have. They came to the conclusion that the majority of maxillofacial prosthodontists and prosthetists fabricate extraoral prosthesis utilizing RTV silicone goods. The most widely used substance was MDX4-4210. Most survey participants used kaolin as an opacifier together with dry earth paints, artist's oil colors, and colored fibers to try and mimic the intrinsic colouring of the skin around the defect. Extrinsic color was often created by combining Medical Adhesive type A substance with xylene as a thinner or solvent, coloring with dry earth pigments or artist's oils, and then immediately applying the combination to the finished prosthesis. With very few instances, manufacturers have shown minimal interest in maxillofacial prosthetic material research due to the niche markets. The need for extra - oral prosthesis treatments will rise as the community gets older. As more maxillofacial prosthodontists and prosthetists provide treatments, the demand for extra oral prosthetic materials will increase. With assistance from the proper authorities and producers, extensive research and development of

materials or combinations of materials that meet the requirements of patients and the profession are required.

2) Pigno MA et al. (1992)2 conducted a study with the following goals: (1) to determine whether fungus growth is connected to the emergence of the black discoloration; (2) to determine whether adding antifungal agents to silicone would stop the fungus from growing in a test tube; and (3) to determine the longevity of the antifungal effect. The findings of this study imply that clotrimazole may be used in nasal prosthesis to stop or delay the growth of fungi, which would stop or delay any accompanying discoloration. The prosthesis would last longer and experience fewer complaints as a result. The effectiveness of clotrimazole in avoiding fungal development and discolouration when the prostheses are subjected to the conditions of daily usage must be determined by clinical research. It might be wise to look at further means of preventing fungus from growing on silicone prosthetics. Microbiologic methods and SEM analyses led to the finding that parts of a nasal prosthesis that were discoloured were linked to a fungus from the species Penicillium. The addition of clotrimazole to in vitro silicone samples worked well to stop the fungus's development. The fact that the clotrimazole samples continued to prevent fungal growth repeatedly shows that they are stable and long-lasting when kept at room temperature. As evidence that regular washing would not significantly lessen the antifungal effect, samples kept in water continued to suppress fungal growth. Under these test settings, nystatin showed no inhibitory effects.

3) A research was conducted by ANDREOPOULOS AG et al. in 19943 to assess different reinforcements for maxillofacial silicone elastomers. The following results were drawn: It was not advised to reinforce silicone elastomers with ultra-high modulus fibres to improve mechanical qualities. 2. Silica loading up to 35% enhanced mechanical strength, which thereafter declined most likely as a result of inadequate dispersion and high network density. The amount of silica and the kind of solvent used had an impact on how much reinforced samples swelled.

4) In a three-part investigation, Haug SP et al. (1997)4 evaluated the impact of colouring agents on the physical characteristics of maxillofacial elastomers in the first part. Five dumbbell-shaped and five trouser-shaped specimens were made for each combination of the three elastomers (Silastic medical adhesive type A, Silastic 4-4210, and Silicone A2186) and the six colourants (dry earth pigments, rayon fibre flocking, artist's oil paints, kaolin, liquid cosmetics, and no-colorants) in this study, for a total of 180 specimens. The specimens in the trouser and dumbbell shapes were used to measure hardness and tear strength, respectively, while the specimens in the dumbbell and trouser shapes were used to measure ultimate tensile strength and % elongation. For each of the 4 physical attributes, elastomer analysis used a one-way ANOVA to examine the 6 colourants. It was discovered that the addition of colouring compounds altered the physical characteristics of maxillofacial elastomers. Artists' oils and liquid cosmetics operated as a liquid phase without connecting to the silicone matrix, while dry earth pigments, kaolin, and rayon flocking served as solid fillers without adhering to the silicone. The investigation came to the

conclusion that none of the experiments in the current study clearly revealed a superior colorant-elastomer combination.

5) In a three-part research, Haug SP et al. (1997)5 evaluated how popular colorant-elastomer combinations' physical qualities changed as a result of exposure to the weather. 540 different species in all were employed in this investigation. For each of the three elastomers, 15 specimens in the shapes of dumbbells and 15 in the form of pants were created (Silastic medical adhesive type A, Silastic 4-4210, and Silicone A-2186). There are six different colourant combinations (dry earth pigments, flocking of rayon fibre, kaolin, liquid cosmetics, and no colours). Each elastomer colourant combination's 15 dumbbell- and trouser-shaped specimens were divided into 5 of each shape for the three test condition groups (control, time passage, and natural weathering). Within a month, control specimens were assessed. Before testing, the time passage group was kept in the dark and enclosed in glass containers for six months. The groups subjected to natural weathering were put on the dentistry school's roof and left there for six months. On specimens that were fashioned like pants, the hardness and rip strength were assessed, and on specimens that were shaped like dumbbells, the ultimate tensile strength and % elongation were assessed. It was discovered that the physical characteristics of several colorant-elastomer combinations have been exposed to weathering and temporal changes, indicating that a clinical prosthesis's attributes might alter with time. The investigation came to the conclusion that colouring the silicones changed how they responded to weathering. Additionally, the silicones were not as stable as had been believed.

6) To assess the colour stability of colorant-elastomer combinations as a result of exposure to weathering, Haug SP et al. (1997)6 carried out a three-part investigation. A total of 270 specimens (18 groups of 15 specimens) were used in this study. Fifteen specimens were made for each of the three elastomers (Silastic medical adhesive type A, Silastic 4-4210, and Silicone A-2186) and the six colourants (dry earth pigments, rayon fibre flocking, artist's oil paints, kaolin, liquid cosmetics, and no colourant). Each elastomer-colorant combination's 15 samples were divided into 3 groups of 5 samples each under the test conditions of control, time passing, and natural weathering. Within a month of production, control specimens were assessed. Before testing, the time passage group was kept in the dark and enclosed in glass containers for six months. The groups subjected to natural weathering were put on the dentistry school's roof and left there for six months. They discovered that several of the colorant-elastomer combinations showed colour variations brought on by weathering. Both coloured and uncolored materials experienced a gradual change in colour over time without being subjected to weathering. According to the study's findings, when subjected to weathering, the addition of colourants may have a stabilising influence on the elastomer's colour.

7) A research on the antifungal effects of origanum oil was undertaken by Manohar et al. (2001)7 in both in vitro and in vivo settings. In broth cultures, a micro dilution approach, and comparative effectiveness tests with origanum oil, carvacrol, nystatin, and amphotericin B were utilised to treat Candida albicans. The growth of Candida albicans in culture was entirely inhibited by origanum oil at a concentration of 0.25 mg/ml. At concentrations of 0.125 mg/ml and 0.0625 mg/ml, respectively, growth inhibitions of 75% and more

than 50% were seen. Additionally, it was discovered that origanum oil and carvacrol suppressed C. albicans germination and mycelial development in a dose-dependent manner. An experimental mouse systemic candidiasis model was used to investigate the therapeutic effectiveness of origanum oil. Six groups of C. albicans-infected mice (n = 6) were fed varied doses of origanum oil in 0.1 ml of olive oil as a final volume (vehicle). In comparison to the group of mice fed olive oil alone, which perished after 10 days, the daily administration of 8.6 mg of origanum oil in 100 l of olive oil/kg body weight for 30 days produced 80% survival with no renal burden of C. albicans. Carvacrol produced outcomes that were comparable. However, mice given origanum oil had a more appealing clinical look than mice treated with carvacrol. The findings of this study suggest more research into origanum oil's effectiveness.

8) Aziz T et al. (2002)8 conducted a research to evaluate the qualities of several silicone rubber maxillofacial materials that are available commercially. In dental flasks, specimens of five frequently utilised maxillofacial materials were created. The following properties were evaluated: hardness, water absorption, water contact angles, % elongation, tear strength, and tensile strength. It was discovered that Factor II, Cosmesil HC, and Nusil all had tear strengths that were equivalent to and much greater than those of Cosmesil St and Prestige (p, 0.001). Cosmesil St and Cosmesil HC were much tougher than the other materials, while Nusil had a significantly greater tensile strength and elongation (p, 0.001). (p, 0.001). Compared to the other materials, Factor II was substantially less wet, whereas Prestige and Cosmsesil St had a significantly greater water absorption. They came to the conclusion that one of

the silicone rubber products that was commercially accessible had the perfect characteristics for usage in maxillofacial prosthetics. Factor II shown better qualities because to its high tear strength, softness, and ease of manipulation.

9) Lai et al. (2003)9 utilised an invite research to evaluate four materials frequently used for gingival flange prosthesis in terms of colour stability, stain resistance, and water sorption. 45 cylindrical discs (15 mm in diameter and 10 mm thick) were made from two heat-polymerized acrylic resins, one silicone (Gingiva moll), and one co-polyamide (QC-20 and Vertex). After being submerged in coffee and tea staining solutions for 7, 14, 30, 60, 120, and 180 days, ten samples of each material were examined by a spectrophotometer. Control samples were submerged in water and were exposed to the air. The variations in colour before and after storage were evaluated. After 56 days, the water sorption of an additional 5 samples of each substance was assessed. It was discovered that the co-polyamide (4.22, 0.40, P.001), two acrylic resins, and coffee solution created the silicone material's greatest discolouration value (7.31, 0.57, P.001). The co-polyamide stained the most in the tea solution (2.74, 0.56), but the other components just slightly changed colour (P.001). When kept in air and water for six months, all the materials under investigation retained their colour. Co-polyamide material had the highest water absorption, whereas silicone had the lowest absorption (P.001). All flange materials showed colour stability in both air and water, it was determined. However, silicone and co-polyamide materials that were kept in coffee solution for 180 days had undesirable colour changes that exceeded 3 NBS (National Bureau of Standards) units.

10) **Han Y et al. (2007)[10]** carried out a study with to evaluate the effect of different concentrations of nanosized oxides of various composition on the mechanical properties of a commercially available silicone elastomer. In this study, nanosized oxides (Ti, Zn, or Ce) were added in various concentrations (0.5%, 1.0%, 1.5%, 2.0%, 2.5%, or 3.0% by weight) to a commercial silicone elastomer (A-2186), commonly used for fabricating extraoral maxillofacial prostheses. Silicone elastomer A-2186 without nanosized oxides served as a control group. Specimens (n=5) were polymerized according to manufacturer's recommendations and tested for tensile strength (ASTM D412) and tear strength (ASTM D624), and percent elongation in a universal testing machine. Uniformity of particle dispersion within the processed elastomer was assessed using scanning electron microscopic imaging. For each property, a 2-way ANOVA was performed evaluating the effect of oxide type and strength, and Fisher's PLSD test was used for pairwise comparisons ($\alpha$=.05). They found that SEM examination indicated that all 3 nanosized oxides distribute evenly throughout the silicone specimens, except for the 3.0% group, which are partly agglomerated. The 2.0% and 2.5% groups of all nanosized oxides demonstrated significantly higher tensile and tear strengths and percent elongation percent among the type of nanosized oxides. It was concluded that overall mechanical properties of the silicone A-2186 maxillofacial elastomer significantly improved with inclusion of Ti, Zn, or Ce nano-oxides at concentrations of 2.0% and 2.5%.

11) **Soylu S et al. (2007)[11]** conducted a study to assess antifungal effects of the essential oils obtained from medicinal plant oregano (Origanum syriacum L. var. bevanii), and fennel (Foeniculum vulgare Mill.) against S. sclerotiorum.

The broad aims of this study were (i) to investigate antifungal effects of the essential oils on hyphal growth on Petri plates and on the viability of sclerotia of S. sclerotiorum in soils, (ii) to assess potential biocontrol capacities of the essential oils against disease suppression in vivo conditions and (iii) to reveal effects of the essential oils on morphological structures of fungal hyphae and sclerotia under light and scanning electron microscopes (SEM). In this study, Inhibitory effects of volatile and contact phases of the essential oils used were determined on hyphae and sclerotia. Both essential oils have a marked antifungal effect against S. sclerotiorum. Soil amendment with essential oils has significant effect on reducing sclerotial viability. Both essential oils significantly inhibited the fungal growth in soil, thereby increasing the number of surviving tomato seedling by 69Æ8% and 53Æ3%, respectively. Light and SEM observations on pathogen hyphae and sclerotia revealed considerable morphological alterations in hyphae and sclerotia. They concluded that the significant reduction in the mycelial growth and germination of sclerotia would greatly reduce the pathogen inoculum source. This may influence the rate of disease development in soil**.**

12) **Hukins et al. (2008)**[12] carried out review of literature to check the application of elevated temperature of the so-called “accelerated aging”, applied to polymers that are associated with medical devices to consider the application of elevated temperature as a means of simulating the aging process, so-called “accelerated aging”, applied to polymers that are associated with medical devices to consider the application of elevated temperature as a means of simulating the aging process, so-called “accelerated aging”, applied to polymers that are associated with medical devices. They concluded that it is

commonly assumed that elevated temperature can be used as a method of accelerated aging. Elevated temperatures have been used to investigate the effects of aging on several polymers that are implanted into the body, especially silicones. The results of different studies on silicones do not all agree but this may well be because different silicones were used in the various investigations. Finally, in vivo aging may involve effects that are not simulated by an in vitro test. Even if samples are maintained in buffered physiological saline solution, they are not subjected to all the chemical interactions (e.g. with fats, proteins, calcium ions, etc.), or with cells that may modify their environment. This chemical environment will also be different in different parts of the body. It is not possible to simulate all the conditions that will be encountered in vivo so that, "the ultimate test of the success of any replacement material must be its long-term performance in the human body".

13) **Han Y et al. (2010)[13]** carried out a research to assess the effect on color stability using nano-oxides in pigmented silicone A-2186 maxillofacial prosthetic elastomers before and after artificial aging. In this study, each of three widely used UV-shielding nano-sized particle oxides (TiO2, ZnO, CeO2), based on recent survey of the industry at 1%, 2%, 2.5% concentrations were combined with each of five intrinsic silicone pigment types (no pigments, red, yellow, blue, and a mixture of the three pigments). Silicone A-2186 without nano-oxides or pigments served as control, for a total of 46 experimental groups of elastomers. In each group of the study, all specimens were aged in an artificial aging chamber for an energy exposure of 450 kJ/m2. CIE L*a*b* values were measured by a spectrophotometer. The 50:50% perceptibility (DE* = 1.1) and acceptability threshold (DE* = 3.0) were used

in interpretation of recorded colour differences. Colour differences after aging were subjected to three-way analysis of variance. Means were compared by Fisher's PLSD intervals at the 0.05 level of significance. They found that yellow pigments mixed with all three nano-oxides at all intervals increased DE* values significantly from 3.7 up to 8.4. When mixed pigment groups were considered, TiO2 at 2%, and 2.5% exhibited the smallest colour changes, followed by ZnO and CeO2, respectively ($p < 0.001$). At 1%, CeO2 exhibited the smallest colour changes, followed by TiO2 and ZnO, respectively ($p < 0.001$). The smallest colour differences, observed for nano-oxides groups, were recorded for CeO2 at 1%, and TiO2 at 2% and 2.5%. When the nano-oxides were tested at all concentrations, CeO2 groups overall had the most colour changes, and TiO2 groups had the least. All DE* values of the mixed pigment groups were below the 50:50% acceptability threshold (DE* = 1.2–2.3, below 3.0) except 2% CeO2 (DE* = 4.2). They concluded that 1% nano-CeO2 and 2% and 2.5% nano-TiO2 used as opacifiers for silicone A-2186 maxillofacial prostheses with mixed pigments exhibited the least colour changes when subjected to artificial aging at 450 kJ/m2. Yellow silicone pigment mixed with all three nano-oxides significantly affected colour stability of A-2186 silicone elastomer.

14) **Souza NAR et al. (2010)[14]** carried out study to check the efficacy of O. vulgare L. and O. majorana L. essential oil in inhibiting the growth and survival of potentially pathogenic fungal strains and also sought to evaluate the possible mechanisms involved in the establishment of the antifungal property of the tested essential oils through assays of osmotic stability and morphogenesis. Test strains included in this study were Candida albicans

ATCC 7645, C. tropicalis LM-14, C. krusei LM-09, Cryptococcus neoformans FGF-5, Aspergillus flavus LM-02, A. fumigatus IPP-21, T. rubrum ATCC 28184, T. mentagrophytes LM-64, Microsporum gypseum ATCC 184, M. canis LM-36 and Cladosporium herbarium ATCC 26362. O. vulgare essential oil presented a MIC value of 80 µL/mL, while for O. majorana this was 160 µL/mL. C. krusei LM-09 was the only strain resistant to all assayed concentrations of both essential oils. O. vulgare and O. majorana essential oil at their MIC values provided a cidal effect against C. albicans ATCC 7645 after 4 h of exposure. O. vulgare essential oil at 80 µL/mLexhibited 100 % inhibition of the radial mycelia growth of T. rubrum ATCC 28184 and M. canis LM-36 for 14 days. Assayed fungus strain protected by sorbitol (osmo-protectant agent) grew in media containing higher concentrations of O. vulgare and O. majorana essential oil in comparison to media without sorbitol, suggesting some specificity of these essential oils for targeting cell wall in the fungi cell. Main morphological changes observed under light microscopy provided by the essential oil of O. vulgare in A. flavus LM-02 were decreased conidiation, leakage of cytoplasm, loss of pigmentation and disrupted cell structure indicating fungal wall degeneration. These results suggested essential oils from Origanum could be regarded as a potential antifungal compound.

15) **Haddad MF et al. (2011)**[15] conducted a research to evaluate the colour stability of a maxillofacial elastomer with the addition of a nanoparticle pigment and/or an opacifier submitted to chemical disinfection and artificial aging. Specimens were divided into four groups (n = 30): group I: silicone without pigment or opacifier, group II: ceramic powder pigment, group III: Barium sulfate (BaSO4) opacifier, and group IV: ceramic powder and BaSO4

opacifier. Specimens of each group (n = 10) were disinfected with effervescent tablets, neutral soap, or 4% chlorhexidine gluconate. Disinfection was done three times a week during two months. Afterward, specimens were submitted to different periods of artificial aging. Colour evaluation was initially done, after 60 days (disinfection period) and after 252, 504, and 1008 h of artificial aging with aid of a reflection spectrophotometer. Data were analyzed by three-way ANOVA and Tukey test (α = 0.05). The isolated factor disinfection did not statistically influence the values of colour stability among groups. The association between pigment and BaSO4 opacifier (GIV) was more stable in relationship to colour change. All values obtained, independent of the disinfectant and the period of artificial aging, were considered acceptable in agreement with the norms presented in literature.

16) **Srivastava A et al. (2013)**[16] carried out a study with an aim to investigate the antifungal activity and properties of a tissue conditioner by incorporating origanum oil. In this study, Origanum oil at varying concentrations was incorporated into a poly (methyl methacrylate) based tissue conditioner (Visco-gel), and its antifungal activity against Candida albicans was evaluated at 1 day and 1 week by using the agar punch well method. The adherence of Candida albicans, surface roughness, tensile strength, and bond strength of the tissue conditioner with an optimized origanum oil concentration were evaluated. The data were subjected to 2-way ANOVA (p=.05). They found that sixty vol% origanum oil in tissue conditioner (Visco-gel) showed a mean inhibitory zone of 21.00 ±1.58 mm at 1 day and 13.44 ±0.88 mm at 1 week. The control group showed 90 ±6.80 yeast cells/mm2 at 1 day and 165 ±7.63 yeast cells/mm2 at 1 week, whereas the group with origanum oil showed 16

±1.15 yeast cells/mm2 at 1 day and 32 ±4.00 yeast cells/mm2 at 1 week. Surface roughness was less with the incorporation of origanum oil. Tensile strength at 1 day was 0.91 ±0.52 N for the control group, whereas the group with origanum oil showed 0.16 ±0.05 N. At 1 day, the bond strength of 3.97 ±0.75 MPa was observed with control specimens, whereas tissue conditioner with origanum oil showed a bond strength of 3.73 ±0.65 MPa. They concluded that within the limitations of this in vitro study, origanum oil can be used as an additive to tissue conditioner to reduce the adherence of Candida albicans without significantly affecting its bond strength to heat-polymerized acrylic resin.

17) **Cevik P et al. (2014)**[17] carried out a study with aim to evaluate the effect of different types of silica and nanosized titanium dioxide addition on the mechanical properties of two RTV silicone elastomers. In this study, A-2000 and A-2006 silicone elastomers were used, and each was divided into four subgroups (n = 5). The first group was the control without additives. Other groups were titanium dioxide, fumed silica, and silaned silica. Each specimen was prepared in compliance with the manufacturer's instructions for the tensile strength, percent elongation, tear resistance, and the hardness tests according to ISO and ASTM standards. A factorial ANOVA with pairwise interaction indicated that the pattern for all four outcomes of the materials was different for A-2000 and A-2006 ($p < 0.05$). Therefore, the average outcome values for the materials within silicone elastomers were then analyzed by Tukey HSD. For the hardness test results, Kruskal-Wallis and Mann-Whitney U test methods were used. The level of statistical significance was $p < 0.05$. They found that there was a statistically significant interaction ($p < 0.05$)

between materials and silicone type for all four tests (tensile strength, tear, hardness, percent elongation). The hydrophobic silica group had significantly higher tensile strength than TiO2 for A-2000. The fumed hydrophilic silica group had significantly higher tensile strength than TiO2 for A-2006. Most of silica specimens had higher tensile strength when compared with the control and TiO2 groups for A-2000 and A-2006 silicones. The TiO2 group had the highest hardness value for A-2000 while the lowest hardness value for A-2006 ($p < 0.05$). There was no significant difference of tear strength among the type of additives ($p > 0.05$) for A-2000. The fumed silica and TiO2 groups had significantly higher tear strength than the control group for A-2006. The fumed silica and the hydrophobic silica groups had significantly higher percent elongation than the control group ($p < 0.05$) for A-2000. The TiO2 group had the lowest percent elongation for A-2006. They concluded that results in this in vitro study may clarify future studies about the effect of different additives on the physical and mechanical properties of maxillofacial elastomers. There is a great interest in the effect of a new-generation hydrophobic silica incorporation into A-2000 silicone as well as the effect of fumed hydrophilic silica incorporation into A-2006 silicone. Future research should be supported with more in vitro trials in different percentages of such additives used in this study.

18) **Hu X et al. (2014)**[18] carried out a study with the purpose to investigate the effects of the type and concentration of intrinsic pigments on the dynamic mechanical properties of a commercially available maxillofacial silicone elastomer over a small range of low frequencies. In this study, ten pigmented mixtures (6 specimens per mixture) were made by using a base silicone

elastomer mixed with each intrinsic silicone pigment (Black, Red, Tan, or Yellow) or all the pigments (Mix All) in a designated high or low concentration. The base elastomer without pigment (Unpigment) was prepared as a control. Dynamic mechanical analysis was performed over 5 low frequencies (0.5, 1.0, 1.5, 2.0, and 2.5 Hz) at room temperature. The storage modulus (E0), loss modulus (E00), and loss tangent in compression were determined. Mixed models for repeated measures were used for the comparisons of E0, E00, and tand among mixtures (a¼.05). They found that the means of E0, E00, and tand of all the pigmented specimens were lower than those of Unpigment. MixAll with high concentration had the lowest values in E0 and E00. The means of E0 and E00 of Red and Yellow in high concentration were lower than those in low concentration, whereas the means of E0 and E00 of Black and Tan in low concentration were significantly lower than those in high concentration; the means of tand for all the mixtures in high concentration were significantly lower than those in low concentration. The means of E0, E00, and tand of all the specimens tested increased as frequency increased from 0.5 to 2.5 Hz (P<0.5). They concluded that within the limitations of this study, it was concluded that the addition of intrinsic silicone pigments into a base maxillofacial elastomer significantly influenced dynamic mechanical properties of the maxillofacial silicone elastomer tested over the low frequencies from 0.5 to 2.5 Hz at room temperature. This effect, which was a quick elastic return to its original shape after deformation during pigmentation or coloration, seems desirable to a certain extent in clinical application. The type and concentration of pigment may influence the elastic and viscous portion of the properties of the maxillofacial elastomeric materials

tested. Low frequencies (0.5 to 2.5 Hz) affect the dynamic viscoelastic properties of the materials.

19) **Nobrega A et al. (2015)[19]** conducted an in vitro study with the purpose to evaluate the influence of adding nanoparticles on the hardness, tear strength, and permanent deformation of a facial silicone. In this study, specimens were made for each test, with 140 for the hardness test, 140 for the permanent deformation test, but 280 for the rupture test. This higher number was due to the fact that the first 140 specimens were ruptured and unusable after the initial reading. ZnO, BaSO4, and TiO2 nanoparticles at concentrations of 1% and 2% of silicone were used, as well as specimens without nanoparticles that consisted of only oil paint and of only silicone. Outcomes were measured before and after 1008 hours of accelerated aging. Data were analyzed by nested analysis of variance (ANOVA) and Tukey honest significant differences test (a=.05). Results showed that the presence of nanoparticles influenced the properties of the assessed groups. The nanoparticles decreased hardness values. The highest values of tear strength were observed for the groups with addition of BaSO4. The 1% ZnO group without oil paint showed the lowest values of permanent deformation. It was concluded that based on the findings of this in vitro study, the use of ZnO nanoparticles is recommended, since they did not negatively affect the properties of the materials evaluated.

20) **Yotova L et al. (2015)[20]** conducted an in vitro study to check the antifungal properties of Oregano against pathogenic yeast and Fungi Imperfecta using standard disc diffusion method In vitro. In vitro antifungal test: Aspergillus

niger, Penicillium claviforme, Saccharomyces cerevisae, Candida albicans 8673 and Candida glabrata 72 were treated for 24 hours with solution of oregano (50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml and 3.125 mg/ml), Chloronitromycin (250 mg/ml). The antifungal activity was assayed by the well diffusion method. Determination of minimum inhibitory concentrations (MICs): The MIC of solution of oregano, that shows antifungal activity, were determined by 2-folddilution methods as described by [8] and MICs were read in g/ml after overnight incubation at 37C. All experiments were made in replicate. Determination of Minimum fungal concentration (MFC): The MFC was carried out to check whether the test microbes were killed or only their growth was inhibited. Potato Dextrose Agar (PDA, Oxoid, Hampshire, UK) was prepared and sterilized at 121 degree C for 15 minutes, the medium was poured into sterile petri dishes and were allowed to cool and solidify. The contents of the MIC in the serial dilution were then sub cultured onto the prepared medium, incubation was made at 37degree C for 24 h, after which each plate was observed for colony growth. The lowest concentration of the extracts without a colony growth was recorded as the MFC. The solutions of oregano had higher antifungal activity than tested antibiotic even from this fourth generation Chloronitromycin. This study has demonstrated that solution of oregano at concentration 12.5 mg/ml for 24 hours notably inhibited growth of S. cerevisae and C. glabrata 72. MIC of solutions of oregano at concentration 6.25 mg/ml for 24 hours notably inhibited growth only of C. albicans 8673. In contrast, MIC of solutions of oregano at concentration 3.125 mg/ml for 24 hours notably inhibited growth of Fungi Imperfecta A. niger and P. claviforme. The probable reason for the higher MIC reported for eukaryotic

microorganisms is the complex structure of their cell. MFC of solutions of oregano at concentration 12.5 mg/ml for 24 hours notably inhibited growth only of S. cerevisae. For Fungi Imperfecta A. niger and P. claviforme, MFC is 3.125 mg/ml. For C. glabrata 72 MFC is 6.25 mg/ml. Based on the results obtained we can conclude that the examined solutions of oregano has bactericidal activity towards both towards both pathogenic yeast and Fungi Imperfecta, but in different concentrations. The results obtained show the existence of antifungal activity of solutions of oregano towards various pathogenic eukaryotic microorganisms. The study demonstrated that oregano represents an economic source of natural mixtures of antifungal compounds that can be as effective as modern medicine to combat pathogenic microorganisms and safe alternative to treat infectious diseases. The solutions of oregano at 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml and 3.125 mg/ml concentrations showed significant antifungal activity on selected pathogens in clinical isolates.

21) **Puskarova A et al. (2016)**[21] reviewed the literature to enumerate the the antibacterial and antifungal activity of six essential oils and their cyto/genotoxicity to human HEL 12469 cells. Six essential oils (from oregano, thyme, clove, lavender, clary sage, and arborvitae) exhibited different antibacterial and antifungal properties. Antimicrobial activity was shown against pathogenic (Escherichia coli, Salmonella typhimurium, Yersinia enterocolitica, Staphylococcus aureus, Listeria monocytogenes, and Enterococcus faecalis) and environmental bacteria (Bacillus cereus, Arthrobacter protophormiae, Pseudomonas fragi) and fungi (Chaetomium globosum, Penicillium chrysogenum, Cladosporium cladosporoides,

Alternaria alternata, and Aspergillus fumigatus). Oregano, thyme, clove and arborvitae showed very strong antibacterial activity against all tested strains at both full strength and reduced concentrations. These essential oils showed different fungistatic and fungicidal activities when tested by direct application and in the vapor phase. The genotoxic effects of these oils on HEL 12469 human embryo lung cells were evaluated using an alkaline comet assay for the first time, revealing that none of the oils induced significant DNA damage in vitro after 24h. This study provides novel approaches for assessing the antimicrobial potential of essential oils in both direct contact and the vapor phase and also demonstrates the valuable properties of the phenol-free arborvitae oil. These results suggest that all the tested essential oils might be used as broad-spectrum anti-microbial agents for decontaminating an indoor environment.

22) **Tyagi S et al. (2016)**[22] conducted a review of existing literature on maxillofacial silicones. They found that the art and science of maxillofacial prosthetics have made significant advances during the past four centuries. Improvements have been made possible through the application of dental techniques and by the availability of newer and more suitable materials. Further improvements will depend upon the discovery and introduction of even more promising materials. But a factor of much greater importance is that of fuller cooperation between the plastic surgeon and the maxillofacial prosthetist. The prostheses can also be considered as definitive prostheses for those patients in different parts of the world who cannot gain access to highly skilled maxillofacial technicians and what has been produced here is much better than available alternatives. Moreover, these prostheses could last for a

long time if they are handled and maintained in a proper way. Colour stability of the prosthesis is an important factor in patient acceptance. Evaluation of colour stability using combinations of pigments, opacifiers, and elastomeric materials allows an understanding of the effects and interactions of each component and aids in identification of the combination of these ingredients that could be used to produce the most colour stable prosthesis.

23) **Bibars A et al. (2017)**[23] carried out an in vitro study to evaluate the effect of adding thixotropic agents on the mechanical properties of 3 commonly used silicone elastomers. In this study, specimens of 3 maxillofacial silicones (M511, Z004; Technovent Ltd, and A2000; Factor II Inc) were prepared according to the manufacturer's instructions. Tear and tensile strength values and percentages of elongation and hardness were evaluated for each material with and without thixotropic agents. Data were analyzed using 1-way ANOVA and the Bonferroni post hoc test (a=.05). They found that that the 3 types of silicone elastomers had significantly different tensile and tear strength and hardness values and percentages of elongation. Z004 silicone showed the highest tensile and tear strength followed by A2000 and M511, regardless of the addition of thixotropic agent. The addition of a thixotropic agent decreased the tear strength ($P<0.01$) but not the tensile strength for all types of silicone. Percentage of elongation was the highest in M511 and the lowest in A2000 and was significantly higher ($P<0.01$) for silicones with no added thixotropic agent. Hardness was highest in A2000 and lowest in M511. Adding a thixotropic agent decreased hardness significantly ($P<0.01$) for Z004 and A2000 only. They concluded that the incorporation of thixotropic agents into the 3 maxillofacial silicone elastomers used in this study reduced some

favourable mechanical properties, particularly tear strength and percentage of elongation. Z004 showed superior mechanical properties among the 3 tested silicones.

24) **Gupta A et al. (2017)**[24] carried out review of literature with the aim is to explain the salient features and the purpose of maxillofacial prostheses. They concluded that the rehabilitation of intraoral and extraoral defects is a challenging aspect of maxillofacial prosthodontics. It requires constant practice of the art to gain confidence and expertise. The goals of the surgeon and prosthetic specialist regarding rehabilitation of the patient are closely allied. The maxillofacial prosthodontist should always try to provide the treatment to the fullest of his ability. Sophistication in the prosthetic reconstruction of structural and functional defects improves the final results, if carefully planned, unbiased rehabilitation regimens are established. It is imperative that the prosthodontists involved either directly or indirectly in prosthetic rehabilitation to be aware of the situations discussed here, so that a more complete and successful service may be rendered to their patients.

25) **Pinheiro J et al. (2017)**[25] conducted a study which investigated the microbial colonization of maxillofacial prostheses and support tissues using the Checkerboard DNA–DNA hybridization method, and the efficacy of 0.12% chlorhexidine gluconate, 10% Ricinus communis solutions, or brushing, on colony forming unit (CFU) reduction in mono species biofilms (Candida glabrata, Staphylococcus aureus, Streptococcus mutans, Escherichia coli, Enterococcus faecalis, and Pseudomonas aeruginosa) formed on two silicones (MDX 4-4210 and Bio-Skin). Biofilm was harvested from 43 maxillofacial

prosthesis wearers for detection of 38 species of microorganisms. The CFU counts of the six above mentioned species were recorded after using the hygiene protocols. All 38 investigated species were identified in prostheses and tissues, with a higher prevalence in the prostheses. 0.12% chlorhexidine gluconate immersion showed the greatest antimicrobial effectiveness, followed by mechanical brushing protocols. MDX 4-4210 silicone produced lower CFU counts than Bio-Skin. They concluded that conclude that: (1) both prostheses and support tissues exhibited moderate to clinically relevant amounts of microorganisms colonizing their surfaces; (2) 0.12% chlorhexidine gluconate immersion showed the greatest antimicrobial effectiveness followed by mechanical brushing protocol; and MDX 4-4210 silicone presented lower CFU counts when compared to Bio-Skin.

26) **Bhat V et al. (2018)**[26] conducted an in vitro study on characterization of herbal antifungal agent, origanum vulgare against oral candida spp. Isolated from patients with candida-associated denture stomatitis. In this study, dry leaves of the plant were purchased and authenticated. Oil extraction was done using Hydro-distillation method. Clinical isolates of Candida from denture wearers was speciated using CHROM agar. Well Diffusion test was used to confirm the antifungal activity. Hydro-distillation & Maceration methods of extraction were compared. MIC/MFC was determined using CSLI guidelines. Infra-Red Spectroscopy was used to identify the active functional group. They found that O.vulgare showed 30±3mm of zone of inhibition as against 19mm for fluconazole. The suitable extraction method was Hydro-distillation. MIC & MFC were found to be 0.024% and 0.097% respectively which was much lesser than for fluconazole (0.25%). The active functional group had

chemically similar structure as Carvacrol, usually found in antifungal herbs. They concluded that within the limitations of the study, it was concluded that (a) O.vulgare is anticandidal for clinical isolates of oral Candida, (b) Hydro-distillation is an effective method as compared to Maceration (c) MIC & MFC are much lower than that of fluconazole (d) the major functional group was structurally similar to Carvacrol.

27) **Bishal A et al. (2018)**[27] carried out an in vitro study with the purpose to evaluate the ability of an oxide nanocoating to prevent colour degradation of maxillofacial silicone elastomers after artificial accelerated aging. In this study, a silicone elastomer with functional intrinsic pigment was tested. Specimens (N=20) were fabricated, and half of them were coated with a nanolayer of titanium dioxide (TiO2) using atomic layer deposition. Both coated and noncoated specimens (control) were exposed to artificial aging at 450 kJ/m2 of total energy. Changes in the colour of all the specimens with and without TiO2 nanocoating were measured before and after the atomic layer deposition coating and before and after aging. The obtained colour data were analyzed by using independent t tests and the 1-sample t test (a=.05). They found that colour change (DE1=3.4 ±1.4) was observed for the silicone elastomers after the specimens were surface coated with TiO2 nanofilm, although this change was not statistically significant (P=.369) compared with the acceptability threshold (DE=3.0). Upon exposure to artificial aging, the noncoated control specimens underwent colour change (DE2=2.5 ±0.7, P=.083 compared with the acceptability threshold). The specimens with TiO2 nanocoated surface experienced the least colour change (DE3=1.4 ±0.6) when subjected to artificial aging, and this change was significantly lower (P than

the established acceptability threshold of DE=3.0. In addition, the chemical analyses confirmed that the TiO2 nanocoating remained on the surface after exposure to artificial aging. They concluded that TiO2 nanocoating was shown to be effective in reducing colour degradation of the silicone elastomer exposed to artificial aging for 120 hours with 450 kJ/m2 of total energy.

28) **Costa I et al. (2018)[28]** conducted a study to evaluate the colour stability of acrylic and bis-acrylic resins after immersion in 3 staining solutions. In this study, forty-eight samples (10 x 2 mm) of each provisional restorative material (Duralay, Dencrilay, Structure 2 and Pro temp 4) were fabricated and distributed into four groups (n = 12): G1 – distilled water (control group); G2 – a cola favoured soft drink; G3 – wine and G4 – coffee. The specimens were immersed for seven days at 37°C in the solutions, which were changed every 24 hours. The colour of all specimens was measured with a spectrophotometer (VITA Easy shade Advance) before (T0) and after immersion (T1), and the colour changes (ΔE) were calculated. Nonparametric Kruskal-Wallis tests were used, followed by Dunn's test with a significance level of 5%. They found that for the acrylic resins (Duralay and Dencrilay), the largest colour change values were obtained in group G4 – coffee, whereas in the bis-acrylic resins (Pro temp 4 and Structure 2), the largest colour difference was observed in groups G3 – wine and G4 – coffee. The acrylic resins showed statistically significantly less colour change than the bis-acrylic resins. They concluded that the coffee and the wine promoted larger colour changes in the provisional prosthetic materials tested in this study. The acrylic resins showed more colour stability than the bis-acrylic resins.

29) **Bansal V et al. (2020)[29]** conducted a study which aimed at detecting the antibacterial and antifungal activity of neem and clove extract against Streptococcus mutans and Candida albicans. In this study, strains of S. mutans and C. albicans and selective media for growing micro-organisms were procured. Antimicrobial activity was assessed using two methods, by determining the minimum inhibitory concentration (MIC) using the broth dilution method and determining the zone of inhibition using well diffusion method on mitis salivarius bacitracin selective for S. mutans and Saboraud's dextrose agar plates for C. albicans. One way ANOVA with post hoc analysis was done to compare the antimicrobial activity of extracts and 0.2% chlorhexidine. They found that the MIC of neem extract was found to be 4.2 mg/ml and 5.0 mg/ml against S. mutans and C. albicans, respectively. While for cloves, it was 5.5 mg/ml for both. Neem had the highest antibacterial activity with a mean zone of inhibition of 11.4 mm followed by chlorhexidine and cloves whereas antifungal activity was highest for chlorhexidine (14.4 mm) followed by neem and clove. They concluded that the result of the study established that both plant extracts possess antimicrobial activity against common microbes present in the oral cavity.

30) **Daivasigamani S et al. (2021)[30]** carried out systematic review of the existing literature on the colour stability of maxillofacial silicone materials after disinfection and ageing procedures. Maxillofacial prosthesis should restore the appearance to near normal so that the affected person should have a normal mental status as well as quality of life. The longevity of any kind of maxillofacial silicone prosthesis is determined by its colour and mechanical properties. To systematically assess the current published literature on the

stability of colour of maxillofacial silicone materials after 10 minutes of disinfection and aging, a literature search on PubMed and Google Scholar was done from January 2000 to December 2020 on the stability of colour of maxillofacial silicone materials after disinfection and aging. In addition, a hand search was performed through standard dental journals for the years 2000 to 2020 using the keywords; colour stability and maxillofacial silicone, maxillofacial silicone and disinfection, maxillofacial silicone, and aging. A total of 52 studies were recognized and 6 in vitro studies were appended for this systematic review. The colour stability of maxillofacial silicone materials was affected by disinfection and aging procedure.

31) **Gupta p et al. (2021)**[31] carried out a systematic review which aimed to identify and interpret results of studies that evaluated the changes in the colour stability of maxillofacial prosthetic materials due to chemical instability of silicones and pigments and the effect of exposure to environmental conditions and aging factors on the same. This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic reviews and Meta-Analyses guidelines (PRISMA). In this study, relevant articles written in English only, before November 15, 2019, were identified using an electronic search in the PubMed/Medline conducted to identify pertinent articles. The relevancy of the articles was verified by screening the title, abstract, and full text, if they met the inclusion criteria. A total of 42 articles satisfied the criteria, from which data were extracted for qualitative synthesis. This review protocol was registered in the International Prospective Register of Systematic Reviews (PROSPERO registration number CRD42019124562). Statistical Analysis Used: Since considerable data heterogenicity was present in all

studies except the ones on incorporation of TiO2 for which meta-analysis using random effects model was performed. They found that the database search resulted in 234 studies, of which 202 articles were excluded due to lack of relevance, duplication, and unavailability of data. The remaining 32 full text articles were assessed for eligibility, out of which 2 articles were excluded. Twelve articles were yielded by manual search. A total of 42 studies were included in the present systematic review. Due to heterogeneous data, meta-analysis could be only carried out with the effect of TiO2 nano particle on colour stability. They found that although there has been extensive amount of research in this field, an ideal maxillofacial silicone exhibiting good colour stability in various human and environmental aging conditions is yet to be identified. Human and environmental aging conditions have an adverse effect on the colour stability and addition of TiO2 nano particle seems to improve the same.

32) **Hosny K et al. (2021)**[32] conducted a study which aimed to produce an oregano essential oil-based nano emulsion (OEO-SNEDD) that would have antibacterial and antifungal effects against oral microbiota and improve oral health. IN this study, several OEO-SNEDDSs were developed using different percentages of OEO (10%, 14%, and 18%), percentages of a surfactant mixture Plura care L64: Lauroglycol FCC (18%, 32%, and 36%), Smix ratios (1:2, 1:1, and 2:1), and hydrophilic-lipophilic balances (HLBs) of the surfactant mixture (8, 10, and 12) using the Box–Behnken design. The optimized concentration of excipients was determined using a pseudoternary phase diagram to obtain the NEs. The formulations were evaluated for their droplet size, stability index, and antibacterial and antifungal activities. They

found that the NEs had a droplet size of 150 to 500 nm and stability index of 47% to 95%, and the produced formulation reached antibacterial and antifungal inhibition zones of up to 19 and 17 mm, respectively. The Box–Behnken design was adopted to get the optimum formulation, which was 18% OEO, 36% Smix, 10.29 HLB of Smix, and a 1.25:1 Smix ratio. The optimized formulation had a lower ulcer index compared with various other formulations evaluated in rats. They concluded that this study illustrated that OEO-SNEDDSs can provide good protection against oral microbial infections.

33) **Janani et al. (2021)**[33] conducted an in vitro study on the efficacy of Oregano essential oil extract in the inhibition of bacterial lipopolysaccharide (LPS)-Induced osteoclastogenesis using RAW 264.7 murine macrophage cell line. In this study, four different concentrations (0 ng/mL, 25 ng/mL, 50 ng/mL, and 100 ng/mL) of oregano essential oil extract were added into 96-well culture plate. Under light microscope, quantification of osteoclast cells was performed. One-way ANOVA with post-hoc Tukey test was carried out on SPSS v21. They found that a significant reduction ($p < 0.001$) in the osteoclast was observed in the experimental groups compared to no oregano essential oil extract (control). A dose-dependent significant reduction ($p < 0.001$) in osteoclast formation was observed among the experimental groups, with lesser osteoclast seen in group IV with 100 ng/mL of oregano essential oil extract. They concluded that oregano essential oil extract can be utilized as a therapeutic agent that can target bacterial LPS-induced osteoclastogenesis. However, randomized controlled studies should be conducted to assess the potential use of this extract in the periapical bone resorption of endodontic origin.

34) **Rashed A et al. (2021)**[34] conducted a review of literature on the antifungal properties of essential oils and their compounds for application in skin fungal infections both the conventional and non-conventional approaches. They concluded that the importance of antifungal agents Essential Oils is widely recognized. Their roles in reducing the severity of fungal infections vary according to species and origin. Based on our review, we strongly believe that EOs should be explored for commercial applications as alternatives to over-the-counter antifungal agents. In addition, commercial applications could be further enhanced with nonconventional strategies in combination with other components, such as fluconazole and Tween 80. Hence, it is vital that efforts continue for the development of EO-based skin antifungal therapies.

35) **Ashraf H et al. (2022)**[35] conducted a study aimed to investigate new tissue conditioner (TC) formulations involving chitosan nanoparticles (CSNPs) and essential oils (EO) for their antifungal potential, release kinetics, and hardness. In this study, CSNPs were synthesized, and the separate solutions of CSNP were prepared with two types of EO, i.e., Oregano oil and Lemongrass. The EO was loaded separately in two concentrations (200 μL and 250 μL). The blank and EO-loaded CSNPs were screened against Candida albicans (C. albicans), and their minimum inhibitory concentration was established. GC Reline™ (GC corporation, USA) TC was considered a control group, whereby the four experimental groups were prepared by mixing CSNPs/EO solutions with TC powder. The antifungal effectiveness (C. albicans) and release kinetics behavior (1–6 h, 24 h, 48 h, and 72 h) was investigated. The Shore A hardness of control and experimental groups was evaluated in dry and wet modes (deionized water and artificial saliva). For statistical analysis, SPSS

version 22 was used to do a one-way ANOVA post-hoc Tukey's test. They found that as compared to the control group, TCs containing blank CSNPs and CSNPs loaded with EO showed 3 and 5 log reductions in C. albicans growth, respectively. A significantly high antifungal effect was observed with TC containing lemongrass essential oil (200 μL). The continuous release of EO was detected for the first 6 hours, whereas completely stopped after 48 hours. Mean hardness values were highest for dry samples and lowest for samples stored in artificial saliva. Regardless of the storage medium, the average and cumulative essential oils release and hardness values of TCs across the measured time points showed a statistically significant variation among and between the study groups. Researchers came to the conclusion that although additional biological investigation must be conducted, TCs with essential-oil-loaded CSNPs appear to be a possible alternative solution for denture-induced stomatitis.

36) **Chotprasert N et al. (2022)**[36] conducted a in vitro study aimed to evaluate the effects of four different disinfection methods on the colour stability of pre-coloured and hand-coloured maxillofacial silicones. Pre-colored and hand-colored silicone samples totaling forty each were prepared. In this investigation, samples were separated into eight groups at random (n = 10) and cleaned using four various disinfection techniques. Six times every day for 60 days were spent disinfecting, approximating one daily cleaning per year. A UV-vis spectrophotometer was used to evaluate color at days 0 and 60. The CIE lab system calculated the changes in color. Two-way ANOVA with post hoc Tukey HSD and t-tests ( 0.05) were used to assess the data. The color stability of maxillofacial silicone can be impacted by disinfectants. The

highest color change in our study was caused by liquid soap and chlorhexidine solution. Greater color stability was demonstrated by pre-colored silicone than by hand-colored silicone.

37) **Habeebulleh Z et al. (2022)**[37] conducted a study with the main objective was to evaluate the tear strength property after disinfection with plant-based disinfection solution after different immersion periods. In this study, fourty specimens of VST-30 maxillofacial silicone were prepared then divided into four main groups (n=10), a control group that was not subjected to disinfection and three experimental groups subjected to disinfection in different immersion time (1 day, 1 month, and 6 months). The experimented groups were immersed in Oregano oil solution. The tear strength test was performed by the use of Universal Testing Machine. Statistical analyzes of the data was performed via One-way ANOVA and Tukey HSD Post-hoc test to determine the significant differences between experimental and control groups at a level of significance ($p \leq 0.05$). They found that the tear strength test showed a non-significant difference between the control group and the experimental groups. Also, there was no significant differences among the three experimental groups. They concluded that the tear strength of VST-30 silicone elastomer was not affected after immersion in 0.4% Oregano solution.

# METHODOLOGY

1. **Study design -** An In vitro study
2. **Study setting -** The study was carried out in the Department of Prosthodontics and Crown and Bridge, KLE V.K Institute of Dental Sciences, Belagavi.
3. **Materials and armamentarium**

   The following materials and armamentarium were used in the procedure

| S. No. | MATERIALS | BRAND NAME |
|---|---|---|
| 1. | Pure Oregano oil | Citispray |
| 2. | MDX-4 Maxillofacial silicone material | Dow Corning Corp, Midland, Mich |
| 3. | 1% Clotrimazole | Glenmark pharmaceuticals |
| 4. | Ethanol | - |
| 5. | Nutrient agar | Hi Media, Belagavi |
| 6. | Sabourad dextrose Agar | Hi Media, Belagavi |
| 7. | Sterile cotton swabs | - |
| 8. | Sterile petri dishes | - |
| 9. | Biological oxygen demand incubator | Remi elektrotechnik ltd, India |
| 10. | Spectrophotometer | X lite Europe |
| 11. | Hot air oven | Labotech,B.D. Instrumentation, India |
| 12. | Digital analytical balance | UniBloc (AUW 220D) And Kern and Sohn Gmbh |
| 13. | Vaccum mixer | EasyMix BEGO |

1. **SOURCE OF DATA/LABORATORY METHOD:**

This study was conducted in-

1) Department of Prosthodontics KLE Academy of Higher Education and Research KLE V. K. Institute of Dental Sciences, Belagavi.
2) Preparation of stock emulsion, serial dilutions of oregano oil was carried out in KLE college of pharmacy, Belagavi
3) Microbiological procedures were carried out in Department of Microbiology, JN Medical College, Belagavi.
4) Determination of colour stability was carried out at the Praj Metallurgical Laboratory, Kothrud, Pune

**Preparation of Origanum oil Emulsion (Stock solution):**

2gms of pure Oregano oil was dispensed in a 100ml beaker. 50ml of Ethanol was added and mixed well. To the above solution 48ml of distilled water was added and stir with a stirrer, to make up the volume to 100ml.

Concentrations of Origanum oil: a) 50mg/ml
b) 25mg/ml
c) 12.5 mg/ml

**Preparation of 1% Clotrimazole solution**

An Active pharmaceutical ingredient (API)- 100% was procured from a pharmaceutical company. 1gm of API was added to 100 ml of Ethanol to make 1% Clotrimazole solution, to be used as a control in the study.

**Determination of Minimum zone of inhibition to evaluate antifungal and antimicrobial efficacy of Origanum oil:**

A total of 33 plates each of Sabarouds dextrose agar and nutrient agar was prepared to assess the mean zone of inhibition of Oregano oil impregnated in MDX-4 maxillofacial silicone comparing with 1% Clotrimazole impregnated in MDX-4 maxillofacial silicone.

↓

A test tube containing peptone broth was taken to which strains of candida albicans and *Staphylococcus aureus* was added. The growth concentration was adjusted to 105 organisms / ml by using 0.5 McFarland's turbidity standard, and incubated to 37°

↓

One loop full of bacterial and fungal suspension was taken and streaked on the surface of nutrient agar plate and sabarouds dextrose agar plate respectively twice or thrice to ensure a uniform confluent growth (Lawn culture).

↓

The agar plates were allowed to dry and three wells each of diameter 6mm was punched out on each plate with a sterile borer in the inoculated agar. A micropipette was used in the wells to pour respective concentrations of *Origanum oil* impregnated in maxillofacial silicone. 1% Clotrimazole impregnated in maxillofacial silicone was used as a positive control.

↓

Colonies was then allowed to grow anaerobically for *Staphylococcus aureus and Candida albicans*. The diameter of zone of inhibition was then measured and the antimicrobial and antifungal activity of the oregano oil was reported accordingly.

## COLOR STABILITY

### Specimen preparation

Thirty-three experimental groups of elastomers were fabricated by variously combining three concentrations of oregano oil with each of three silicone pigments (red yellow, blue). The control group were silicone MDX-4 with no pigment added.

The specimens (10-mm diameter 2-mm thick) were processed in metal moulds using a mixture of silicone MDX-4 (RTV). Each material was handled following manufacturer's instructions.

First, the oregano oil with three concentrations was mixed with the base by hand using metal spatula on a glass plate with pressure for 20 min. Then catalyst was then added into mixtures to achieve the recommended ratio of 10:1. Each group of these materials was then mixed well and divided into three groups for each pigment. The three pigments consist's of red, yellow and blue. Then, each mixture of silicone, Oregano oil and pigments were mixed by hand with a spatula until the colour was evenly distributed.

The moulds will be placed in vacuum chamber for 30 min to remove the air bubbles incorporated in the mixture. Then the materials will be allowed to polymerize at room temperature for 24 h. After the materials are cured, the specimens will be removed from the moulds. All specimens will be trimmed and cut-marked to classify the groups[6].

**COLOR ANALYSIS**

The colour measurements were determined by a Spectrophotometer (X-rite Europe). This device consists of a light illuminating unit which is located in the head of the measuring unit and this measuring unit is connected to a computer that has the software to run this device and which generates CIE Lab measurements i.e L*, a*, b* and △E automatically.

Measuring characteristics of the spectrophotometer were standard illuminant D50; illuminating / viewing geometry d/8 degrees and standard observer's angle of 2 degrees. The spectrophotometer has no gloss trap to exclude the specular component. Before each measurement session, the spectrophotometer will be calibrated according to the manufacturers recommendations by using the supplied white calibration standard.

Colour measurements will be made under dark conditions. Colour changes (△E) will be calculated by measuring tristimulus values at several wavelengths in the visual spectrum with the use of CIE Lab colour space. The CIE Lab is a colour system representing three – dimensional colour space with the components of lightness (L), red-green (a), yellow -blue (b).

The colour differences (△E) between the measurements ( before and after aging ) in terms of L, a , b were calculated from the following equations:

$$\triangle E^* = (\triangle L^*)^2 + (\triangle a^*)^2 + (\triangle b^*)^2)^{1/2}$$

In which △L, △a, and △b are the differences of L, a, b values before and after aging.

Specimen placement will be standardised by using a thermocol and making hole in it (without perforating it) to provide space for the placement of specimen. Two marking will be marked on periphery of the hole and on each specimen with marker pen.

The marking on the specimen should coincide with markings on thermocol every time they were placed on the thermocol and at this position the first readings was recorded. This procedure was undertaken in order to obtain the calorimetric values L*, a*, b* at the same spots before and after aging.

For the second reading the specimen was placed on same spot as that were of first readings. The readings were charted and the mean for every three readings was recorded. The spectrophotometer automatically generated the CIE Lab measurements i.e L*, a*, b* and $\triangle E$.

To relate the colour differences ($\triangle E$) to a clinical environment, the colour data was quantified by the National Bureau of standards (NBS) units through formula NBS units = $\triangle E^{*}0.92$

**Accelerated Aging**

Application of elevated temperature as a means of simulating the aging process is called accelerated aging. A common approach is to assume that the rate of aging is increased by a factor: $f = 2T/10$

In Eq. (1), $T = T - Tref$, where Tref is a reference temperature, at which the effects of aging are to be determined, and T is an elevated temperature used to accelerate these effects.

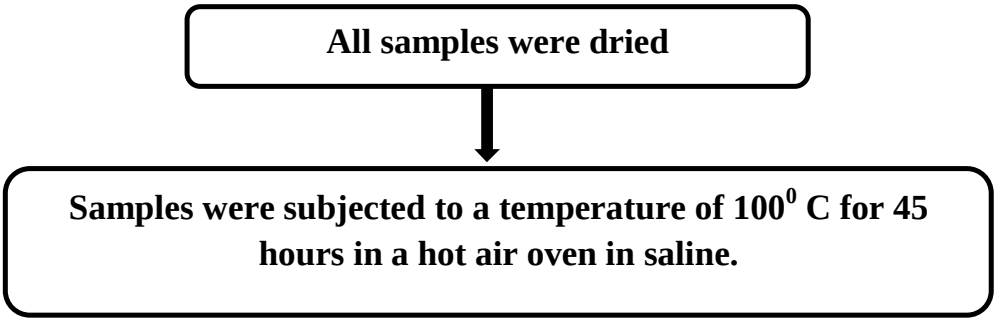
Silicones appear to retain their mechanical properties after being heated to 150°C for 30 days according to Eq. (1), this is equivalent to approximately 7 years of aging at body temperature.

Considering the above principle,

All specimens were dried and measured before and after artificial aging using a spectrophotometer CIELAB colour values were obtained. Specimens were placed in a Hot air oven and exposed to controlled temperatures. All L*, a*, and b* values were collected for calculation of colour change as shown in flowchart below.

**ACCELERATED AGING**

a) Application of elevated temperature as a means of simulating the ageing process is called accelerated aging




b) Provision was made for keeping the samples immersed in the saline in a sealed container in the hot air oven

c) These conditions were equivalent in maintaining the materials in the body for 7 – 10 years

d) Colour analysis was done before and after artificial aging using a spectrophotometer, CIELAB colour values will be obtained

e) All L* a* b* values were collected for calculation of colour change.

**1) Eligibility criteria**

**A) Inclusion criteria**

1) Homogenous mix free of voids and deformities of oregano oil and maxillofacial silicone in microbiological study.

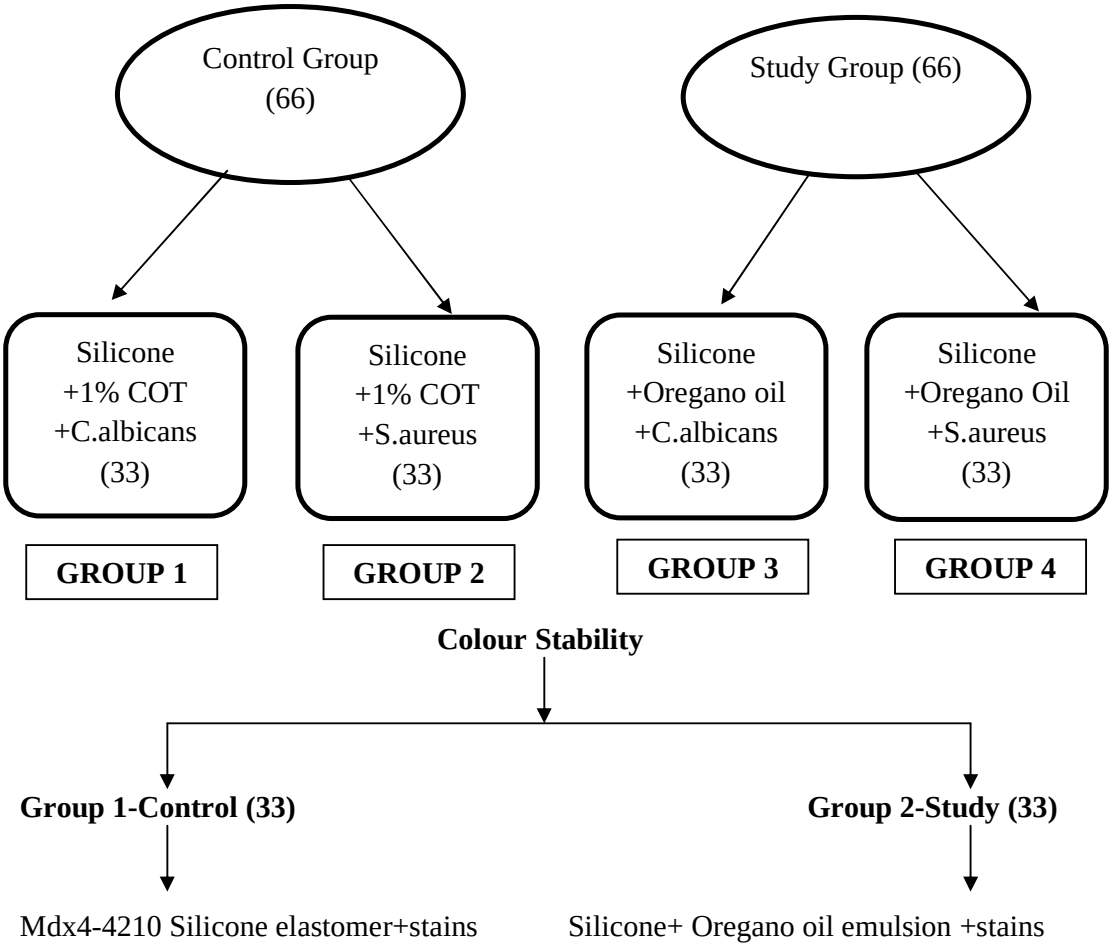
2) Specimens with identical size and shape for color stability tests.

**B) Exclusion criteria**

1) Non homogenous mix of oregano oil and maxillofacial silicone.

2) Specimens with surface defects, porosities and deformities.

**Antimicrobial properties against *Candida albicans* and *Staphylococcus aureus***



**2) SAMPLE SIZE**

Sample size (n) estimation was done using the following formula below

$N=\frac{2S^2(Z_{1-\alpha} + Z_{1-\beta})^2}{(X_1-X_2)^2} = 33$

By substituting the values for

$Z_{1-\alpha=}$ 1.96 at 5% α error

$Z_{1-\beta=}$ 0.842 at 20% β error

$d = X_1 - X_2 =$ Margin of error = 2.53

Standard deviation in the 1st group S1=2.17

Standard deviation in the IInd group S2= 3.92

Mean difference between Ist and IInd sample = 2.53

Effect size = 0.830870279146141

Alpha error (%) = 5

Power (%) = 80

Sided = 2

The final sample in each group was (n) = 33

**3) STATISTICS ANALYSIS:**

All the data was entered in Microsoft excel. Descriptive characteristics of the sample was carried out in terms of mean and standard deviation. Normality of data was explored by Kolmogorov – Smirnov and Shapiro wilk test with *p* value <0.05 as the reference for using parametric or non – parametric tests.

Independent sample t test was used to compare the means of groups independent of each other and Unpaired t test or Mann – Whitney U test was used for intergroup comparison. All the data analysis was carried out in SPSS (statistical product service solution / statistical package for social sciences) version 25.

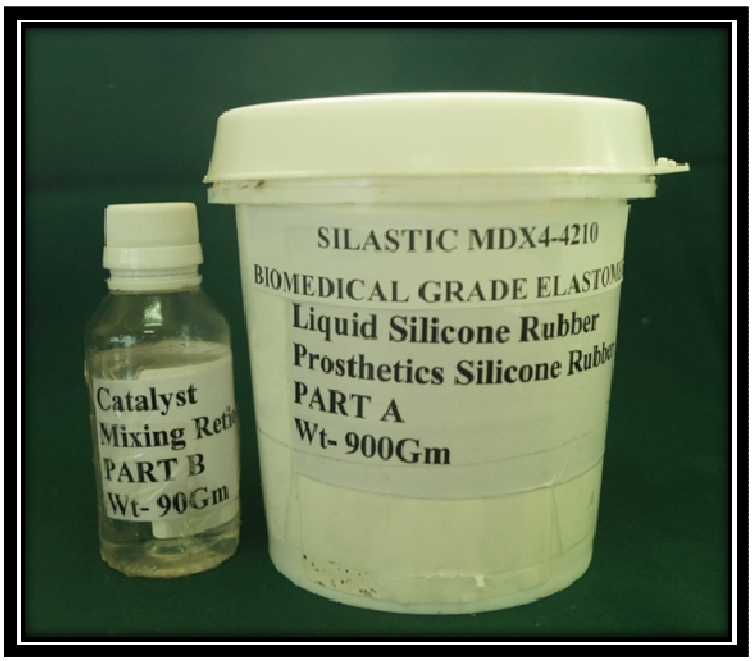



**Figure 1: Silastic Mdx4-4210**


**Figure 2: Origanum Oil Emulsion**

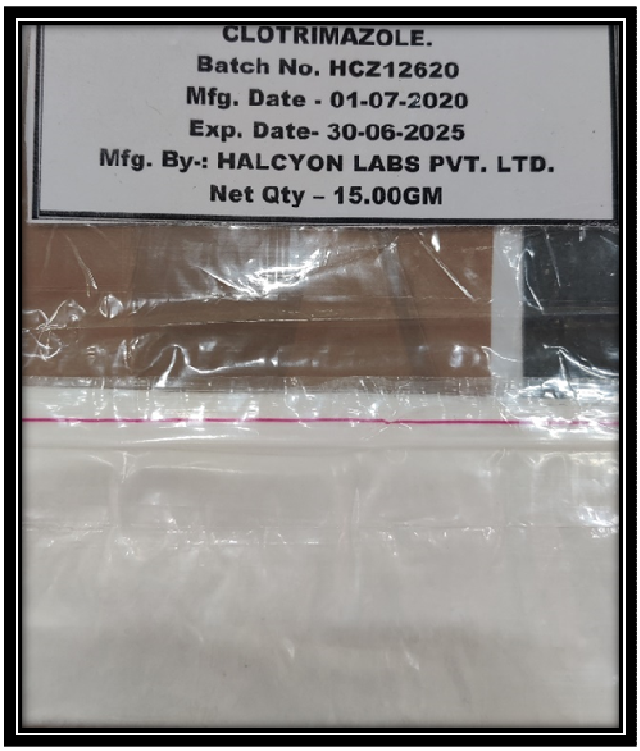



**Figure 3: API Clotrimazole**


**Figure 4: 1% Clotrimazole**


**Figure 5: Candida albicans ATCC90028**

**Figure 6: Staphylococcus aureus strain**

**Figure 7: Incubator for incubation of culture plates**

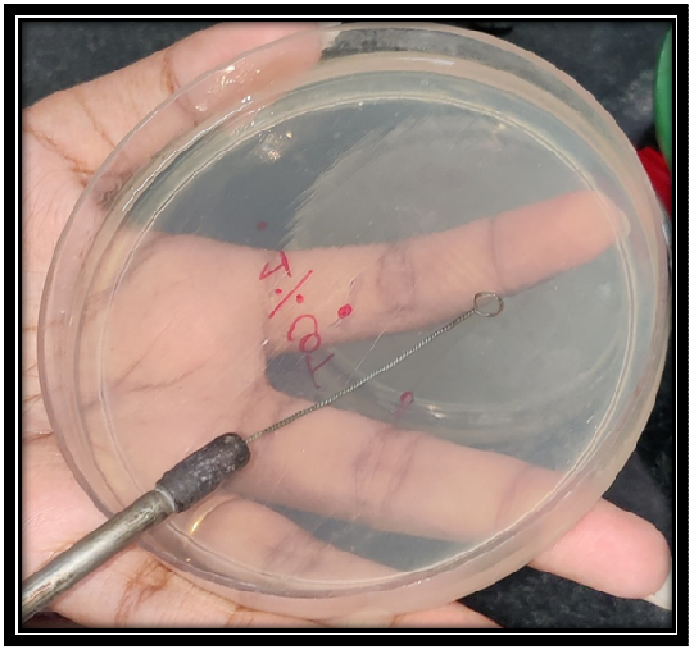


**Figure 8: Streaking of culture media with the fungal and bacterial strains**

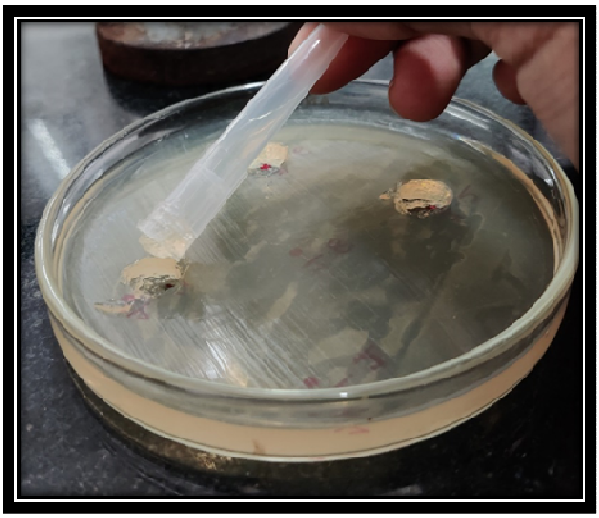


**Figure 9: Digging of wells, for well- diffusion method for determination of minimum zone of inhibition**

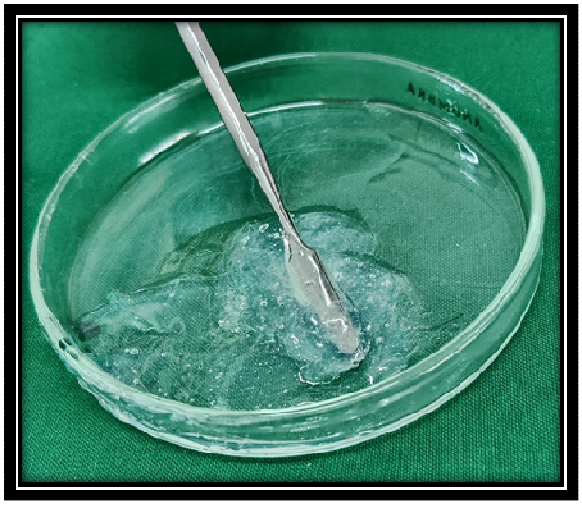


**Figure 10: Mixing of silicone with Origanum oil emulsion to be put in wells.**

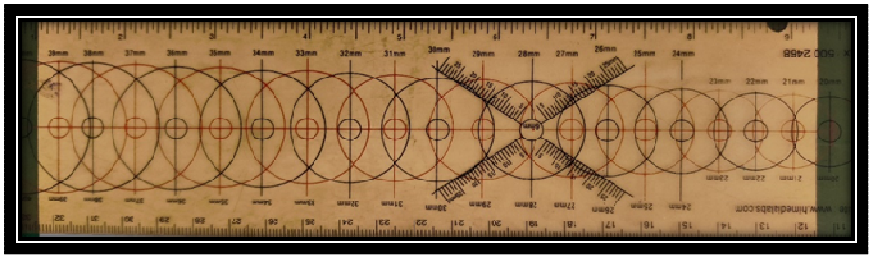


**Figure 11: Scale for measuring minimum zone of inhibition**


**Figure 12: Culture plate of Sabaroud's dextrose agar showing minimum zone of inhibition in Candida albicans with different concentrations of Origanum oil and COT as control**

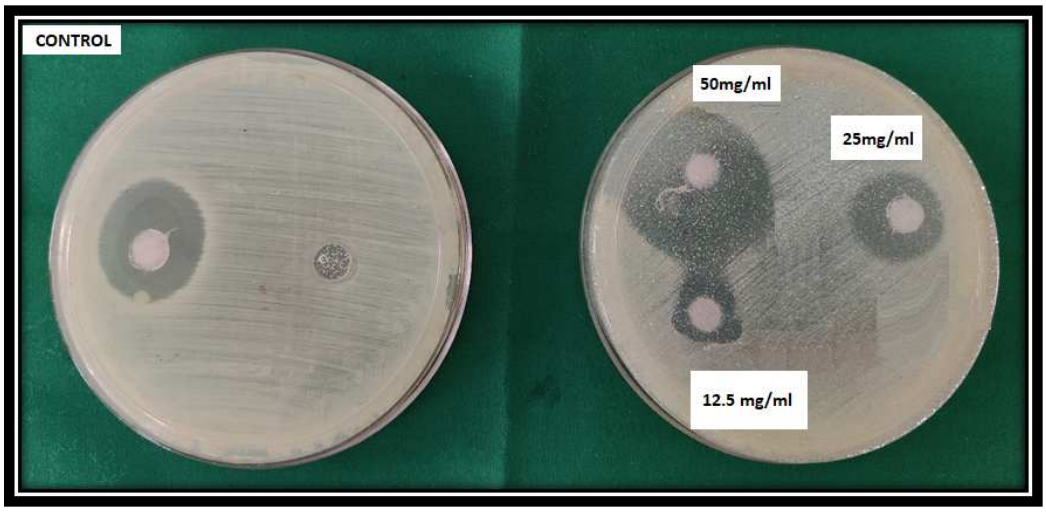



**Figure 13: Culture plate of Nutrient agar showing minimum zone of inhibition in Staphylococcus aureus with different concentrations of Origanum oil and COT as control.**

**Figure 14: Mould for specimen preparation for determining colour stability**


**Figure 15 : Maxillofacial silicone intrinsic color stains**

**Figure 16: Coloured specimens of maxillofacial silicone to be used for the determination of color stability**


**Figure 17: Spectrophotometer used for the determination of color stabilty of testing samples**


**Figure 18: Spectrophotometer showing L*a*b* values**


**Figure 19: High temperature chamber used for accelerated aging showing specimens being subjected to 100 degree celsius**

# RESULTS

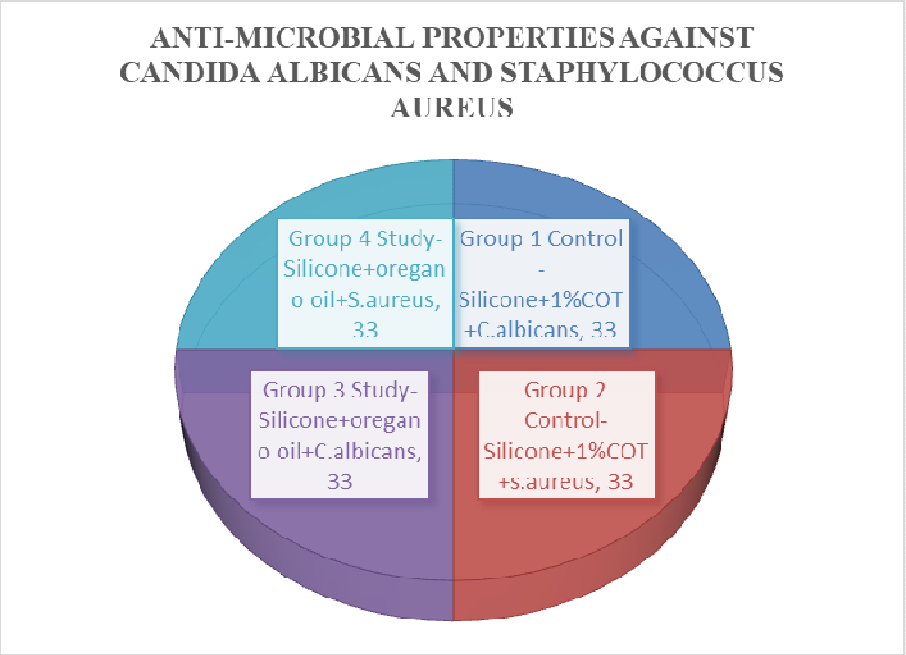
In this study, the results and data analyzed has been presented in the form of tables.

## 1. Descriptive study details

Homogenous mix free of voids and deformities of Oregano oil and maxillofacial silicone and specimens with identical size and shape for color stability tests were included as shown in **graph 1 and graph 2.**

### 1. a) Antimicrobial properties against *Candida albicans, staphylococcus aureus*

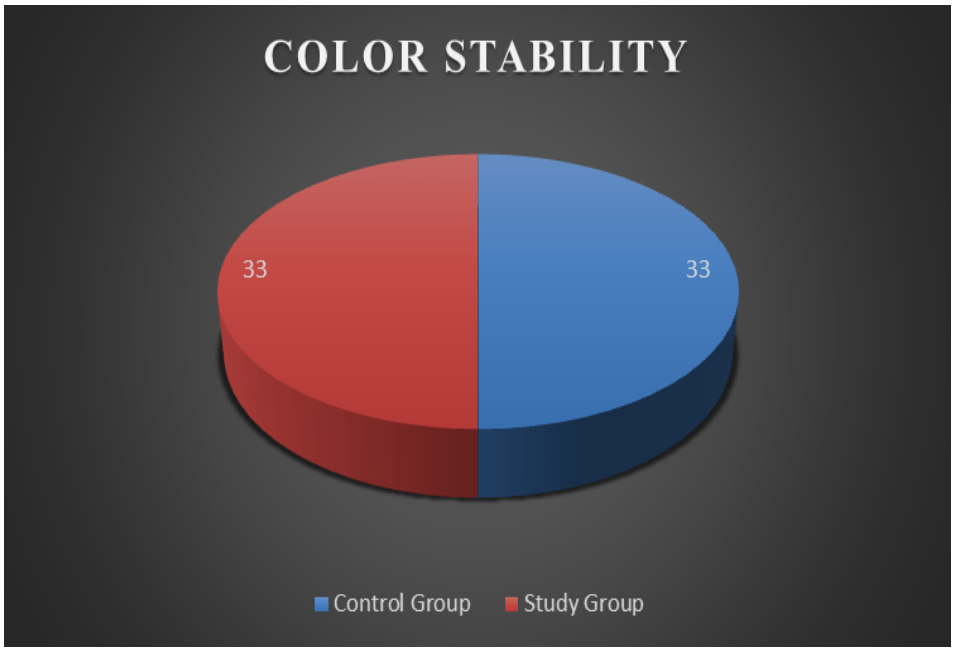
Antimicrobial properties against *Candida albicans*, *Staphylococcus aureus***,** both the study group (n=66) and control group (n=66) had 66 numbers in each group. Control group had two groups: Group 1 of Silicone+ 1%COT+ C. albicans (n=33) and Group 2 of Silicone +1% COT + S. aureus (n=33). While study groups: Group 3 of Silicone + Oregano oil + C. albicans (n=33) and Group 4 of Silicone + Oregano oil + s. aureus (n=33) as shown below in **graph 1.**




**Graph 1: Showing included samples for antimicrobial properties against candida albicans staphylococcus aureus**

**1.b) Color Stability-**

For color stability, both the study group and control group has 33 (n=33) in each group. Group 1 of Mdx-4 4210 silicone elastomer +color pigments had (n=33) numbers while Group 2 under study group of silicone + oregano oil emulsion + color pigments had (n=33) numbers as shown in **graph 2 below.**




**Graph 2: Showing included samples for color stability**

1. **Test for Normality**

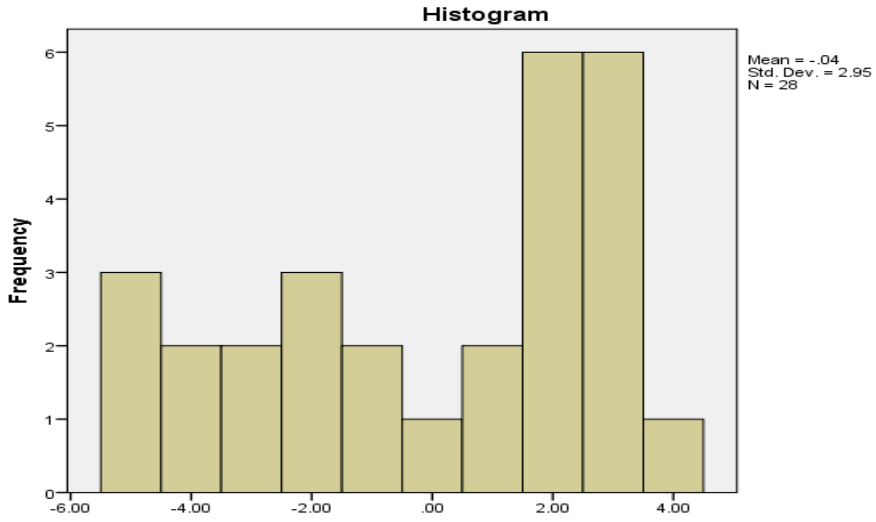
For testing, whether the data is normally distributed or not, we used both kolmogorov - smirnov test and shapiro - wilk test for checking the normal distribution of data with the p value or the alpha value 0.05 as the standard as shown in **table 1 below**. It was seen that the value of both kolmogorov - smirnov test and shapiro - wilk test is > 0.05, which means that the data is normally distributed over the given parameters from the sample.

**Tests of Normality**

**Table 1: Showing data distribution normality statistics**

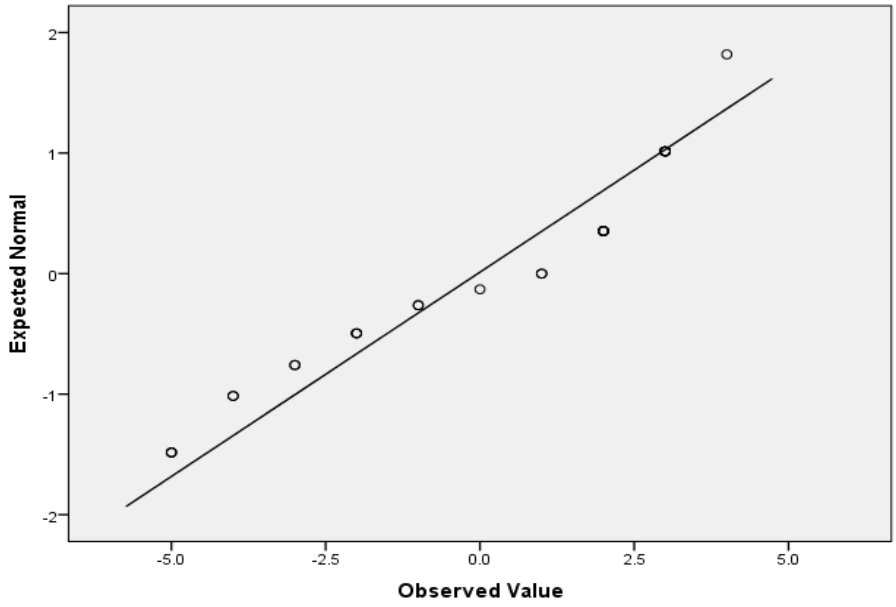
| Values | Kolmogorov-Smirnov[a] | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|
| | Statistic | df | Sig. | Statistic | df | Sig. |
| l. | .219 | 28 | .001 | .887 | 28 | .006 |
| a. | .149 | 28 | .113 | .909 | 28 | .019 |
| b. | .140 | 28 | .169 | .920 | 28 | .034 |

The distribution of the data was observed for all the given parameters through histograms and normal Q-Q plot and through error bars. For all the parameters, it was seen that the mid points of **histograms as shown in graph 3 below** for all parameters when joined, forms a linear line of normal distribution with no presence of kurtosis and skewness or no deviation of data to the extremes were evident.



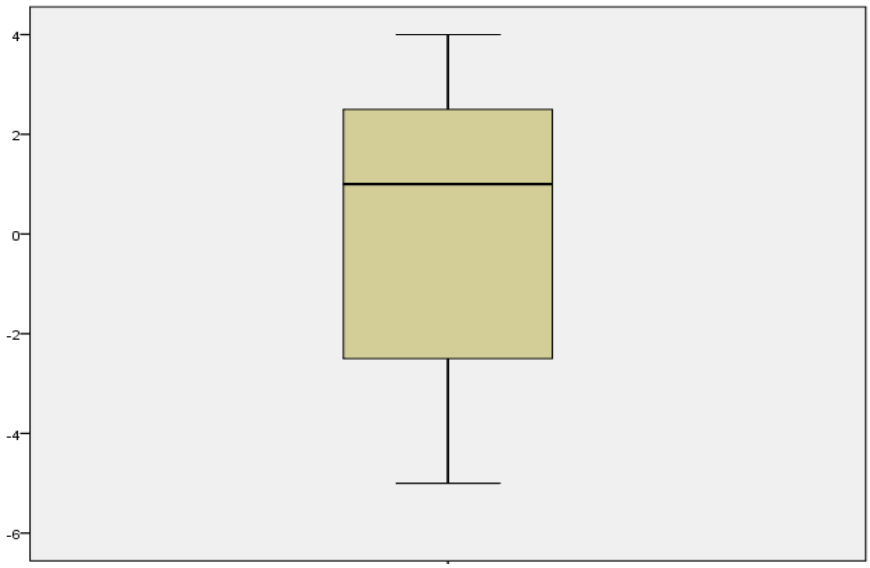

**Graph 3: Showing normality data distribution through histogram**

The mean value of the parameters were not showing deviation towards the extreme while the standard deviation values were less, as depicted in **Q-Q plot as shown in graph 4 below** with the data from samples following the line of distribution towards the mean or the expected value did not deviate more from the approximate or the original value.




**Graph 4: Showing normality data distribution through Q-Q plot**

The error bar **as shown in graph 5 below** for all parameters, did not show more variance towards the extreme as the median value do not show more deviation towards the upper ($25^{th}$ percentile) and lower value ($75^{th}$ percentile)




**Graph 5: Showing normality data distribution through error bar diagram**

**Table 2 (a): Showing baseline control group with three observed values using independent sample t test**

**Group Statistics**

| | values | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Baseline control values | a | 6 | 3.5833 | 2.69772 | 1.10134 |
| | B | 6 | 12.2000 | 9.69041 | 3.95609 |

**Independent Samples Test**

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | F | Sig. | T | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | | Lower | Upper |
| Baseline control values | Equal variances assumed | 13.211 | 0.005 | -2.098 | 10 | 0.062 | -8.61667 | 4.10653 | -17.76659 | 0.53326 |
| | Equal variances not assumed | | | -2.098 | 5.77 | 0.083 | -8.61667 | 4.10653 | -18.76271 | 1.52938 |

*p* value <0.05 – statistically significant. The comparative effect of Origanum oil and 1% Clotrimazole was same and no significant difference was seen.

As shown in above table, the independent sample t test was used to carry out the difference in the values in the control group at the baseline. Three values of l,a, and b were compared individually with each other by keeping *p* value <0.05 as statistically significant. Group statistics was carried out between values at baseline values. No significant difference was observed by comparing the means between the values, (*p* >0.05).

**Table 2 (a1): showing baseline control group with three observed values using independent sample t test**

**Group Statistics**

| | values | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Baseline control values | l | 6 | 68.5500 | 1.88335 | .76887 |
| | a | 6 | 3.5833 | 2.69772 | 1.10134 |

**Independent Samples Test**

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | | Lower | Upper |
| Baseline control values | Equal variances assumed | 2.222 | 0.167 | 48.368 | 10 | .000* | 64.96667 | 1.34317 | 61.97389 | 67.95944 |
| | Equal variances not assumed | | | 48.368 | 8.938 | .000* | 64.96667 | 1.34317 | 61.925 | 68.00833 |

p value <0.05 – statistically significant. Comparative effect of Origanum oil on antibacterial efficacy was superior to 1% Clotrimazole with a significant statistically difference.

As shown in above table, the independent sample t test was done to carry out the difference in the values in the control group at the baseline. Two values of l, and a were compared individually with each other by keeping *p* value <0.05 as statistically significant. Group statistics was carried out between values at baseline values. A statistically significant difference was observed (*p* <0.05) when the means between the values were compared.

**Table 2 (b): Showing baseline experimental group with three observed values using independent sample t test**

**Group Statistics**

| | values | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Baseline experimental value | l | 12 | 65.3333 | 3.28144 | .94727 |
| | a | 12 | 3.4917 | 1.58485 | .45751 |

**Independent Samples Test**

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | | Lower | Upper |
| Baseline experimental value | Equal variances assumed | 9.281 | 0.006 | 58.787 | 22 | .000* | 61.84167 | 1.05197 | 59.66002 | 64.02331 |
| | Equal variances not assumed | | | 58.787 | 15.867 | .000* | 61.84167 | 1.05197 | 59.61008 | 64.07326 |

*P* value <0.05 – statistically significant, for experimental group at baseline the comparative effect of Origanum oil on antibacterial efficacy was superior to 1% Clotrimazole with a significant statistical difference.

As shown in above table, the independent sample t test was used to carry out the difference in the values in the control group at the baseline. Two values of l, and b were compared individually with each other by keeping *p* value <0.05 as statistically significant. Group statistics was carried out between values at baseline values. By comparing the means between the values, a statistically significant difference was observed (*p* <0.05).

**Table 2 (b1): Showing baseline experimental group with three observed values using independent sample t test**

**Group Statistics**

| | values | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Baseline experimental value | a | 12 | 3.4917 | 1.58485 | .45751 |
| | b | 12 | 13.9417 | 12.40487 | 3.58098 |

**Independent Samples Test**

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | | Lower | Upper |
| Baseline experimental value | Equal variances assumed | 53.74 | 0 | -2.895 | 22 | 0.008 | -10.45 | 3.61009 | -17.9369 | -2.96314 |
| | Equal variances not assumed | | | -2.895 | 11.359 | 0.014 | -10.45 | 3.61009 | -18.3652 | -2.53479 |

*P* value <0.05 – statistically significant, for experimental group at baseline the comparative effect of Origanum oil on antibacterial efficacy was equal to 1% Clotrimazole with a non-significant statistically difference. Both are equal more or less likely.

As shown in above table, the independent sample t test was used to carry out the difference in the values in the experimental or study group at the baseline. Two values of a, and b were compared individually with each other by keeping *p* value <0.05 as statistically significant. Group statistics was carried out between values at baseline values. By comparing the means between the values, no statistically significant difference was observed (*p* >0.05).

**Table 2 (b2): Showing baseline experimental group with three observed values using independent sample t test**

**Group Statistics**

| | values | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Baseline experimental value | l | 12 | 65.3333 | 3.28144 | .94727 |
| | b | 12 | 13.9417 | 12.40487 | 3.58098 |

**Independent Samples Test**

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | | Lower | Upper |
| Baseline experimental value | Equal variances assumed | 35.935 | 0 | 13.874 | 22 | .000* | 51.39167 | 3.70415 | 43.70973 | 59.0736 |
| | Equal variances not assumed | | | 13.874 | 12.532 | .000* | 51.39167 | 3.70415 | 43.35885 | 59.42448 |

*P* value <0.05 – statistically significant, for experimental group at baseline the comparative effect of Origanum oil on antibacterial efficacy was superior to 1% Clotrimazole with a significant statistical difference.

As shown in above table, the independent sample t test was used to carry out the difference in the values in the experimental or study group at the baseline. Two values of l and b were compared individually with each other by keeping *p* value <0.05 as statistically significant. Group statistics was carried out between values at baseline values. By comparing the means between the values, a statistically significant difference was observed ($p < 0.05$).

**Table 3 (a): Showing after conditioning control group with three observed values using independent sample t test**

**Group Statistics**

| | values | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Conditioning control values | l | 3 | 63.7333 | 2.00333 | 1.15662 |
| | a | 3 | 4.1000 | 2.08806 | 1.20554 |

**Independent Samples Test**

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | | Lower | Upper |
| Conditioning control values | Equal variances assumed | 0.021 | 0.891 | 35.694 | 4 | .000* | 59.6333 | 1.67066 | 54.9948 | 64.2718 |
| | Equal variances not assumed | | | 35.694 | 3.993 | .000* | 59.6333 | 1.67066 | 54.9917 | 64.275 |

*P* value <0.05 – statistically significant, after conditioning in control group, the comparative effect of Origanum oil on antibacterial efficacy was superior to 1% Clotrimazole with a significant statistically difference.

As shown in above table, the independent sample t test was used to carry out the difference in the values in the control group after conditioning. Two values of a., and l were compared individually with each other by keeping *p* value <0.05 as statistically significant. Group statistics was carried out between values at baseline values. By comparing the means between the values, a statistically significant difference was observed (*p* <0.05).

**Table 3 (a1): Showing after conditioning control group with three observed values using independent sample t test**

**Group Statistics**

| | values | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Conditioning control values | l | 3 | 63.7333 | 2.00333 | 1.15662 |
| | b | 3 | 13.5333 | 10.67911 | 6.16559 |

**Independent Samples Test**

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | | Lower | Upper |
| Conditioning control values | Equal variances assumed | 7.292 | 0.054 | 8.002 | 4 | .001* | 50.2 | 6.27313 | 32.783 | 67.617 |
| | Equal variances not assumed | | | 8.002 | 2.141 | .012* | 50.2 | 6.27313 | 24.8385 | 75.5615 |

*P* value <0.05 – statistically significant, the comparative effect of Origanum oil on antibacterial efficacy was superior to 1% Clotrimazole with a significant statistical difference.

As shown in above table, the independent sample t test was used to carry out the difference in the values in the control group after conditioning. Two values of b, and l were compared individually with each other by keeping *p* value <0.05 as statistically significant. Group statistics was carried out between values at baseline values. By comparing the means between the values, a statistically significant difference was observed (*p* <0.05).

**Table 3 (a2): Showing after conditioning control group with three observed values using independent sample t test**

**Group Statistics**

| | values | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Conditioning control values | a | 3 | 4.1000 | 2.08806 | 1.20554 |
| | b | 3 | 13.5333 | 10.67911 | 6.16559 |

**Independent Samples Test**

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | | Lower | Upper |
| Conditioning control values | Equal variances assumed | 7.92 | 0.054 | 8.002 | 4 | .001* | 50.2 | 6.7313 | 32.783 | 67.617 |
| | Equal variances not assumed | | | 8.002 | 2.141 | .012* | 50.2 | 6.27313 | 24.385 | 75.5615 |

*P* value <0.05 – statistically significant, the comparative effect of Origanum oil on antibacterial efficacy was similar to 1% Clotrimazole with a non- significant statistically difference.

As shown in above table, the independent sample t test was used to carry out the difference in the values in the control group after conditioning. Two values of b and a were compared individually with each other by keeping *p* value <0.05 as statistically significant. Group statistics was carried out between values at baseline values. By comparing the means between the values, a no statistically significant difference was observed (*p* >0.05).

**Table 3 (b): Showing after conditioning experimental group with three observed values using independent sample t test**

**Group Statistics**

| | values | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Conditioning experimental values | l | 6 | 57.5333 | 2.87309 | 1.17294 |
| | a | 6 | 2.9167 | 1.69873 | 0.6935 |

**Independent Samples Test**

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | | Lower | Upper |
| Conditioning experimental values | Equal variances assumed | 1.91 | 0.197 | 40.082 | 10 | .000* | 54.6167 | 1.36262 | 51.5806 | 57.6528 |
| | Equal variances not assumed | | | 40.082 | 8.115 | 0.0008 | 54.6167 | 1.36262 | 51.4822 | 57.7511 |

*P* value <0.05 – statistically significant, after conditioning in experimental group, the comparative effect of Origanum oil on antibacterial efficacy was similar to 1% Clotrimazole with a non- significant statistical difference. Both had a more or less similar effect.

As shown in above table, the independent sample t test was used to carry out the difference in the values in the control group after conditioning. Two values of a, and l were compared individually with each other by keeping *p* value <0.05 as statistically significant. Group statistics was carried out between values at baseline values. By comparing the means between the values, a statistically significant difference was observed (*p* <0.05).

**Table 3 (b1): Showing after conditioning experimental group with three observed values using independent sample t test**

**Group Statistics**

| | values | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Conditioning experimental values | l | 6 | 57.5333 | 2.87309 | 1.17294 |
| | b | 6 | 13.35 | 12.2195 | 4.98857 |

**Independent Samples Test**

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | | Lower | Upper |
| Conditioning experimental values | Equal variances assumed | 19.271 | 0.001 | 8.622 | 10 | .000* | 44.18333 | 5.12461 | 32.76499 | 55.60167 |
| | Equal variances not assumed | | | 8.622 | 5.551 | .000* | 44.18333 | 5.12461 | 31.39395 | 56.97272 |

*P* value <0.05 – statistically significant, after conditioning in experimental group, the comparative effect of Origanum oil on antibacterial efficacy was superior to 1% Clotrimazole with a significant statistical difference. Oregano oil showed superior effect in comparison to 1% clotrimazole

As shown in in above table, the independent sample t test was used to carry out the difference in the values in the control group after conditioning. Two values of a and l were compared individually with each other by keeping *p* value <0.05 as statistically significant. Group statistics was carried out between values at baseline values. By comparing the means between the values, a statistically significant difference was observed (*p* <0.05).

**Table 3 (b2): Showing after conditioning experimental group with three observed values using independent sample t test**

**Group Statistics**

| | values | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Conditioning experimental values | a | 6 | 2.9167 | 1.69873 | 0.6935 |
| | b | 6 | 13.35 | 12.2195 | 4.98857 |

**Independent Samples Test**

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | | Lower | Upper |
| Conditioning value experimental group | Equal variances assumed | 25.414 | 0.001 | -2.072 | 10 | 0.065 | -10.4333 | 5.03654 | -21.6555 | 0.78879 |
| | Equal variances not assumed | | | -2.072 | 5.193 | 0.091 | -10.4333 | 5.03654 | -23.2367 | 2.36999 |

*P* value <0.05 – statistically significant, after conditioning in experimental group, the comparative effect of Origanum oil on antibacterial efficacy was similar to 1% Clotrimazole with a non-significant statistical difference. Oregano oil showed more or less equal effect in comparison to 1% clotrimazole

As shown in above table, the independent sample t test was used to carry out the difference in the values in the control group after conditioning. Two values of a., and b were compared individually with each other by keeping *p* value <0.05 as statistically significant. Group statistics was carried out between values at baseline values. By comparing the means between the values, a no statistically significant difference was observed (*p* >0.05).

# DISCUSSION

The purpose of the present in-vitro study is to evaluate Origanum oil, as an additive to a maxillofacial silicone prosthesis and assess its effect on mechanical property like colour stability by its incorporation into a maxillofacial silicone. The null hypothesis stated no significant effect of addition of oregano oil on antimicrobial properties and colour stability of maxillofacial silicone elastomer.

Currently, silicone elastomer is used for maxillofacial prosthetics because of its mechanical qualities, clinical dimensional stability, biocompatibility, and ease of manipulation[3]. Silicone, also popularly known as polydimethylsiloxane, is a currently widely used polymer in the biomedical industry[4].The use of silicone prosthesis was first presented by Barnhart in 1960[5]. Maxillofacial prosthesis main objective is to improve the patient's appearance so that self-esteem/confidence can be raised and the patients can lead a normal life. It implies that creating a prosthesis with the best possible physical characteristics and aesthetics, as well as maintaining those characteristics during the prosthesis life span, are of greatest priority. However, silicone prosthetic care and durability remain a concern[6].

The longevity of a facial prosthesis made of silicone is 14.5 to 36 months. The main justification for replacing a facial prosthesis is a decline in appearance brought on by changes in physical characteristics and color [6]. The silicone elastomers discoloration is the most frequent cause for replacing facial prosthesis[7]. UV (ultraviolet) radiation seems to have a considerable impact[9]. The main causes of color deterioration are weather conditions and external environmental factors like solar radiation, temperature, and moisture.[9]

Black staining of the inside surfaces of prosthesis following prolonged usage is a concern that has emerged as a result of using such materials. The issue mainly affects nasal prostheses that are more prone to contamination due to the continuous flow of moist air and secretions through nasal apertures. The main causes for replacing a facial prosthesis are color degradation and wearing on the borders of the prosthesis, which are both attributable to fungus growth. [11] The presence of porosities, together with the modification of the anatomy of the facial tissues as a result of the lesion, may compromise the natural balance of the microbial flora, favoring microbial colonization [12].

The frequency of significant fungal infections has grown in recent years. Opportunistic mycoses primarily affect people with immune suppression, which is primarily brought on by degenerative diseases. Oregano's antibacterial properties have been effectively used to stop the growth of a number of foodborne pathogenic bacteria. Its inhibitory impact has also been shown to be effective against fungi that are foodborne, preventing the development of aflatoxins and spores in addition to fungal growth. Several developing parameters that affect the concentration of essential oil and chemical make-up of oregano plants have an impact on the monoterpene concentration, the main molecules with biological activity. [19]

Particularly carvacrol and thymol, have shown to be responsible for the antimicrobial impact of oregano as well as other individual components, are two volatile chemicals present in its essential oil that are primarily responsible for the antibacterial activity of oregano. Thymol and -terpinene were found to be the two primary chemical constituents in Bonfanti et al. (2012) research on the chemical makeup of O. vulgare spp. essential oil. The two primary substances with antibacterial

activity were carvacrol (66%) and p-cymene (14%). Depending on the concentration, the survival/inactivation dynamics displayed bacteriostatic or bactericidal effects; in subminimal inhibitory concentrations. Cell death demonstrated right away after exposure to the highest tested concentrations.

Oregano essential oil and extracts have been shown to be among the most potent antioxidants of all the popular herbs and spices in studies on their antioxidant activity. There are further reports on oregano antioxidants with phenolic and glucosidic structures that are distinct from thymol and carvacrol (Avila-Sosa et al., 2010a).

According to studies, the inclusion of colors, opacifiers, nanoparticles, and numerous human and environmental conditions can impact the color stability of maxillofacial silicones; as a result, all these fields need to be researched. A single "ideal" maxillofacial prosthetic material which probably can survive the effects of various human and environmental variables on color changes and stability has not yet been found despite much research. Currently utilized maxillofacial silicones are reported to hardly last 6 to 24 months before needing to be replaced. Their physical characteristics could alter even during the retention period, causing color variations and stability. Although it has been theorized that adding nanoparticles to silicones used in the craniofacial region may benefit patients, there is still no conclusive human research to support this. Prospective, randomized controlled trials (RCTs) are the only types of studies that can provide credible evidence.

In this study, homogenous mix free of voids and deformities of Oregano oil and maxillofacial silicone and specimens with identical size and shape for color

stability tests were included. For antimicrobial properties against *Candida albicans Staphylococcus aureus***,** study group (n=66) and control group (n=66) both had 66 numbers in each group. Control group had two groups: Group 1 of Silicone+ 1%COT+ C. albicans (n=33) and Group 2 of Silicone +1% COT + S. aureus (n=33). While study groups: Group 3 of Silicone + Oregano oil + C. albicans (n=33) and Group 4 of Silicone + Oregano oil + s. aureus (n=33) while for color stability, both the study group and control group had 33 (n=33) in each group. Group 1 of Mdx- 4 4210 silicone elastomer+ color pigments had (n=33) numbers while Group 2 under study group of silicone + oregano oil emulsion + color pigments had (n=33) numbers.

Results were carried out at baseline and after conditioning at $100^0$ C temperature for 45 hours both for the control and experimental groups. Three specimens were included in the study: Silicone+ 50mg/ml oil emulsion+ red, Silicone+25mg/ml oil emulsion+ yellow and Silicone +12.5 mg/ml oil emulsion+ blue. The observed values of l ,a and b for the samples were compared both at the baseline and after conditioning. For the baseline, it was seen that the observed values both in the control as well as in the experimental groups were statistically insignificant or both were more or less likely equally while after conditioning, statistically significant difference was seen by comparing the means of the observed values for the three specimens by employing independent sample t test both at the baseline and at after conditioning.

**As shown in table 2(a)**, difference in the values in the control group at the baseline was assessed. Three values of l, a and b were compared individually with each other by keeping *p* value <0.05 as statistically significant. Group statistics was

carried out between values at baseline values. By comparing the means between the values, no significant difference was seen ($p$ >0.05).

As shown in **table 2(a1),** the independent sample t test was used to carry out the difference in the values in the control group at the baseline. Two values of l , and a were compared individually with each other by keeping $p$ value <0.05 as statistically significant. Group statistics was carried out between values at baseline values. By comparing the means between the values, a statistically significant difference was observed ($p$ <0.05).

As shown in table **2(b)**, the independent sample t test was used to carry out the difference in the values in the control group at the baseline. Two values of l, and b were compared individually with each other by keeping $p$ value <0.05 as statistically significant. Group statistics was carried out between values at baseline values. By comparing the means between the values, a statistically significant difference was seen ($p$ <0.05).

As shown in table **2(b1)**, the independent sample t test was used to carry out the difference in the values in the experimental or study group at the baseline. Two values of a., and b were compared individually with each other by keeping $p$ value <0.05 as statistically significant. Group statistics was carried out between values at baseline values. By comparing the means between the values, a no statistically significant difference was observed ($p$ >0.05).

As shown in table **2(b2)**, the independent sample t test was used to carry out the difference in the values in the experimental or study group at the baseline. Two values of l, and b were compared individually with each other by keeping $p$ value

<0.05 as statistically significant. Group statistics was carried out between values at baseline values. By comparing the means between the values, a statistically significant difference was observed ($p$ <0.05).

As shown in table **3(a)**, the independent sample t test was used to carry out the difference in the values in the control group after conditioning. Two values of a, and l were compared individually with each other by keeping $p$ value <0.05 as statistically significant. Group statistics was carried out between values at baseline values. By comparing the means between the values, a statistically significant difference was observed ($p$ <0.05).

As shown in table **3(a1)**, the independent sample t test was used to carry out the difference in the values in the control group after conditioning. Two values of b, and l were compared individually with each other by keeping $p$ value <0.05 as statistically significant. Group statistics was carried out between values at baseline values. By comparing the means between the values, a statistically significant difference was observed ($p$ <0.05).

As shown in table **3(a2)**, the independent sample t test was used to carry out the difference in the values in the control group after conditioning. Two values of b, and a were compared individually with each other by keeping $p$ value <0.05 as statistically significant. Group statistics was carried out between values at baseline values. By comparing the means between the values, a no statistically significant difference was observed ($p$ >0.05).

As shown in table **3(b)**, the independent sample t test was used to carry out the difference in the values in the control group after conditioning. Two values of a, and l

were compared individually with each other by keeping *p* value <0.05 as statistically significant. Group statistics was carried out between values at baseline values. By comparing the means between the values, a statistically significant difference was observed (*p* <0.05).

As shown in table **3(b1)**, the independent sample t test was used to carry out the difference in the values in the control group after conditioning. Two values of a and l were compared individually with each other by keeping *p* value <0.05 as statistically significant. Group statistics was carried out between values at baseline values. By comparing the means between the values, a statistically significant difference was observed (*p* <0.05).

As shown in table **3(b2)**, the independent sample t test was used to carry out the difference in the values in the control group after conditioning. Two values of a, and b were compared individually with each other by keeping *p* value <0.05 as statistically significant. Group statistics was carried out between values at baseline values. By comparing the means between the values, a no statistically significant difference was observed (*p* >0.05).

Similarly, Gupta et al. in their systematic review discovered that numerous investigations have been carried out on the color stability of maxillofacial prosthetic materials published in 2021. The variations between the studies also were addressed. Despite extensive research over the last few years, it appears that the one "ideal" material for maxillofacial prosthetics has not yet been discovered. Furthermore, the serviceability and durability of facial prostheses continue to be an issue for maxillofacial prosthodontists across the globe. Numerous studies have been carried out using opacifiers, pigments, and nanoparticles in a variety of settings, including

perspiration, sebum, and disinfectants. It has been noted that both natural and artificial aging have a negative impact on color stability. According to reports, physiological factors like sweat and sebum also influence to color deterioration. However, a high risk of bias was seen because of an absence of standardization, an insufficient sample size, problems with randomization, examiner blindness, inferential statistics, and estimated effect sizes. The durability of maxillofacial silicone elastomers in Asian nations, particularly those with hot and humid climates, have received very little research. TiO2 inclusion was the only factor that, when subjected to meta analysis, indicated increased color stability. Randomized control trials with appropriate research designs are required for the remaining variables in order to get a definite conclusion. Furthermore, it is essential that the scientific community continuing its investigation into maxillofacial silicones and the necessary changes to them in order to improve color stability and reduce clinical issues.

Likewise, in a systematic review conducted by Daivasigamani et al 2021, they concluded that maxillofacial silicone silastic MDX4-4210 with different pigmentations after disinfection and aging, ceramic powder showed more colour stability compared with makeup and colourless after disinfection and 252, 504,1008 hrs aging period. Maxillofacial elastomer mixed with an opacifier and/or a nanoparticle underwent artificial aging and disinfection. The samples with BaSO4 opacifier and ceramic nanoparticles were the most stable material on colour, without intent for aging and disinfection. The colour stability MDX4-4210 maxillofacial silicone after disinfection and accelerated aging was tested and mentioned that chlorhexidine showed more colour change in the facial silicone compared to neutral soap and Efferdent tablet. Also, they mentioned that accelerated aging had a significant effect on the colour stability of all kinds of silicone materials. The barium

sulfate opacifier was more stable than titanium dioxide. The colour stability of Silastic 732 RTV and MDX 4-4210 after disinfection with neutral soap and Efferdent tablet and concluded that neutral soap showed the least effect of colour stability than Efferdent tablets.

The results of our in vitro study, it can be concluded that the origanum oil had a significant effect overall as an additive to maxillofacial silicone prosthesis and also its effect on mechanical property of colour stability.

## SCOPE FOR FUTURE RESEARCH

1) Future research is necessary to determine the effectiveness of Origanum oil when applied to various tissue conditioners to assess its clinical use.

2) Further research is required because the colour change of these materials is a problem that professionals must closely study and control in order to achieve a successful dental rehabilitation.

# LIMITATIONS

1. In the study setting, in vitro research caused the lack of homogeneity in the methodology .
2. It also limited the application of the concepts of evidence- based dentistry.
3. Specimens preparation in general lacked methodological homogeneity.
4. In between studies comparison is not possible.
5. Poor external validity or generalizability of the results.
6. Dissemination of results not possible.

# CLINICAL IMPLICATIONS

1. Given its mechanical qualities, biocompatibility, clinical inertness, and convenience of manipulation, the material of choice for maxillofacial prostheses is silicone elastomer.
2. Origanum oil inhibits fungal adherence and colonization without materially impairing its physical qualities and has improved susceptibility to *Candida albicans* adhesion.
3. Many foodborne pathogenic bacteria have been effectively stopped in their tracks by using the antibacterial properties of oregano. Adding oregano oil in increasing the antibacterial characteristics and color stability of maxillofacial silicone elastomer has been proved against foodborne fungi, by the development of aflatoxins and spores and suppressing fungal growth.

# CONCLUSION

A Perfect material for maxillofacial prostheses is silicone elastomer because of its mechanical qualities, biocompatibility, clinical inertness, and flexibility of handling. Origanum oil lowers fungal adherence and colonization without significantly impairing the material's physical qualities and has improved resistance to *Candida albicans* adhesion. Oregano's antibacterial properties have been effectively used to stop the growth of a number of foodborne pathogenic bacteria. Its inhibitory impact has also been shown to be effective against fungi that are foodborne, preventing the development of aflatoxins and spores in addition to fungal growth.

# SUMMARY

The present research was conducted with the aim to evaluate and compare the effect of Oregano oil on antimicrobial efficacy against Candida albicans, Staphylococcus Aureus and colour stability of maxillofacial silicone after incorporating Oregano oil in it.

Homogenous mix free of voids and deformities of oregano oil and maxillofacial silicone and specimens with identical size and shape for color stability tests were included. Sample size estimation was done using G Power* software and a total of 33 samples were included in each group.

a) For antimicrobial properties against *Candida albicans ,Staphylococcus aureus* both the study group (n=66) and control group (n=66) had 66 numbers in each group. Control group had two groups.

Group 1 of Silicone+ 1%COT+ C. albicans (n=33)

Group 2 of Silicone +1% COT + S. aureus (n=33).

Group 3 of Silicone + Oregano oil + C. albicans (n=33)

Group 4 of Silicone + Oregano oil + s. aureus (n=33)

b) For colour stability

For color stability, both the study group and control group had 33 (n=33) in each group.

Group 1 of Mdx4- 4210 silicone elastomer (n=33) numbers

Group 2 under study group of silicone + oregano oil emulsion + color pigments (n=33) numbers

To compare the means of the study groups after obtaining the values, they were subjected to descriptive statistics, group statistics and independent sample t test for group wise comparison with p value < 0.05 as statistically significant.

On analysing data, the comparative effect of Origanum oil on antibacterial efficacy was superior to 1% clotrimazole with a significant statistical difference. Origanum oil showed superior effect in comparison to 1% clotrimazole.

Within the limitations of the study, the study and its findings have indicated that silicone elastomer is the material of choice for maxillofacial prosthesis because it is clinically inert, biocompatible, mechanical properties and ease of manipulation. Origanum oil showed better resistance to the adhesion of Candida Albicans, and also reduced fungal adherence and colonization without seriously compromising the physical properties of the material. Oregano's antibacterial properties have been effectively used to stop the growth of a number of foodborne pathogenic bacteria. Its inhibitory impact has also been shown to be effective against fungi that are foodborne, preventing the development of aflatoxins and spores in addition to fungal growth.

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# ANNEXURE – I

## ETHICAL CLEARANCE

**Research and Ethics Committee**
**KLE V K INSTITUTE OF DENTAL SCIENCES**
**KLE University**
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Sl. No. : 1455

**CERTIFICATE**

*This is to Certify that the synopsis titled*

Comparative evaluation of effect of origanum vulgare on the antimicrobial efficacy and color stability of maxillo-facial silicone An-in vitro study

*Submitted by*

*Dr.* **REG. NO-IM0220005** _____ *P. G. Student / Staff, Guided by* _____ *from Department of* Prosthodontics & Crown & Bridge *has been critically evaluated by committee members and granted ethical clearance to conduct the above mentioned study*

**Date :** 5/5/21

**Member Secretary**
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

**Chairman**
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

# ANNEXURE – II

**A) Baseline**

| Sr. No. | Sample ID | | Control Group | | |
|---|---|---|---|---|---|
| | | | Observed Values | | |
| | | | L | a | b |
| 1 | Silicon + 50 mg | No.1 | 67.9 | 5.8 | 9.6 |
| | | No.2 | 67.6 | 4.5 | 7.0 |
| 2 | Silicon + 25 mg | No.1 | 69.4 | 0 | 24.6 |
| | | No.2 | 68.3 | 0.3 | 24.2 |
| 3 | Silicon + 12.5 mg | No.1 | 71.8 | 5.6 | 4.2 |
| | | No.2 | 66.3 | 5.3 | 3.6 |

| Sr. No. | Sample ID | | Experimental Group | | |
|---|---|---|---|---|---|
| | | | Observed Values | | |
| | | | L | a | b |
| 1 | Silicon + 50 mg | No.1 | 68.0 | 3.3 | 6.7 |
| | | No.2 | 69.4 | 4.3 | 9.3 |
| | | No.3 | 68.6 | 3.8 | 8.2 |
| | | No.4 | 60.3 | 3.7 | 6.8 |
| 2 | Silicon + 25 mg | No.1 | 64.9 | 1.7 | 29.5 |
| | | No.2 | 66.6 | 1.7 | 30.1 |
| | | No.3 | 69.7 | 0.9 | 31.5 |
| | | No.4 | 66.5 | 1.9 | 31.0 |
| 3 | Silicon + 12.5 mg | No.1 | 60.8 | 4.9 | 4.0 |
| | | No.2 | 63.6 | 5.4 | 3.4 |
| | | No.3 | 62.5 | 5.1 | 3.6 |
| | | No.4 | 63.1 | 5.2 | 3.2 |

**B) After Conditioning**
(Temp. 100° C for 45 hours)

| Sr. No. | Sample ID | | Control Group | | |
|---|---|---|---|---|---|
| | | | Observed Values | | |
| | | | L | a | b |
| 1 | Silicon + 50 mg | No.1 | 63.0 | 5.5 | 9.7 |
| 2 | Silicon + 25 mg | No.1 | 62.2 | 1.7 | 25.6 |
| 3 | Silicon + 12.5 mg | No.1 | 66.0 | 5.1 | 5.3 |

| Sr. No. | Sample ID | | Experimental Group | | |
|---|---|---|---|---|---|
| | | | Observed Values | | |
| | | | L | a | b |
| 1 | Silicon + 50 mg | No.1 | 61.0 | 3.8 | 8.3 |
| | | No.2 | 54.9 | 2.4 | 5.4 |
| 2 | Silicon + 25 mg | No.1 | 60.3 | 0.9 | 29.2 |
| | | No.2 | 57.0 | 1.1 | 28.8 |
| 3 | Silicon + 12.5 mg | No.1 | 53.8 | 4.7 | 3.7 |
| | | No.2 | 58.2 | 4.6 | 4.7 |

**CONTROL**

| Silicone+1% COT + Candida | Silicone+ 1% COT + Staphylococcus |
|---|---|
| 6.2 | 7 |
| 6 | 7.2 |
| 5.5 | 7 |
| 5 | 6.8 |
| 6 | 6.5 |
| 6.2 | 7 |
| 5.3 | 7.2 |
| 5 | 6.9 |
| 6.5 | 7 |
| 6 | 6.5 |
| 6.1 | 6.8 |
| 6.3 | 6.9 |
| 6 | 6.5 |
| 5.8 | 6.4 |
| 6.3 | 7 |
| 5 | 7.2 |
| 5.3 | 7.3 |
| 6.2 | 7 |
| 6 | 6.9 |
| 5 | 6.5 |
| 5.2 | 6.8 |
| 6.1 | 7 |
| 6.2 | 7.2 |
| 6 | 7.1 |
| 5.8 | 6.6 |
| 5.2 | 6.9 |
| 5 | 6.8 |
| 6.2 | 6.3 |
| 6 | 7 |
| 5.5 | 7.2 |
| 6 | 7 |
| 6.2 | 7 |
| 6 | 7 |

**STUDY**

| | CANDIDA | STAPHYLOCOCCUS AUREUS |
|---|---|---|
| 50 mg/ml | 5.2 | 6 |
| | 5.5 | 5.8 |
| | 4.8 | 5.5 |
| | 5 | 6.1 |
| | 5.5 | 5.5 |
| | 5.2 | 5.8 |
| | 5.1 | 5.8 |
| | 4.5 | 6 |
| | 5 | 5.9 |
| | 5.2 | 5.5 |
| | 5.5 | 6 |
| 25 mg/ml | | |
| | 4.8 | 5 |
| | 4.5 | 4.9 |
| | 4.4 | 4.5 |
| | 4.5 | 5.2 |
| | 4.2 | 5.1 |
| | 4.7 | 5 |
| | 4.5 | 4.8 |
| | 4 | 4.5 |
| | 4.2 | 4.8 |
| | 4.5 | 5 |
| | 4.8 | 5 |
| 12.5 mg/ml | | |
| | 3 | 3.2 |
| | 2.8 | 3.8 |
| | 2.9 | 3.1 |
| | 3.2 | 3.5 |
| | 3.1 | 3 |
| | 2.8 | 3.6 |
| | 3 | 3.1 |
| | 3 | 3.5 |
| | 3 | 3 |
| | 3.2 | 3.2 |
| | 2.6 | 3 |
| | | |