ORIGINAL ARTICLE

The effect of antifungal activities of auto-polymerized silicone soft denture liner material containing *origanum vulgare* gel against *Candida albicans* over 14-day period: an in vitro study

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ABSTRACT

Introduction: The greatest disadvantage of silicone soft denture liner (SDL) materials is the difficulty in keeping them clean due to their incompatibility with conventional denture cleaning solutions. As a result, chemical methods are often recommended as effective alternatives to mechanical cleaning. Natural products have emerged as promising substitutes for synthetic chemical agents. *Origanum vulgare,* in particular, has shown significant antifungal activity against *Candida albicans*. This study aims to analyze the effect of antifungal activities of auto-polymerized silicone soft denture liner material containing *Origanum vulgare* gel against *Candida albicans* over a 14 days period. **Methods**: The antifungal activity was evaluated using the disc diffusion method. The samples were divided into three groups: (1) SDL without antifungal agent addition, (2) SDL with nystatin addition, and (3) SDL with *Origanum vulgare* gel addition. Data were analyzed using one-way ANOVA and Dunnett's T3 for usage duration of 7 and 14 days. **Results**: The addition of *Origanum vulgare* gel showed significant antifungal activity, with minimum inhibitory concentration (MIC) 3.12% and minimum fungicidal concentration (MFC) 6.25%. One way ANOVA analyses the inhibition zone of *Candida albicans* was significantly affected by the usage duration (7 and 14 days), with $p<0.001$ respectively. Post-hoc analyses using Dunnett's T3 test for the groups at 7 days and 14 days revealed significant difference in the antifungal effect between each group with MIC and MFC. **Conclusion**: Nystatin was found to be less effective than *Origanum vulgare* gel in inhibiting the growth of *C. albicans.* The addition of *Origanum vulgare* gel at a concentration of 6.25% to auto-polymerized silicone SDL is recommended, as it effectively inhibits the growth of *Candida albicans* for up to 14 days.

KEYWORDS

Soft denture, essential oil, *origanum vulgare*, *inhibition zone*, *candida albicans*

INTRODUCTION

A complete denture is a fixed or removable dental prosthesis that replaces the entire dentition and associated structures of the maxilla and mandible.[1] According to the World Health Organization (WHO), the prevalence of complete denture use is highest among individuals aged 65 years and older, at 5.8%.[2] Over time, complete denture may become loose due to alveolar ridge resorption, which can cause pain

or damage to the denture-bearing tissue. One approach to addressing this problem is the denture relining using a SDL, which is applied to the anatomical surface of the complete denture base.[3] Soft denture liner materials can be categorized as either plasticized acrylic resins or silicone elastomers. Silicone elastomers, composed of dimethyl-siloxane polymers, are free from leachable plasticizers and retain their elastic properties over extended periods, making them the term permanent liners.[4]

Auto-polymerized silicone SDL have the benefit of becoming naturally soft over time and are the most widely used material for this purpose. Due to the absence of a plasticizer, silicone liners outperform acrylic resin liners in terms of resilience and long-term cushioning maintenance.[5] Despite these advantages, soft denture liners are more difficult to clean than hard denture bases because they cannot be effectively cleaned using mechanical brushing.

Additionally, they exhibit reduced resilience and water sorption, are prone to degradation, and facilitate the accumulation of microorganisms. These factors can contribute to the progression of pathological processes, limiting treatment options for existing infections. Soft denture liners that lack antifungal properties allow the formation of biofilm, which are difficult to remove even with denture cleansers. The increased growth of *Candida albicans* can lead to denture stomatitis.[6] Epidemiological studies indicate that 30–50% of complete denture wearers suffer from denture stomatitis.[7]

Antifungal agents can be used to prevent *Candida* colonization. Incorporating antifungal agents into SDL is an inexpensive method with a high success rate. This method has garnered significant attention because it does not require patient cooperation. The addition of antifungal agents into resilient denture liners has been widely adopted and has proven effective and viable both in *in vitro* and *in vivo* studies.[8]

However, antifungal such as nystatin can have side effects, including toxicity, drug interactions, and the emergence of drug-resistant fungal species. As a result, herbal antifungals are increasingly being used to mitigate the side effects associated with chemical antifungals.[9,10] The incorporation of herbal antifungals to SDL is a promising strategy for inhibiting *Candida albicans.* Oregano essential oil, in particular, has demonstrated superior antifungal activity.

Origanum vulgare essential oil contains carvacrol and thymol as its the main constituents, which provides analgesic, antifungal, antiseptic, antitoxic, antiviral and bactericidal properties.[10] Ariyani et al.,[11] was found the MIC of oregano essential oil to be 3.125% and determined the zone inhibition of SDLs with gel-based oregano essential oil and SDL with 10% nystatin, which indicated the lower inhibition zone of nystatin group than oregano group.[11] Oregano essential oil has been studied as an antifungal agent in tissue conditioners, effectively reducing fungal colonization.[12]

Bhat et al.,[13] determined the MIC and MFC of *Origanum vulgare at* concentrations of 0.024%, 0.048%, 0.097%, 0.195%, 0.39%, 0.781%, 1.56%, 3.12%, 6.25%, 12.5%, 25%, 50%. According to research by Godil et al.,[14] the MIC of an antifungal agent can be determined by evaluating the inhibition zone, with a diameter greater than 15 mm. Tarigan et al.[15] defined the minimum concentration that kills >99% of the fungal population, which was found to be 6.25%. This was evidenced by the absence of visible fungal growth on the *Sabouraud dextrose agar* (SDA) plate.[15]

However, there is a lack of research on the incorporation of *Origanum vulgare* essential oil into auto-polymerized silicone SDL and its effect on the inhibition zone of *Candida albicans*. The null hypothesis states there is no effect of incorporating *Origanum vulgare* gel at its MIC of 3.12% and MFC of 6.25% over durations of 7 and 14 days. This study aims to analyze the effect of antifungal activities of auto-polymerized silicone soft denture liner material containing *Origanum vulgare* gel against *Candida albicans* over a 14 days period.

METHODS

The antifungal activity of the oil was assessed using the agar diffusion technique. The *Candida albicans* ATCC 10231™ strain was subcultured on Sabouraud dextrose agar (SDA) and incubated at 37°C for 24 hours. A *Candida albicans* inoculum was prepared by growing a pure culture of *Candida albicans* ATCC®10231™ in sterile distilled water to a density visually equivalent to 1×10^6 CFU/ml (McFarland 0.5) standard.

Sterile paper discs (6 mm diameter) imbibed with 10 µl of Origanum vulgare essential oil (Happy Green, Indonesia) were placed in the center of the seeded dishes, and the plates were then incubated at 37°C for 24 hours.

After 24 hours of incubation at 37°C, the inhibition zones were measured (in millimeters). The MIC of *Origanum vulgare* essential oil was determined before evaluating the *Candida albicans* inhibition zone using the disc diffusion method with varying concentrations of *Origanum vulgare* essential oil (50%, 25%, 12,5%, 6,25%, 3,125%, 1,562%, 0,781%, 0,39%, 0,195%, 0,097%, 0,048%, and 0,024%). (Figure 1) [8][9] (Figure 1).

The MFC of *Origanum vulgare* essential oil was determined using the total plate count method with varying concentrations of *Origanum vulgare* essential oil (50%, 25%, 12,5%, 6,25%, and 3,12%). All experiments were performed in duplicate (Figure 2).

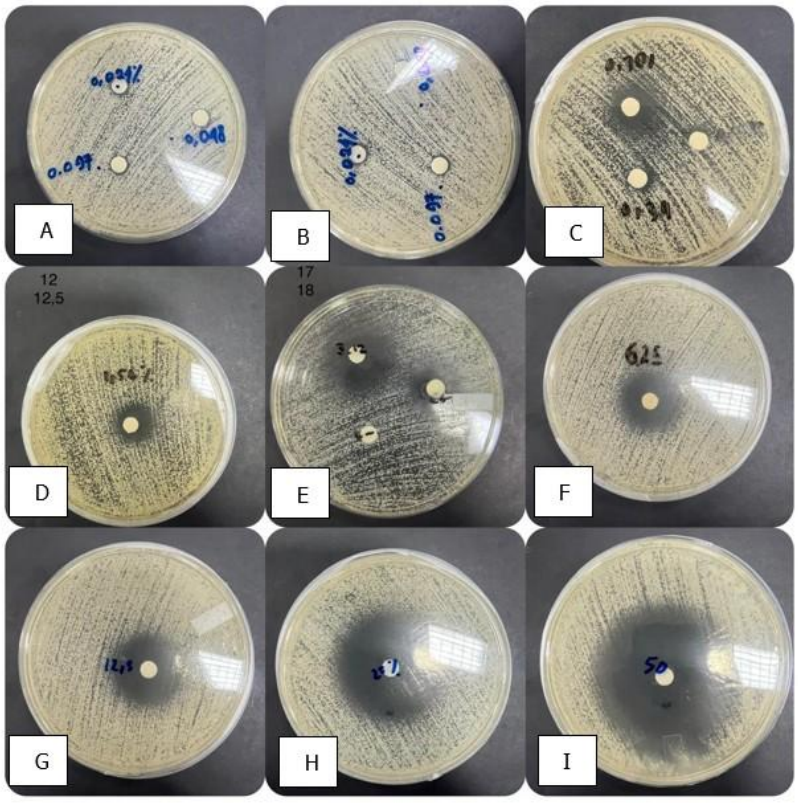

Figure 1. Minimum Inhibition Concentration (MIC) test of *Origanum vulgare* essential oil using disc diffusion with different concentration of *Origanum vulgare* essential oil :(A)0,097%, 0,048%, and 0,024%;(B)0.195%;(C)0.39%;(D)0.781%; (E)1.562%and 3.125%;(F)6.25%;(G)12.5%;(H)25%;(I)50%

Test materials used for the study were divided into the following four groups, with seven samples each: Group A consisted of auto-polymerized silicone SDL without the addition of antifungal agent; Group B included auto-polymerized silicone SDL with the addition of 10% nystatin gel (Taisho Mycostatin® Nystatin Oral Suspension, Japan); Group C comprised auto-polymerized silicone SDL with the addition of Origanum vulgare gel at the minimum inhibitory concentration (MIC)

of 3.12%; Group D contained auto-polymerized silicone SDL with the addition of Origanum vulgare gel with MFC of 6.25%.

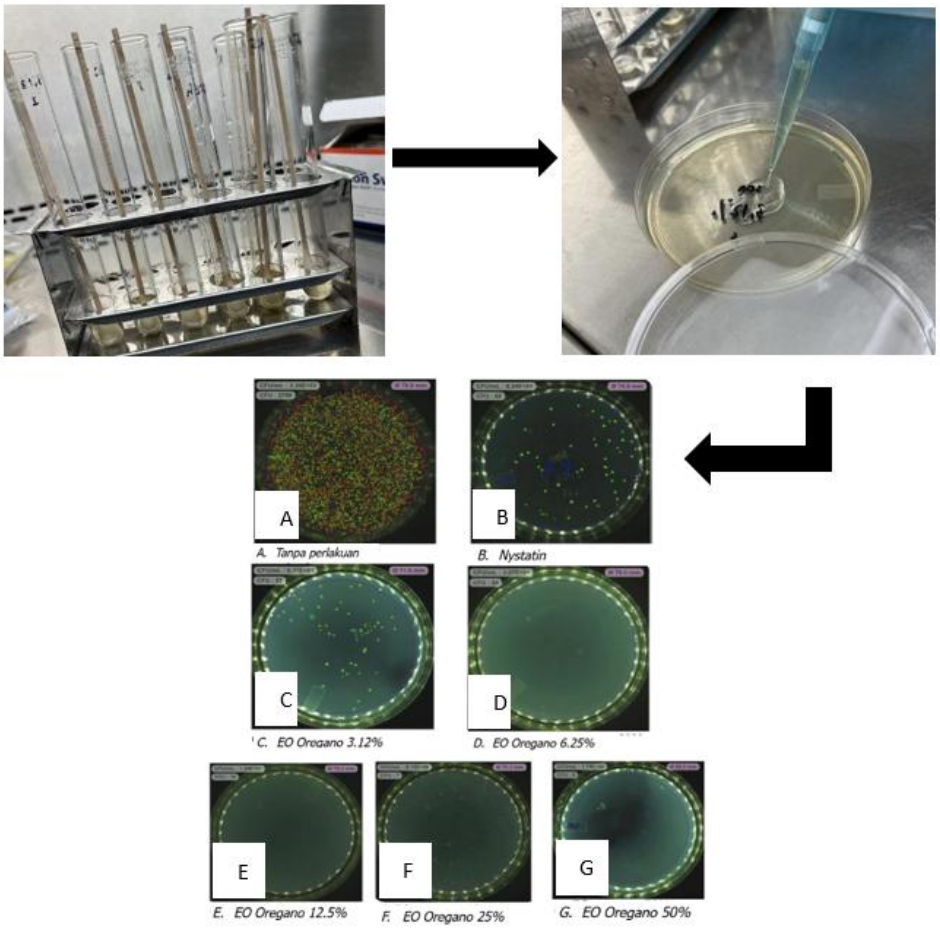

Figure 2. MFC test of *Origanum vulgare* essential oil using total plate count method with different concentration of *Origanum vulgare* essential oil :(A)Control; (B)Nystatin;(C)3.125%;(D)6.25%;(E)12.5% and;(F)25%;(G)50%

The antifungal herbal gel with the minimum inhibitory concentration (MIC) was prepared by heating 40.14% (40.14 grams) of *aqua destillata* (distilled water) until it reached boiling point, after which it was transferred into a beaker. Next, 0.05% (0.05 grams) of nipagin was dissolved into the heated distilled water and stirred until fully dissolved.

Subsequently, 0.75% (0.75 grams) of sodium carboxymethyl cellulose (CMC Na) and 0.75% (0.75 grams) of hydroxypropyl methylcellulose (HPMC) were added, and the mixture was then stirred until homogeneous with a gel-like consistency beginning to form. Following this, 5% (5 grams) of propylene glycol was added slowly while continuously stirring until the mixture became homogeneous. Finally, a mixture of hydrogenated castor oil (HCO) at 1.75% (1.75 grams) and *Origanum vulgare* essential oil (Happy Green, Indonesia) at 1.56% (1.56 grams) was incorporated. The resulting essential oil gel was stored in a sterile plastic container.[16]

The antifungal herbal gel with MFC was prepared by heating 38.575% (38.575 grams) of distilled water until it reached boiling point, after which it was transferred into a beaker. Next, 0.05% (0.05 grams) of nipagin was dissolved into the heated distilled water and stirred until it fully dissolved. Subsequently, 0.75% (0.75 grams) of CMC Na and 0.75% (0.75 grams) of HPMC were added, and the mixture was then stirred until homogeneous, with a gel-like consistency beginning to form. Following this, 5% (5 grams) of propylene glycol was added slowly while continuously stirring until the mixture became homogeneous, and then it was

transferred into a mortar. Finally, a mixture of HCO at 1.75% (1.75 grams) and *Origanum vulgare* essential oil (Happy Green, Indonesia) at 3.12% (3.12 grams) was incorporated. The resulting essential oil gel was stored in a sterile plastic container.[16]

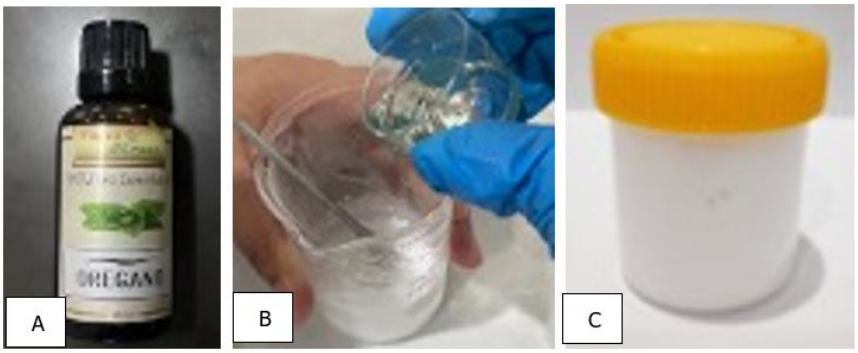

Figure 3. (A) essential oil oregano;(B)mixture HCO with essential oil oregano; (C)store antifungal gel at sterile plastic pot

Discs with a diameter of 6 mm and a thickness of 1 mm were prepared from auto-polymerized silicone SDL (Mollosil Detax GmbH, Ettlingen, Germany), with nine discs used for each group in this study. The preparation of the sample began with mixing the base and catalyst in a 1:1 ratio, following the manufacturer's instructions, until a homogeneous mixture was achieved for Group A. For Groups B, C, and D, 10% antifungal agents were added to the base and catalyst before mixing. The mixture was then applied to the mold and pressed using a hydraulic press until it set. Excess material was removed using a sterile scalpel.

The *Candida albicans* ATCC®10231™ strain was subcultured on Sabouraud dextrose agar (SDA) and incubated at 37°C for 24 hours. A *Candida albicans* inoculum was prepared by growing a pure culture of *Candida albicans* ATCC®10231TM in sterile distilled water to a density visually equivalent to 1 × 10^6 CFU/ml (McFarland 0.5) standard. The *Candida albicans* suspension was streaked onto SDA media in a Petri dish using a sterile cotton swab. The sterile sample discs were then placed on the agar plates and incubated at 37°C. The inhibition zones of *Candida albicans* were measured three times at 7 days and 14 days using digital calipers, and the results were averaged (Figure 4 & 5).

Figure 4. Inhibition zones of 7 samples after 7 Days.

Figure 5. Inhibition zones of 7 samples for after 14 Days.

The data were analyzed using the Statistical Package for Social Sciences (SPSS) version 21. A univariate test was conducted to determine the values of the *Candida albicans* inhibition zones. A One-way ANOVA test was used to analyze the effect of *Origanum vulgare* gel addition, and post-hoc Dunnett's T3 was applied to analyze the differences between each group.

RESULTS

The experimental results show that the *Origanum vulgare* essential oil have very good activity against the strain *Candida albicans*. The minimum inhibition concentration (MIC) of *Origanum vulgare* essential oil was determined to be 3.12%, as it produced an inhibition zone greater than 15 mm. Additionally, 6,25% was identified as MFC, as it was the lowest concentration capable of killing >99% of the fungal population on SDA plates.[13,14]

The effect of *Origanum vulgare* gel addition was analyzed using a One-way ANOVA test, and the antifungal activities between the groups were compared using post-hoc Dunnett's T3. The results, as shown in Table 1, indicated that no inhibition zone was observed in group A for any duration, confirming the absence of antifungal activity. In contrast, Group C exhibited the largest inhibition zone compared to Group A and B at both 7 days and 14 days.

There was a significant effect of the addition of *Origanum vulgare* gel at the MIC of 3.12% on inhibition zone, based on duration of 7 days and 14 days $p<0.001$ ($p<0.05$). Post-hoc analyses using Dunnett's T3 test for the groups at 7 days and 14 days revealed significant differences in the antifungal effects between each group (Table 1).

Table 1. Effect of the addition of Origanum vulgare essential oil gel at the MIC of 3.12% concentration on the inhibition zone of Candida albicans in a silicone soft denture line based on duration 7 and 14 days

Duration	Group	*Candida albicans* Inhibition Zone Mean value ± SD	*p-Value*
	Group A7	0	
7 days	Group B7	9.5 ± 0.44	<0.001*
	Group C7	12.6 ± 0.85	
	Group A14	0	
14 days	Group B14	9.06 ± 0.31	<0.001*
	Group C14	11.54 ± 0.94	
Dunnett's T3 test			
Group A7 and B7			<0.001*
Group A7 and C7			<0.001*
Group B7 and C7			<0.001*
Group A14 and B14			<0.001*
Group A14 and C14			<0.001*
Group B14 and C14			<0.001*

Table 2 shows that the inhibition zone of Group D was the largest compared to Group A and B at both 7 days and 14 days. Based on One-way ANOVA analysis, there was a significant effect of the addition of *Origanum vulgare* gel at the MFC of 6.25% on inhibition zone at durations of 7 days and 14 days with $p<0,001$ ($p<0.05$). Post-hoc analyses using Dunnett's T3 test for the groups at 7 days and 14 days revealed significant differences in the antifungal effect between each group (Table 2).

Table 2. Effect of the addition of *Origanum vulgare* essential oil gel at the MFC of 6.25% concentration on the inhibition zone of *Candida albicans* in a silicone soft denture liner based on duration 7 and 14 days

Duration	Group	*Candida* Inhibition Zone	*p-Value*
		Mean value ± SD	
7 days	Group A7	0	
	Group B7	9.5 ± 0.44	<0.001*
	Group D7	15.67 ± 0.55	
14 days	Group A14	0	
	Group B14	9.06 ± 0.31	<0.001*
	Group D14	15.23 ± 0.62	
Dunnett's T3 test			
Group A7 and B7			<0.001*
Group A7 and D7			<0.001*
Group B7 and D7			<0.001*
Group A14 and B14			<0.001*
Group A14 and D14			<0.001*
Group B14 and D14			<0.001*

DISCUSSION

The result demonstrated that the inhibition zones of auto-polymerized silicone SDLs with the addition of *Origanum vulgare* gel at the MIC of 3.12% and the MFC of 6.25% were larger than those with the addition of nystatin at both 7 days and 14 days. Antifungal activity has also been seen in the studies conducted by Rawat P et al.,[17] with finding the minimum inhibitory concentration of oregano oil it is 2.5 µg/ml and similar decreasing trends in the antifungal activity have also been seen in the studies. Decrease in zones of inhibition at the end of fourteen days was due to the regrowth of the fungus.[18]

Table 1 and table 2 showed *Origanum vulgare* gel exhibited the largest inhibition zone compared to nystatin and control at both 7 days and 14 days. *Origanum vulgare* oil, an essential oil derived from the dried leaves of the oregano plant, contains key constituents such as 4-terpineol, g-terpinene, thymol, and carvacrol. These phenolic compounds disrupt membrane-embedded proteins, alter the ion transport processes across the cell membrane, modify calcium channel activity, and inhibit cellular respiration. These mechanisms increase cell permeability, leading to the release of vital intracellular components. As a result, *Origanum vulgare* oil exhibits a strong antifungal effect against the *Candida albicans*.

This *in vitro* study demonstrated that oregano or *Origanum vulgare* oil had higher antifungal activity compared to nystatin.[19] Nystatin was found to be less effective than *Origanum vulgare* gel in inhibiting the growth of *C. albicans* in this *in vitro* study. The mechanism of nystatin, when added to soft denture liners, involves its action as a polyene fungicidal agent. Nystatin and molecules are insoluble in water and possess the broadest spectrum of activity among available antifungals agents.

As a polyene, nystatin binds to ergosterol, a major component of the fungal cell membrane, causing membrane destabilization. At sufficient concentrations, it forms pores in the membrane, leading to potassium leakage, acidification, and disruption of cellular function. The inhibition zone values were greater with the addition of *Origanum vulgare* essential oil gel due to its mechanism of actions: while nystatin increases fungal cell wall permeability, the carvacrol and thymol in *Origanum vulgare* essential oil gel denature protein structure, enhancing its antifungal efficacy.[15,20]

The use of *Origanum vulgare* gel as an antifungal agent incorporated into auto-polymerized silicone liners (SDLs) has not been reported in previous studies. The results demonstrated a significant difference in antifungal effects between each group, which efficacy sustained for up to 14 days of use with MFC. Bhat et al.,[13]

found the MIC of *O. vulgare* was 0.024% and MFC was observed as 0.097% when it was tested against *Candida*. Previous study showed that *O. vulgare* can be fungicidal on *C. albicans* from mice with a MIC of 0.125% and MFC of 0.25%. In their study, they also observed that a daily dose of 1.0 µl of *O. vulgare* oil for 30 days can cure systemic candidiasis in mice.[13]

Origanum vulgare essential oil exhibits strong bactericidal and fungicidal activity against various pathogens, due to its high concentration of carvacrol and thymol, which are phenolic components.[15,19,20] The antifungal activity of these compounds is attributed to their ability to alter the permeability of microbial cells membranes. Specifically, carvacrol eliminates *Candida albicans* growth by denaturing proteins and reducing surface tension, thereby increasing permeability. Protein denaturation disrupts nutrient absorption and metabolic processes, leading to enzyme damage, protein coagulation, and ultimately, cell death.[15]

A previous study reported that carvacrol was one of the most important bioactive compounds for antifungal activity. The antifungal activities could vary due to the synergistic and antagonistic interactions among components within essential oil. Several researchers documented synergistic activity between carvacrol and thymol. Additionally, *p*-cymene was shown to increase the antimicrobial activity of both thymol or carvacrol.[18,23] The addition of 60% oregano oil to the tissue conditioner demonstrated an initial zone of *Candida* inhibition, but the size of the inhibition zone decreased over time.[17,24] Higher concentrations of herbal ingredients added to the soft denture liner resulted in larger inhibition zones against *Candida albicans.*

However, over extended use, the antifungal activity of the inhibition zone decreased.[25] *In vitro* antifungal activity testing using the disc diffusion method demonstrated a concentration–dependent inhibition of *Candida albicans* growth.[26] This phenomenon occurs because higher concentrations of essential oil result in greater antimicrobial efficacy due to the bioactive components in the extract. The effectiveness of an antifungal agent is directly influenced by the concentration of its active ingredients; as the concentration increases, so does its ability to inhibit microbial growth.[27]

Nevertheless, further studies are recommended to evaluate its impact on the physical and chemical properties of auto-polymerized silicone soft denture liners, such as tensile bond strength, modulus of elasticity and surface roughness. Additionally, long term *in vitro* and *in viv*o studies are required before this product can be commercialized.

A limitation of this study is that it could not be conducted for more than 14 days due to the risk of contamination in the Sabouraud dextrose agar (SDA). Additionally, the results of this study cannot be directly applied to clinical settings, and further research is needed to validate these findings.

CONCLUSION

The addition of *Origanum vulgare* gel at a concentration of 6.25% to silicone soft denture liner is recommended, as it effectively inhibits the growth of *Candida albicans* for up to 14 days of use, outperforming the addition of nystatin. However, the inhibition zone decreases over time. *Origanum vulgare* gel has been demonstrated to be an effective natural antifungal agent. The practical implication of these findings is the use of *Origanum vulgare* at a MFC of 6.25% for a duration of 14 days.

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