ORIGINAL RESEARCH

An *In Vitro* Comparative Evaluation of Conventional and Novel *Thymus vulgaris* Derived Herbal Disinfectant Solutions against Pathogenic Biofilm on Maxillofacial Silicones and Its Impact on Color Stability

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Abstract

Aim: This study aims to assess the antimicrobial efficacy and impact on color stability of *Thymus (T.) vulgaris* solution compared to conventional disinfectants on maxillofacial silicones.

Materials and methods: Various solutions were evaluated, including *T. vulgaris* solutions at 5 and 10%, saline (control), chlorhexidine (4%), and soap water. The substrates were MDX4-4210 silicone elastomers, and the microorganisms tested were *Candida (C.) albicans* and *Staphylococcus (S.) aureus*. The viability of microorganisms was determined through an 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) reduction assay, and color stability was measured using a spectrophotometer with X-Rite Europe software. Statistical analysis was performed using the Kruskal–Wallis test, Mann–Whitney *U post hoc* test, and Wilcoxon Signed Rank test.

Results: Soap water demonstrated superior disinfectant action against both microorganisms, while *T. vulgaris* solutions at 5 and 10% exhibited comparable antimicrobial efficacy. Chlorhexidine and 10% *T. vulgaris* solution showed minimal color changes in the silicone material. In contrast, soap water and the 5% *T. vulgaris* solution resulted in clinically unacceptable color alterations.

Conclusion: This study underscores the potential of *T. vulgaris* as an herbal disinfectant for combating microbial biofilms on maxillofacial silicones, particularly at concentrations of 5 and 10%. The importance of maintaining color stability is emphasized, with Chlorhexidine and the 10% *T. vulgaris* solution demonstrating effective preservation of esthetics. These findings suggest the viability of considering *T. vulgaris* as an alternative disinfectant in clinical settings for maxillofacial silicone prostheses.

Clinical significance: Maxillofacial silicones are vital in restoring aesthetic features for individuals with facial trauma, congenital deformities, or post-surgical interventions. Yet, biofilm-related infections jeopardize their durability and visual integrity. Clinically, *T. vulgaris* signifies a potential advance in prosthodontic care, offering valuable insights for improving antimicrobial performance and aesthetic durability in maxillofacial prostheses.

Keywords: *Candida albicans*, Color stability, Disinfection, Maxillofacial silicones, *Staphylococcus aureus*.

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Introduction

Maxillofacial silicones represent specialized materials employed in facial prosthetics to enhance or restore the appearance of individuals who have undergone facial surgery, experienced facial trauma, or were born with facial abnormalities. These materials play a significant role in enhancing the overall quality of life for these individuals.[1] They are commonly used with adhesives, implant-retained bars, and magnets being the most common retention methods.[2] However, the use of these prostheses can lead to complications if not properly disinfected, with Whear et al., reporting an infection rate of 16%.[3] Disinfection methods such as chlorhexidine solution and liquid soap can affect the color stability of the silicone, with pre-colored silicone showing higher stability than hand-colored silicone.[4] Furthermore, silicones used for maxillofacial prostheses can harbor microorganisms, with silicone being more vulnerable to microbial adhesion due to its larger porosities.[5] Therefore, proper disinfection and maintenance of maxillofacial silicone prostheses are crucial to prevent complications.

It is imperative to sterilize and disinfect maxillofacial silicones to ensure cleanliness, sterility, and patient safety. This process

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serves to eliminate *bacteria, viruses*, and various other types of microorganisms that might inhabit the silicone surface.[6] Effective cleaning procedures not only reduce the risk of infections but also extend the lifespan of prostheses and help maintain their aesthetic appeal. Therefore, the implementation of proper disinfection protocols for maxillofacial silicones is of paramount importance.[4]

A significant concern arises from the proliferation of pathogenic biofilms on maxillofacial silicones. Microorganisms such as *Candida (C.) albicans* and *Staphylococcus (S.) aureus* play a role in infections associated with these biofilms. Specifically, *C. albicans* exhibits adhesion to silicone surfaces and undergoes morphological changes that promote the development of biofilms. On the other hand, *S. aureus* employs virulence factors in the formation of biofilms, leading to increased resistance to antimicrobial treatments and immune responses. A comprehensive understanding of these mechanisms is imperative for the development of effective strategies to both prevent and address biofilm-related infections on maxillofacial silicones.[7,8]

Conventional hygiene practices for maxillofacial silicones involve rinsing with warm water, applying mild soap or a non-abrasive cleanser, and gentle scrubbing with a soft brush or sponge. Despite these methods, limitations arise, as mild soap may struggle to remove persistent stains or biofilm, potentially heightening infection susceptibility. Hard-to-reach areas may not be effectively cleaned with soft-bristled brushes, and air drying or cloth drying could leave residual moisture, fostering microbial growth. Given these challenges, specialized antimicrobial agents or disinfectants become imperative for thorough silicone disinfection.[9]

Thymus vulgaris, commonly known as thyme, is a perennial herb renowned for its versatile pharmacological properties and culinary applications. This herb contains various bioactive compounds, including phenolic compounds, flavonoids, and terpenoids which contribute to its noteworthy antioxidant, antimicrobial, and anti-inflammatory attributes. Thymol and carvacrol are the major phenolic compounds present in *T. vulgaris*.[10] Flavonoids such as luteolin, apigenin, and naringenin are also present.[11] It is also rich in terpenoids, including *p*-cymene, γ-terpinene, and thymol along with Rhamnogalacturonan I-type polysaccharides.[10,12]

Thyme demonstrates robust antimicrobial effects against *bacteria, fungi,* and even *viruses*, including drug-resistant strains. Its antioxidant activity serves to safeguard cells from oxidative damage, consequently diminishing the risk of chronic diseases. Moreover, thyme possesses anti-inflammatory properties and bolsters the immune response.[13,14] Furthermore, extracts derived from *T. vulgaris* have exhibited promising anticancer potential by inducing cell death, restraining tumor growth, and suppressing cancer cell proliferation across various cancer types. It stands as a valuable herb with substantial medicinal properties. Notably, Thymus essential oil has demonstrated superior antimicrobial and antifungal qualities *in vitro*, surpassing the performance of chemical solutions for root canal irrigation. In a separate *in vitro* investigation, *T. vulgaris* essential oil exhibited heightened antifungal effectiveness when compared to chlorhexidine for disinfecting removable orthodontic appliances. These appliances, with their more hydrophobic surface, tend to increase salivary Candida carriers, particularly of the albicans species, by facilitating Candida binding through hydrophobic interactions.[15] This study aims to delve into the therapeutic potential of *T. vulgaris* and its standardized extracts as alternative methods for disinfecting maxillofacial silicones.[16]

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The preservation of color stability in maxillofacial silicones holds significant importance in maintaining the enduring aesthetic appeal of facial prostheses. These silicones are meticulously designed to harmonize with natural skin tones, thus creating a seamless and lifelike appearance. Their formulation incorporates specific pigments and additives to counteract fading or discoloration induced by various factors such as UV radiation, heat, and moisture.[17]

This study introduces a novel approach to managing biofilms on maxillofacial silicones by employing previously unexplored herbal disinfectant solutions derived from *T. vulgaris*. The primary objective of this investigation is to assess the antimicrobial efficacy of these herbal solutions and their impact on color stability in comparison to conventional disinfectants when applied to maxillofacial silicone materials.

Materials and Methods

The present study was conducted as an *in vitro* investigation and adhered to the guidelines of good laboratory practice (GLP). It received approval from the Institutional Review Board (IRB No: EC:1364/2021). To create the experimental specimens, 100 disk-shaped samples were fashioned, each measuring 9 mm in diameter and 4 mm in thickness. These specimens were crafted using MDX4-4210 room-temperature vulcanization elastomer enclosed within a metallic matrix. Notably, MDX4-4210 is a silicone material commonly used in maxillofacial applications.

During the fabrication process, intrinsic pigments were incorporated into the silicone to achieve the desired coloration. The silicone elastomer was prepared following the manufacturer's instructions, which entailed mixing the base and catalyst at a ratio of 10:1. The resulting mixture was applied to the matrix and allowed to polymerize at room temperature for a period of three days. Subsequently, the specimens were delicately removed, any irregularities were trimmed, and sterilization was carried out using ethylene oxide. No additional finishing or polishing procedures were conducted to preserve a smooth surface conducive to cell attachment.

The standard fresh strains of *S. aureus* – MTCC 12598 and *C. albicans* – MTCC 2091, were procured from Dr Prabhakar Kore Basic Science Research Center, Belagavi, India. The specimens were divided into two distinct groups, with 35 specimens each. One group was intentionally contaminated with *S. aureus*, while the other group was contaminated with *C. albicans*. To assess cell viability, the 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay was used. Subsequently, biofilms of *C. albicans* and *S. aureus* developed on the surfaces of these silicone specimens. The next step involved placing these contaminated specimens into two separate, pre-sterilized 24-well microtiter plates. To each well, 2 µL of standardized cell suspensions were added, along with 980 mL of Sabouraud medium for *C. albicans* and Brain-heart infusion medium for *S. aureus*. Subsequently, the microtiter plates were incubated under aerobic conditions in an orbital shaker at 37°C, with an agitation speed of 75 rpm, for a duration of 72 hours. This incubation period allowed for the formation of biofilms on the surface of the silicone material.

The sample size for the five groups was determined through a power and effect size analysis using Cohen's statistical power analysis.[18] The estimation of power and effect size relied on data obtained from the pilot study, with a fixed *p*-value of 0.05 (alpha = 0.05, power = 80%). The assumed effect size was set at 0.5. Consequently, the calculated sample size for each group was determined to be 35. Following the formation of biofilms, each group of 35 specimens for both microorganisms was further subdivided into five distinct groups. In the control group (Group I), the specimens were immersed in a saline solution for a duration of 10 minutes. In the experimental groups, various disinfection methods were used. This included submerging the specimens in 4% chlorhexidine solution (Group II), soap water solution (Group III), and *T. vulgaris* solutions at concentrations of 5% (Group IVa) and 10% (Group IVb), all for a 10 minute duration.[19] Commercially available thyme oil was procured and authenticated from Surajbala Exports Private Limited, New Delhi (HSN Code: 3301). The essential oil was made into a disinfectant solution at KLE Pharmacy College, Belagavi. Previous studies established the minimum inhibitory concentration (MIC) of *T. vulgaris* essential oil specifically for *S. aureus* and *C. albicans*.[14,20,21] Considering these findings, the concentrations for *T. vulgaris* disinfectant were established at 5 and 10%. The contents of the disinfectants included thyme oil as the active ingredient, Tween 80 and Span 60 were the emulsifying agents, and water was used as the solvent. The composition of the *T. vulgaris* disinfectant solutions at concentrations of 5 and 10% is detailed in Table 1.

To evaluate the influence of disinfection protocols on biofilm viability, the MTT reduction assay was employed, using L929 mouse fibroblast cell lines sourced from the National Centre for Cell Science in Pune. This assay quantifies the conversion of MTT into 'Formazan blue' by viable cells. Following the formation of biofilms, the specimens were rinsed with phosphate buffered saline (PBS) and transferred to new wells containing PBS enriched with glucose and MTT. The plates were then incubated in darkness at 37°C for a duration of 3 hours. After incubation, 300 µL from each well was transferred to a fresh plate, and 100 µL of DMSO was added to dissolve the Formazan crystals. Optical density (OD) measurements were conducted at 492 nm using an ELISA reader. The impact of the disinfection solutions on biofilm metabolic activity was determined by calculating the percentage of cell viability relative to the control group, which represented 100% viability. Optical density data were transformed into numerical values that represented cell viability, with the equation used being % of cell viability = [OD (test specimen) × 100%]/[OD (control specimen)].

In the second part of the study, 30 disk-shaped specimens were manufactured using MDX4-4210 silicone elastomer, employing the same procedure as previously described for specimen fabrication. These specimens shared the same dimensions, measuring 9 mm in diameter and 4 mm in thickness. The evaluation was centered on assessing the color stability of conventional disinfectants applied to the silicone elastomer. Color assessment was carried out at two specific time points: initially, at the commencement of the disinfection procedure, and subsequently, after a 30-day duration of disinfection. The color analysis was conducted with the utilization of the Spectrophotometer X-Rite Europe software. Color changes were quantified utilizing the CIE Lab × color system, employing the formula $\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$.[13] The ΔE values obtained were categorized into three clinically relevant intervals: ΔE <1 (indicating undetectable color alteration), 1 < ΔE < 3.3 (representing color changes clinically considered acceptable), and ΔE > 3.3 (indicating color changes clinically regarded as unacceptable.

Table 1: Composition of *Thymus vulgaris* disinfectant at various concentrations

Ingredients	*Concentrations*		*Function*
Thyme oil	2.5% (2.5 mL)	5% (5 mL)	Active ingredient
Tween 80	1.2 gm	2.0 gm	Emulsifying agent
Span 60	0.5 gm	0.75 gm	Emulsifying agent
Water	100 mL	100 mL	Vehicle/Solvent

Values are expressed in percentages (%); gm, grams; mL, milliliter

Statistical Analysis

Data collection was conducted using MS Excel, and data analysis was performed with IBM-SPSS® Statistics, Version 21 (Armonk, NY, USA: IBM Corp., 2012). For intragroup comparisons, the Kruskal–Wallis Test was employed, followed by the Mann–Whitney *U post hoc* test intergroup comparisons. Additionally, pairwise comparisons were carried out using the Wilcoxon Signed Rank test. The statistical significance level was set at $p \leq 0.05$ for all conducted tests.

Results

The comparison of mean absorbance value after the MTT assay for *C. albicans* and *S. aureus* biofilms is presented in Table 2.

Disinfectant Action on *Candida albicans*

The mean absorbance value was highest in the control (saline) group (1.78 ± 0.04) and lowest in the soap water group (0.33 ± 0.02). A statistically significant difference in the mean absorbance values was observed among the five groups, as determined by the Kruskal–Wallis Test ($p > 0.001$). When the Mann–Whitney *U post hoc* test was applied for intergroup comparisons, statistically significant differences in the mean absorbance values were found (Table 2).

Table 2: Comparison of absorbance (mean optical density) among the control and intervention groups using MTT assay

	Candida albicans (n = 35)			*Staphylococcus aureus (n = 35)*		
Disinfectant	*N*	*Absorbance (OD) Mean ± SD*	*p-value*	*N*	*Absorbance (OD) Mean ± SD*	*p-value*
Saline (control)	7	1.78 ± 0.04	0.001*	7	1.64 ± 0.01	0.001*
Chlorhexidine	7	$0.85 \pm 0.02^{\alpha}$		7	$0.58 \pm 0.02^{\alpha}$	
Soap water	7	$0.33 \pm 0.02^{\alpha\beta}$		7	$0.23 \pm 0.02^{\alpha\beta}$	
Thymus vulgaris 5%	7	$0.54 \pm 0.02^{\alpha\beta\gamma}$		7	$0.27 \pm 0.03^{\alpha\beta\gamma}$	
Thymus vulgaris 10%	7	$0.64 \pm 0.03^{\alpha\beta\gamma\delta}$		7	$0.33 \pm 0.02^{\alpha\beta\gamma\delta}$	

OD, optical density; All values are expressed as mean ± standard deviation (SD). The statistical test used: Kruskal–Wallis Test; Mann–Whitney *U* test (*post hoc* test). Level of significance: *$p \leq 0.05$ is considered statistically significant; statistically significant difference with $^{\alpha}$control, $^{\beta}$chlorhexidine, $^{\gamma}$soap water, and $^{\delta}$*Thymus vulgaris* 5%

Cell viability of *C. albicans* was highest in the control group, followed by the Chlorhexidine group. Both the 5 and 10% concentrations of *T. vulgaris* exhibited cell viability of 48.17 and 52.34%, respectively, which were comparable to the soap water group, which had the lowest cell viability at 44.6% (Fig. 1). This observation underscores the comparative efficacy of different disinfectants against *C. albicans*. Notably, the disinfectant action of soap water emerged as the most potent, demonstrating a pronounced inhibitory effect on the growth or viability of *C. albicans*. Subsequent to soap water, *T. vulgaris* exhibited varying degrees of efficacy, with the 5% concentration demonstrating a substantial impact, followed by a slightly diminished effect at the 10% concentration.

Disinfectant Action on *Staphylococcus aureus*

The mean absorbance value and cell viability of *S. aureus* followed a similar trend as that of *C. albicans*. The highest mean absorbance value was observed in the control group (1.64 ± 0.01), and the lowest was found in the soap water group (0.23 ± 0.02). The Kruskal–Wallis test revealed a statistically significant difference in the mean absorbance values among the five groups ($p > 0.001$). Intergroup comparisons using the Mann–Whitney *U post hoc* test also showed statistically significant differences (Table 2).

Cell viability of *S. aureus* was observed highest in the control group, followed by the Chlorhexidine group. Both the 5 and 10% concentrations of *T. vulgaris* exhibited cell viabilities of 33 and 31%, respectively, which were comparable to the soap water group, with the lowest cell viability at 29% (Fig. 2). These findings imply a hierarchy in the efficacy of disinfectants against *S. aureus*, with soap water exhibiting the most pronounced effect. The robust disinfectant action of soap water suggests its potential as an effective antimicrobial agent against *S. aureus*. Following this, *T. vulgaris* demonstrated varying degrees of efficacy, with the 5 and 10% concentrations showcasing a more substantial impact comparable to the soap solution.

Color Stability of the Silicones

The evaluation of color stability in the silicones subjected to various disinfectants was conducted after a 30-day period. The results revealed that both chlorhexidine ($p = 0.553$) and *T. vulgaris* at a 10% concentration ($p = 0.345$) exhibited color alterations that were clinically undetectable. In contrast, saline ($p = 0.015$) and *T. vulgaris* at 5% ($p = 0.018$) exhibited color changes that were considered clinically acceptable. On the other hand, soap water ($p = 0.014$) led to color alterations that were deemed clinically unacceptable (Table 3). This indicates that both chlorhexidine and *T. vulgaris* showed superior color stability among all the tested disinfectants.

Discussion

Silicone is a widely favored material for maxillofacial prostheses due to its comfort and flexibility. However, the porous nature of these prostheses, along with moisture accumulation and the acidic pH of the skin, especially in individuals with limited dexterity, creates a favorable environment for the growth of bacteria and fungi.[22] In order to manage and prevent infections in individuals

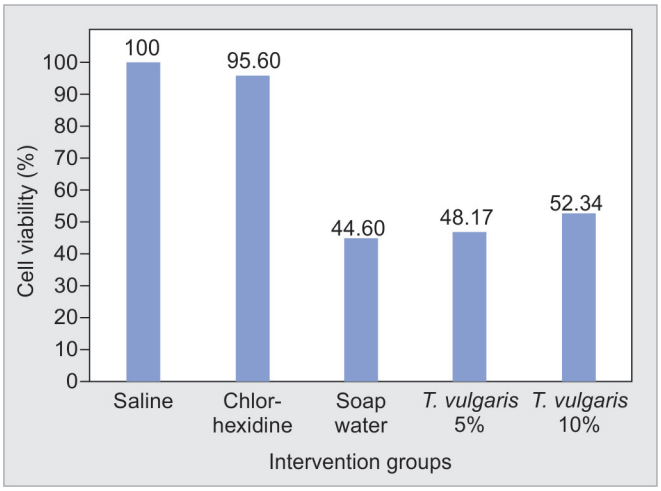

Fig. 1: Comparison of cell viability of *Candida albicans* among the intervention groups

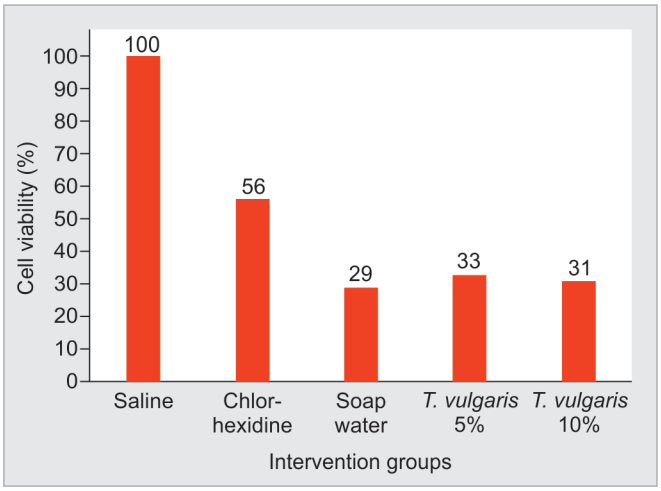

Fig. 2: Comparison of cell viability of *Staphylococcus aureus* among the intervention groups

Table 3: Comparison of color stability among the control and intervention groups

Disinfectant	*N*	*Baseline*	*30 days*	*Mean difference*	*p-value*
Saline (control)	6	58.03 ± 0.85	61.21 ± 0.09	3.18	0.015*
Chlorhexidine	6	57.83 ± 1.14	58.07 ± 0.15	0.24	0.553
Soap water	6	55.85 ± 0.43	60.47 ± 0.43	4.62	0.014*
Thymus vulgaris 5%	6	57.36 ± 0.69	59.59 ± 0.70	2.23	0.018*
Thymus vulgaris 10%	6	58.71 ± 0.46	59.02 ± 0.54	0.31	0.345

All values are expressed as mean ± standard deviation (SD). The statistical test used: Wilcoxon Signed Rank test for pairwise comparison; Level of significance: *$p \leq 0.05$ is considered statistically significant

using maxillofacial prostheses, strict adherence to proper hygiene practices and thorough disinfection of the prosthesis is essential.[23] The current study aimed to assess the effectiveness of *T. vulgaris* solution, a herbal disinfectant for maxillofacial prostheses, and its impact on color stability, addressing the critical need for improved disinfection methods in this context.

In this study, the antifungal activity of five disinfectants against *C. albicans* was assessed using the MTT assay. Soap water exhibited the highest antifungal action, followed by the 5% and 10% concentrations of *T. vulgaris* solution, respectively. Treatment with these solutions resulted in a significant reduction in *C. albicans* viability, on par with the effect of soap water. While soap water demonstrated the highest antifungal activity, the efficacy of *T. vulgaris* was nearly as potent and even surpassed that of chlorhexidine. These findings indicate that *T. vulgaris* solution holds promise as an effective disinfectant for maxillofacial prostheses, particularly in combating *C. albicans* infections. These results are consistent with those of Naseri et al., where a 2% *T. vulgaris* solution exhibited favorable antimicrobial activity against *C. albicans*.[15] Additionally, Jafri and Ahmad demonstrated that *T. vulgaris* is effective in treating biofilms associated with *C. albicans*, supporting the findings of this study.[24] It was also found to be effective against removable orthodontic appliances infected with *C. albicans* as reported by Kavianirad et al.[25]

A similar trend was observed in this study against *S. aureus*. Soap water exhibited the highest antibacterial activity, followed by *T. vulgaris* solutions at 5 and 10%, respectively. The cell viability of the herbal disinfectants was comparable to that of soap water and superior to chlorhexidine. Notably, chlorhexidine demonstrated stronger antimicrobial activity against *S. aureus* compared to *C. albicans*. These results indicate that the *T. vulgaris* disinfectant possesses significant antibacterial effects against *S. aureus*, on par with soap water and superior to chlorhexidine. These findings align with those reported by Guiotti et al. and Galgano et al.[18,26] It was found to be effective in treating *S. aureus*, including methicillin-resistant *S. aureus*. It has been found to inhibit biofilm formation, and disrupt preformed biofilms.[27] It also demonstrated synergistic effects when combined with other antimicrobial compounds, enhancing its antibacterial activity.[28] These findings suggest that *T. vulgaris* can be effective in treating *S. aureus* infections.

Saline solution, being a neutral and isotonic substance without inherent antimicrobial properties, serves as a baseline for assessing disinfectant effects. Its similarity to body fluid sodium levels ensures its suitability for biological research.[29] In contrast, a 4% chlorhexidine solution, known for its biocompatibility and comprehensive disinfection capabilities, has consistently proven effective in disinfecting dental impression materials and prostheses using both spray and immersion methods.[30]

Color stability is recognized as a pivotal characteristic when assessing the functionality of a facial prosthesis from the patient's perspective. It is a common parameter used to evaluate the longevity and aesthetic appeal of materials. In a study by Cantor et al., methods for assessing the aesthetics and color matching of prosthetic facial materials with human skin using reflectance spectrophotometry were explored.[31] In the present study, the chlorhexidine group exhibited the least color alterations among the tested disinfectants, followed by *T. vulgaris* at a 10% concentration. These findings were in line with a study conducted by Chamaria et al.,[19] although they contradicted the results of Chotprasert et al.[4] Conversely, other disinfectants demonstrated substantial color alterations after a 30-day period, including *T. vulgaris* at a 5% concentration and the soap water group.

The observed color changes in the maxillofacial silicones indicate that conventional disinfectants such as soap may have a mild adverse effect on the aesthetic quality of the prostheses over time.[9] The specific concentration of soap for disinfecting maxillofacial silicones is not explicitly detailed in the provided studies. However, the study by Goiato et al. recommends the use of neutral soap for cleansing maxillofacial prostheses. It's important to note that the process of disinfecting specimens using neutral soap involves applying digital friction, which may gradually remove the superficial layer of silicone.[32] Therefore, careful consideration must be given to selecting a cleaning solution that can achieve both disinfection and preservation of the prosthetic material's aesthetic quality simultaneously.

Numerous environmental factors, including sunlight, humidity, air pollutants, facial secretions, and disinfection methods, can impact the color stability of silicone prostheses. Research conducted by Goiato et al. and Mancuso et al. underscores how external factors and chemical cleansers can lead to discoloration and the alteration of material properties.[32,33] The influence of UV light exposure, as documented in studies by Lemon et al., Haug et al., Eleni et al., and Hatamleh et al. has been observed to induce significant changes in coloration. These changes are attributed to processes like cross-linking and interaction with fatty acids.[34–37] Additionally, research by Hatamleh et al. and Polyzois et al. has shown that exposure to simulated sebum solutions can result in color changes and the decomposition of elastomeric materials.[37,38]

Despite soap water's superior antimicrobial efficacy, it did not exhibit higher color stability on maxillofacial silicones in this study. In contrast, the herbal disinfectant proved more effective in preserving color while still providing adequate antimicrobial protection. When choosing chemical disinfectants, it is crucial to consider both their antimicrobial properties and compatibility with preserving the material's physical characteristics. The minimal impact of the *T. vulgaris* disinfectant (10%) on color stability is promising, indicating that antimicrobial treatment with Thymus does not compromise the aesthetic quality of maxillofacial silicones. Herbal disinfectants, with their bioactive phytochemical composition, demonstrate potent antimicrobial activity against pathogens, offering a more sustainable and cost-effective solution compared to synthetic counterparts. Conducting clinical trials with patients having maxillofacial defects could provide a more comprehensive and scientifically rigorous assessment of the efficacy of *T. vulgaris* disinfectant.

Limitations and Recommendations

This study has certain limitations and should be acknowledged for a comprehensive understanding of its scope. First and foremost, the research predominantly centers on monocultures, thereby overlooking the intricate interactions that may arise in mixed-species scenarios. The exclusive utilization of a singular type of silicone elastomer further restricts the generalizability of the findings, as variations in physical properties across different elastomers remain unexplored. Moreover, the absence of assessments in simulated environments, such as those mimicking artificial aging or exposure to sebum solutions, represents another limitation. The inclusion of such simulated conditions could have offered valuable insights into the material's performance in more diverse and realistic settings, enhancing the overall robustness and applicability of

the study's outcomes. Additionally, it is noteworthy that this study does not specifically address the MIC of the compounds under investigation.[9,17,19] The study's findings open avenues for future research in maxillofacial prosthetics. Subsequent investigations may explore the efficacy of *T. vulgaris*-derived herbal disinfectant solutions against diverse microbial strains and in mixed-species biofilm models. Future directions could involve developing hybrid disinfectant formulations addressing both pathogenic biofilm and color stability in maxillofacial silicones. Additionally, research might focus on more realistic simulated environments, like extended use or exposure to environmental factors. These considerations will contribute to refining and advancing the practical applicability of disinfectant solutions for the care and longevity of maxillofacial prosthetic materials.

Clinical Relevance

The findings of the study carry significant implications for clinicians involved in maxillofacial prosthetics. The research underscores the importance of carefully considering disinfectant solutions in the maintenance and care of maxillofacial silicones, as they play a crucial role in combating pathogenic biofilm. Clinicians are advised to evaluate and select disinfectants judiciously, considering not only their antimicrobial efficacy but also their impact on the color stability of silicone materials. The study suggests that the novel *T. vulgaris*-derived herbal disinfectant solution is a potential alternative for maxillofacial silicones. These considerations will contribute to informed decision-making in clinical practice, ensuring the optimal care and longevity of maxillofacial prosthetic materials.

Conclusion

This study highlights *T. vulgaris* as a promising herbal disinfectant against microbial biofilms on maxillofacial silicones, particularly at 5 and 10% concentrations, though not surpassing the efficacy of conventional soap water. This suggests *T. vulgaris* is a potential alternative in clinical settings, emphasizing its compatibility with silicone materials. Additionally, the study underscores the importance of color stability in maxillofacial prosthetics, with Chlorhexidine and 10% *T. vulgaris* effectively preserving color, aiding practitioners in selecting disinfection agents that ensure both microbial control and visual integrity.

IRB Approval

Obtained from KLE V.K. Institute of Dental Sciences Institutional Review Board (Ref. No: EC:1364/2021).

Statement of Approval by Authors

The manuscript has been read and approved by all the authors, the requirements for authorship have been met and each author believes that the manuscript represents honest work.

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References

1. Gupta P, Deshpande S, Radke U, et al. The color stability of maxillofacial silicones: A systematic review and meta-analysis. J Indian Prosthodont Soc 2021;21(2):138–149. DOI: 10.4103/jips.jips_253_19.
2. Hatamleh MM, Haylock C, Watson J, et al. Maxillofacial prosthetic rehabilitation in the UK: A survey of maxillofacial prosthetists' and technologists' attitudes and opinions. Int J Oral Maxillofac Surg 2010;39(12):1186–1192. DOI: 10.1016/j.ijom.2010.08.002.
3. Whear NM, Cousley RR, Liew C, et al. Post-operative infection of Proplast facial implants. Br J Oral Maxillofac Surg 1993;31(5):292–295. DOI: 10.1016/0266-4356(93)90062-2.
4. Chotprasert N, Shrestha B, Sipiyaruk K. Effects of disinfection methods on the color stability of precolored and hand-colored maxillofacial silicone: An in vitro study. Int J Biomater 2022;2022:7744744. DOI: 10.1155/2022/7744744.
5. Kumar A, Seenivasan MK, Inbarajan A. A literature review on biofilm formation on silicone and poymethyl methacrylate used for maxillofacial prostheses. Cureus 2021;13(11):e20029. DOI: 10.7759/cureus.20029.
6. Cevik P, Yildirim-Bicer AZ. Effect of different types of disinfection solution and aging on the hardness and colour stability of maxillofacial silicone elastomers. Int J Artif Organs 2018;41(2):108–114. DOI: 10.5301/ijao.5000659.
7. Cevik P, Akca G, Asar NV, et al. Antimicrobial effects of nano titanium dioxide and disinfectants on maxillofacial silicones. J Prosthet Dent 2023;S0022-3913(23)00135-X. DOI: 10.1016/j.prosdent.2023.03.001.
8. Mat-Rani S, Chotprasert N, Srimaneekarn N, et al. Fungicidal effect of lemongrass essential oil on candida albicans biofilm pre-established on maxillofacial silicone specimens. J Int Soc Prev Community Dent 2021;11(5):525–530. DOI: 10.4103/jispcd.JISPCD_63_21.
9. Babu AS, Manju V, Gopal VK. Effect of chemical disinfectants and accelerated aging on maxillofacial silicone elastomers: An in vitro study. Indian J Dent Res 2018;29(1):67–73. DOI: 10.4103/ijdr.IJDR_272_16.
10. Doghish AS, Shehabeldine AM, El-Mahdy HA, et al. Thymus vulgaris oil nanoemulsion: synthesis, characterization, antimicrobial and anticancer activities. Mol Basel Switz 2023;28(19):6910. DOI: https://doi.org/10.3390/molecules28196910.
11. Hossain MA, AL-Raqmi KAS, AL-Mijizy ZH, et al. Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown Thymus vulgaris. Asian Pac J Trop Biomed 2013;3(9):705–710. DOI: 10.1016/S2221-1691(13)60142-2.
12. Banerjee P, Mukherjee S, Bera K, et al. Polysaccharides from Thymus vulgaris leaf: Structural features, antioxidant activity and interaction with bovine serum albumin. Int J Biol Macromol 2019;125:580–587. DOI: 10.1016/j.ijbiomac.2018.11.117.
13. Karpiński TM, Ożarowski M, Seremak-Mrozikiewicz A, et al. Anti-candida and antibiofilm activity of selected lamiaceae essential oils. Front Biosci (Landmark Ed) 2023;28(2):28. DOI: 10.31083/j.fbl2802028.
14. Sateriale D, Forgione G, De Cristofaro GA, et al. Towards green strategies of food security: Antibacterial synergy of essential oils from thymus vulgaris and syzygium aromaticum to inhibit escherichia coli and staphylococcus aureus pathogenic food isolates. Micro 2022;10(12):2446. DOI: 10.3390/microorganisms10122446.
15. Naseri N, Kalantari Khandani A, Baherimoghadam T, et al. The effect of thymus vulgaris essential oil and chlorhexidine on candida albicans accumulated on removable orthodontic appliance: A clinical trial. J Dent Shiraz Iran 2022;23(1 Suppl):190–197. DOI: 10.30476/DENTJODS.2021.89317.1404.

16. Oliveira AS, Rolo J, Gaspar C, et al. Chemical characterization and bioactive potential of Thymus×citriodorus (Pers.) Schreb. preparations for anti-acne applications: Antimicrobial, anti-biofilm, anti-inflammatory and safety profiles. J Ethnopharmacol 2022;287:114935. DOI: 10.1016/j.jep.2021.114935.
17. Mehta S, Nandeeshwar DB. A spectrophotometric analysis of extraoral aging conditions on the color stability of maxillofacial silicone. J Indian Prosthodont Soc 2017;17(4):355–360. DOI: 10.4103/jips.jips_87_17.
18. Guiotti AM, Cunha BG, Paulini MB, et al. Antimicrobial activity of conventional and plant-extract disinfectant solutions on microbial biofilms on a maxillofacial polymer surface. J Prosthet Dent 2016;116(1):136–143. DOI: 10.1016/j.prosdent.2015.12.014.
19. Chamaria A, Aras MA, Chitre V, et al. Effect of chemical disinfectants on the color stability of maxillofacial silicones: An in vitro study. J Prosthodont 2019;28(2):e869–e872. DOI: 10.1111/jopr.12768.
20. Fani M, Kohanteb J. In vitro antimicrobial activity of thymus vulgaris essential oil against major oral pathogens. J Evid-Based Complement Altern Med 2017;22(4):660–666. DOI: 10.1177/2156587217700772.
21. Amatiste S, Sagrafoli D, Giacinti G, et al. Antimicrobial activity of essential oils against Staphylococcus aureus in fresh sheep cheese. Ital J Food Saf 2014;3(3):1696. DOI: 10.4081/ijfs.2014.1696.
22. Chong WX, Lai YX, Choudhury M, et al. Efficacy of incorporating silver nanoparticles into maxillofacial silicone against Staphylococcus aureus, Candida albicans, and polymicrobial biofilms. J Prosthet Dent 2022;128(5):1114–1120. DOI: 10.1016/j.prosdent.2021.01.010.
23. de Azevedo MN, Marques NT, Fonseca MFL, et al. Disinfectant effects of Brazilian green propolis alcohol solutions on the Staphylococcus aureus biofilm of maxillofacial prosthesis polymers. J Prosthet Dent 2022;128(6):1405–1411. DOI: 10.1016/j.prosdent.2021.03.025.
24. Jafri H, Ahmad I. Thymus vulgaris essential oil and thymol inhibit biofilms and interact synergistically with antifungal drugs against drug resistant strains of Candida albicans and Candida tropicalis. J Mycol Méd 2020;30(1):100911. DOI: 10.1016/j.mycmed.2019.100911.
25. Kavianirad F, Bahador N, Naseri N, et al. The antifungal effect of thymus vulgaris on isolated candida albicans from the surface of removable orthodontic appliances. Herb Med J Herb Med J 2019;4(2):55–64. DOI: https://doi.org/10.22087/hmj.v4i2.718.
26. Galgano M, Capozza P, Pellegrini F, et al. Antimicrobial activity of essential oils evaluated in vitro against escherichia coli and staphylococcus aureus. Antibiotics (Basel) 2022;11(7):979. DOI: 10.3390/antibiotics11070979.
27. Kot B, Wierzchowska K, Grużewska A, et al. The effects of selected phytochemicals on biofilm formed by five methicillin-resistant Staphylococcus aureus. Nat Prod Res 2018;32(11):1299–1302. DOI: 10.1080/14786419.2017.1340282.
28. Vaillancourt K, LeBel G, Yi L, et al. In vitro antibacterial activity of plant essential oils against Staphylococcus hyicus and Staphylococcus aureus, the causative agents of exudative epidermitis in pigs. Arch Microbiol 2018;200(7):1001–1007. DOI: 10.1007/s00203-018-1512-4.
29. van der Waal SV, Oonk CAM, Nieman SH, et al. Additional disinfection with a modified salt solution in a root canal model. J Dent 2015;43(10):1280–1284. DOI: 10.1016/j.jdent.2015.07.015.
30. Alqarni H, Jamleh A, Chamber MS. Chlorhexidine as a disinfectant in the prosthodontic practice: A comprehensive review. Cureus 2022;14(10):e30566. DOI: 10.7759/cureus.30566.
31. Cantor R, Webber RL, Stroud L, et al. Methods for evaluating prosthetic facial materials. J Prosthet Dent 1969;21(3):324–332. DOI: 10.1016/0022-3913(69)90295-9.
32. Goiato MC, Zucolotti BCR, Mancuso DN, et al. Care and cleaning of maxillofacial prostheses. J Craniofac Surg 2010;21(4):1270–1273. DOI: 10.1097/SCS.0b013e3181e1b431.
33. Mancuso DN, Goiato MC, Santos DM dos. Color stability after accelerated aging of two silicones, pigmented or not, for use in facial prostheses. Braz Oral Res 2009;23(2):144–148. DOI: 10.1590/s1806-83242009000200009.
34. Lemon JC, Chambers MS, Jacobsen ML, et al. Color stability of facial prostheses. J Prosthet Dent 1995;74(6):613–618. DOI: 10.1016/s0022-3913(05)80314-2.
35. Haug SP, Andres CJ, Moore BK. Color stability and colorant effect on maxillofacial elastomers. Part III: Weathering effect on color. J Prosthet Dent 1999;81(4):431–438. DOI: 10.1016/s0022-3913(99)80010-9.
36. Eleni PN, Krokida MK, Polyzois GL, et al. Effect of different disinfecting procedures on the hardness and color stability of two maxillofacial elastomers over time. J Appl Oral Sci 2013;21(3):278–283. DOI: 10.1590/1679-775720130112.
37. Hatamleh MM, Watts DC. Effect of extraoral aging conditions on color stability of maxillofacial silicone elastomer. J Prosthodont 2010;19(7):536–543. DOI: 10.1111/j.1532-849X.2010.00627.x.
38. Polyzois GL, Tarantili PA, Frangou MJ, et al. Physical properties of a silicone prosthetic elastomer stored in simulated skin secretions. J Prosthet Dent 2000;83(5):572–577. DOI: 10.1016/s0022-3913(00)70017-5.