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Evaluate and Compare the Antifungal Effects of Herbal Extracts and Commercially Available Denture Cleanser on Heat-Polymerized Acrylic Denture Base Resin

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KEYWORDS

Sodium Perborate, Fittydent, Origanum Oil, C. Albicans, Sabouraud Dextrose Broth, Herbal Extracts, Acrylic Denture Base Resin

ABSTRACT: Aim: This study aims to evaluate and compare the antifungal effects of origanum oil as a denture cleanser.

Materials and Methods: The sample size for each group is 13. A total of 39 heat-polymerized acrylic samples were divided into 3 groups with 13 samples in each group. Specimens with the specific dimensions were obtained from the modeling wax. Wax patterns were invested into the flask and dewaxing was performed conventionally. Finishing was carried out using fine-grit sandpaper and the sodium perborate tablet "fittydent" was dissolved in distilled water. Eight pre-cultured C. albicans were standardized and the commercial denture cleanser, ie. fittydent tablets and the herbal extracts, Origanum oil were diluted in distilled water. Agar plates were incubated accordingly. The denture samples were immersed in Sabouraud dextrose broth containing C. albicans. The specimens were washed and stained with crystal violet, the dentures were washed with 70% methanol to remove and the adhered C. albicans and optical densities of the samples were measured using a spectrophotometer.

Statistical Analysis and Results: Each group contains an equal number of samples, specifically 13 samples. The percentages indicate that each group contributes equally to the total sample size, with each group comprising 33.33% (n=13) of the total sample population. Mean ± SD of optical densities in group 1, 2 and 3 were 0.069 ± 0.017, 0.272 ± 0.263 and 0.213 ± 0.065 respectively. Minimum and maximum values of optical densities in group 1 were 0.043 and 0.094, in group 2 were 0.015 and 0.690 and; in group 3 were 0.015 and 0.690.

Conclusion: Within the limitations of the study the authors concluded that the antifungal effects of Origanum oil exhibits comparable efficacy to the commercial denture cleanser Fittydent tablet on heat-polymerized acrylic denture base resin. They also concluded that the potential of Origanum oil as a viable alternative to conventional denture cleansers for managing denture stomatitis devoid of adverse effects.

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Introduction

A view of dentistry revolves around maintaining the oral health of geriatric people. Older people are prone to several oral mucosal diseases and it has a multifactorial etiology. Tooth loss is a condition that is noticed prevalently among older adults. Replacement of teeth is of importance as it tends to increase the quality of life. The most commonly chosen cost-effective and practical replacement for total tooth loss is complete dentures.[1,2] Candida species occur in a yeast form (blastospore) or a mycelial form (pseudohyphae).[3] However, most of the lesions of generalized simple or granular type denture stomatitis seem to be induced by Candida species, although trauma may be a significant predisposing factor.[4] Although the tissues appear raw and inflamed, the patient is rarely aware of the condition and seldom complains of soreness. “Denture stomatitis” and “chronic atrophic candidiasis” have been suggested as more appropriate descriptions of the condition.[5,6] Denture trauma, allergy, poor oral hygiene, pH level of saliva, age, sex, smoking, and immune system deficiency are generally regarded as etiologic factors in denture stomatitis. The significant causes of denture stomatitis are trauma and infection with Candida species.[7] Acrylic resins have inherent properties like hydrophilicity and high free surface energy. These properties aid in favor of fast microbial colonization on the fitting surface of the denture.[8-10] A denture cleanser must be effective and capable of removing plaque from not only the polished surfaces of prosthesis but more importantly from the unpolished tissue surfaces.[11-12] Commercial denture cleansers that are widely available in the market are generally made of synthetic chemicals such as alkaline peroxide, sodium hypochlorite, and chlorhexidine gluconate. Herbal-based denture cleansers have not been widely available in the market, even though many medicinal plants grow around us. It is an extract of the perennial plant (oregano) of the Lamiaceae family and is found in Europe, the Mediterranean, and southern and central Asia. Although it contains over 22 compounds, 4-terpineol, carvacrol, and thymol are the main ingredients which impart analgesic, antifungal, antiseptic, antitoxic, antiviral, and bactericidal properties. Both thymol and carvacrol were found to be effective against Candida albicans. [13-15] Manohar et al. observed that origanum oil completely inhibited the growth of Candida albicans. Further, it was also able to inhibit the germination and mycelial growth of Candida albicans.[16] Although Origanum oil was found to provide good antifungal protection, its use in dental applications has not been investigated. Therefore, the present study was conducted to evaluate and compare the antifungal effects of origanum oil as a denture cleanser.

Materials and Methods

This study was conducted in the Department of Prosthodontics and Crown & Bridge at I.T.S Centre for Dental Studies and Research, Delhi – Meerut Road, Ghaziabad and Department of Centre for Advanced Research at I.T.S – Centre for Dental Studies and Research.

Materials and Equipments

Origanum oil, Fittydent tablets, Modeling wax, Type III gypsum product, Heat cure acrylic resin, Flask, Fine-grit sandpaper (220 grit), Acrylic polishing burs, Pumice, Distilled water, 4% hypochlorite, Sabouraud dextrose broth, Saline solution, Crystal violet, 70% methanol, Cotton, Tissue papers, Sterile Gloves (Walden), Mouth Mask (Walden), Test tubes, Test tube stand, Dropper, Spectrophotometer.

Methodology

The study was conducted in the Department of Prosthodontics and Crown & Bridge, I.T.S dental college, Ghaziabad to evaluate to and compare the antifungal effects of herbal extracts and commercially available denture cleanser on heat-polymerized acrylic denture base resin. Ethical approval was obtained from the Institutional Ethics Committee at the ITS Dental college, Ghaziabad. (ITSCDSR/IIEC/2021-24/PROSTHO/01). With the help of literature survey, it was revealed that the expected S.D and mean difference of parameter of group 1 & group are 2.4 and 2.7 respectively and mean difference is 3.0 of two groups for variables. Using the above formula we have found the sample size for each group is 13. A total of 39 heat-polymerized acrylic samples was divided into 3 groups with 13 samples in each group:

GROUP 1	Origanum oil
GROUP 2	Distilled water
GROUP 3	Fittydent tablet

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Sample Preparation

Specimens with the dimensions of 10×10×2 mm3 were obtained from the modeling wax. Wax patterns were invested into the flask and dewaxing was performed conventionally. Acrylic resin (DPI Heat-cure Improved; DPI India, India) was then packed and trial closure and final closures were done traditionally. Bench curing was done for 30 minutes and polymerization was carried out according to the manufacturer's instruction. Finishing was carried out using fine-grit sandpaper following wet sandpapering and polishing was done on a wet felt cone and rag wheel with pumice slurry to obtain a well-polished surface.

Preparation of Denture Cleanser "Fittydent"

Following the instructions on the packaging, the sodium perborate tablet "fittydent" was dissolved in distilled water. The tablet was placed in 100 mL of warm water (55°C) and allowed to dissolve completely until the tablet appeared to be fully dispersed.

Minimal Inhibitory Concentration (MIC) Test

This test was performed as per the Clinical and Laboratory Standards Institute guidelines. Eight pre-cultured C. albicans were standardized to have a concentration of 1–2 × 108 cells/mL. The commercial denture cleanser, ie. fittydent tablets and the herbal extracts, Origanum oil were diluted in distilled water. The dilutions had concentrations of 50, 75, 100, and 150 µL. Agar plates were incubated at 37 °C for 48 hours. After incubation, the zone of inhibition was calculated for the MIC required for C. albicans.

Antifungal Activity Test

Sterile specimens were washed with 4% hypochlorite before the commencement of the antifungal activity test. The denture samples were immersed in Sabouraud dextrose broth containing C. albicans for 16 hours at 37 °C for inoculation; this period of immersion simulated the duration of the denture in the mouth; then the specimens were washed with a saline solution, and were immersed in a denture cleanser solution. They were prepared by mixing origanum oil with 75 µL/mL MIC value with the required amount of distilled water. The specimens were immersed for 8 hours at room temperature to simulate the duration of the denture in water overnight. Finally, the specimens were washed and stained with crystal violet, the dentures were washed with 70% methanol to remove and the adhered C. albicans and optical densities of the samples were measured using a spectrophotometer at 595 nm.

Figure 1: Mold for fabrication of samples

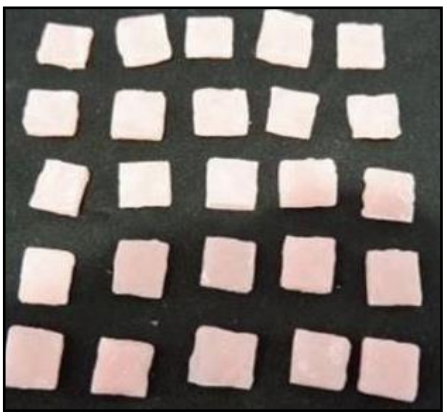

Figure 2: Acrylic plate samples

Figure 3: Origanum Oil

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Figure 4: Fittydent cleansing Tablets

Figure 5: Immersing plates in fittydent solution and distilled water

Figure 6: Crystal violet

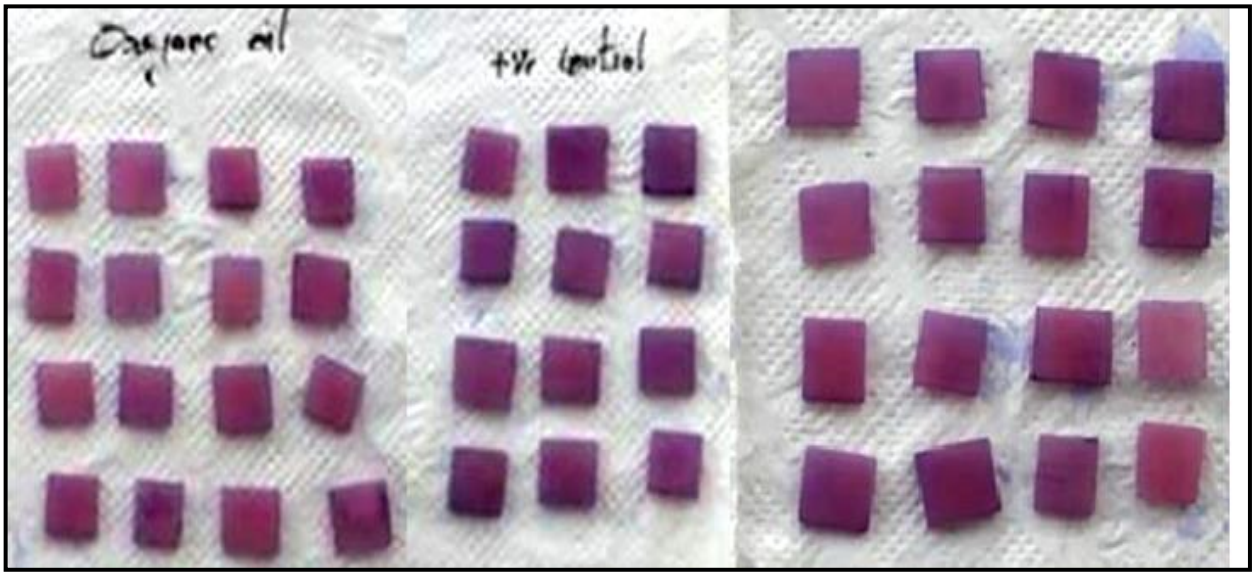

Figure 7: Samples stained with crystal violet, Left to Right (a) Origanum oil, (b) Control and (c) Fittydent

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Figure 8: Candida albicans in Sabouraud dextrose agar plate

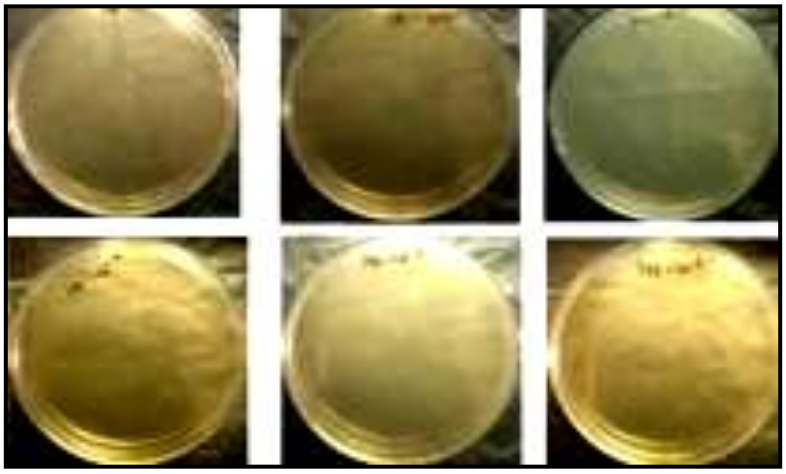

Figure 9: Candida albicans colony after treatment

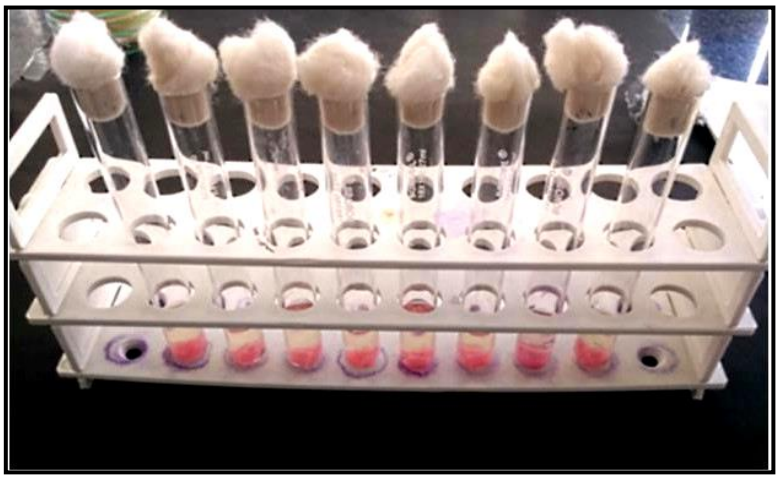

Figure 10: Serial dilution of colony counting

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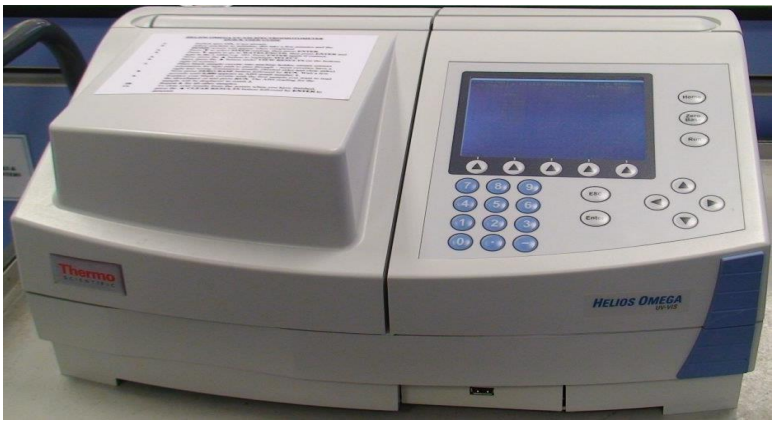
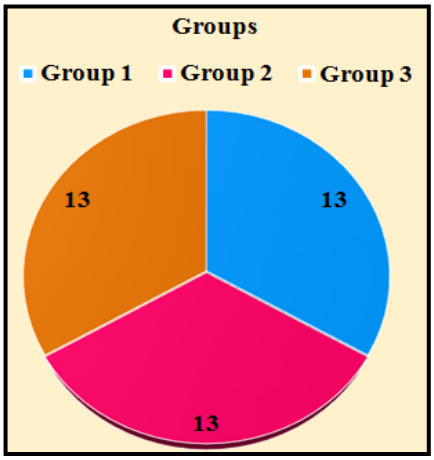


Figure 11: Spectrophotometer

Statistical Analysis and Results

Data was entered in Microsoft Excel 365 for Windows. Mean, standard deviation, minimum and maximum values of optical densities in different groups were calculated. For comparison of optical densities between different groups, one-way ANOVA was applied. P value <0.05 was considered statistically significant. Data analyses were performed using version 23.0 of the Statistical Package for Social Sciences (IBM Corporation, Armonk, New York, USA). The level of significance and confidence interval was 5% and 95% respectively, i.e. p< 0.05.



Graph 1: Distribution of samples in different groups

Results

Table 1: Distribution of samples in different groups

Groups	**n (%)**
Group 1	13 (33.33%)
Group 2	13 (33.33%)
Group 3	13 (33.33%)
Total	39 (100.00%)

Each group contains an equal number of samples, specifically 13 samples. The percentages indicate that each group contributes equally to the total sample size, with each group comprising 33.33% (n=13) of the total sample population. The graph represents a visual depiction of the data presented in each group (Group 1, Group 2, and Group 3) may be represented by a distinct bar or segment corresponding to the number of samples in each group.

Table 2: Comparison of optical densities between different groups

Groups	**Optical Density**	
	Mean ± SD	Min-Max
Group 1	0.069 ± 0.017	0.043-0.094
Group 2	0.272 ± 0.263	0.015-0.690

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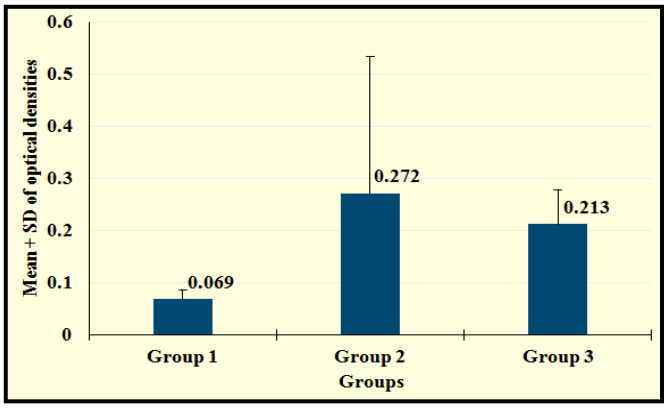
Group 3	0.213 ± 0.065	0.115-0.312
One way ANOVA	F = 5.766, P = 0.007 (<0.01), Highly significant	

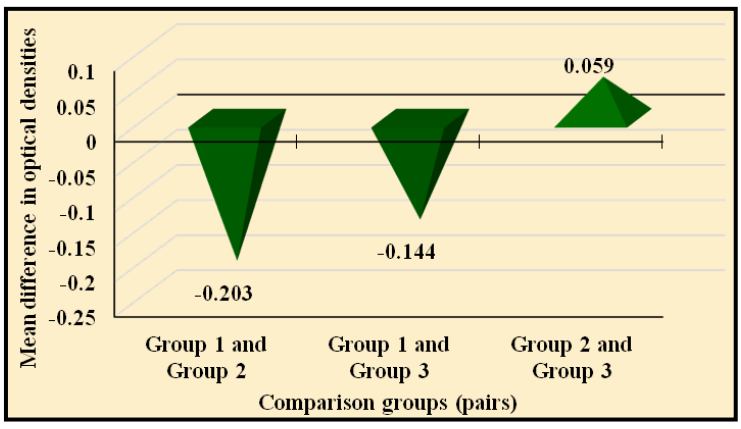


Graph 2: Comparison of optical densities between different groups

Table 2 and graph 2 show a comparison of optical densities between different groups. Mean ± SD of optical densities in group 1, 2 and 3 were 0.069 ± 0.017, 0.272 ± 0.263 and 0.213 ± 0.065 respectively. Minimum and maximum values of optical densities in group 1 were 0.043 and 0.094, in group 2 were 0.015 and 0.690 and; in group 3 were 0.015 and 0.690. One way ANOVA showed significant difference between different groups for optical densities (F = 5.766, P <0.01, highly significant). After the significant result of one-way ANOVA, Tukey HSD post hoc test was applied for pairwise comparison.

Table 3: Pairwise comparison of optical densities between different groups using Tukey HSD post hoc test

Comparison groups (Pairs)	Mean difference	P value
Group 1 and Group 2	-0.203	0.006 (<0.01), Highly significant
Group 1 and Group 3	-0.144	0.062 (>0.05), Not significant
Group 2 and Group 3	0.059	0.612 (>0.05), Not significant



Graph 3: Pairwise comparison of optical densities between different groups

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Table 3 and graph 3 show a pairwise comparison of optical densities between different groups using Tukey HSD post hoc test.

The Tukey HSD post hoc test showed following observations:

1. Optical density in group 1 was significantly lower than group 2 (mean difference = -0.203, P<0.01, highly significant).

2. There was no significant difference for optical densities between group 1 and group 3 (mean difference = -0.144, P>0.05, not significant) and; group 2 and group 3 (mean difference = 0.059, P>0.05, not significant).

Discussion

Heat-cured acrylic material is extensively utilized for fabricating removable denture prostheses, serving as a pivotal tool for rehabilitating cases of tooth loss due to its manifold advantages. Candida species occur in a blastospore (yeast) form or a mycelial form.[3] Origanum oil is highlighted for its natural antifungal properties, broad-spectrum antimicrobial activity, minimal risk of resistance development, and favorable safety profile which comes in agreement with the study done by Cleff, Meinerz.[8] The same findings were reported by the study done by Kaskatepe, Aslan et al.[21] where the comparison were made between antifungal and anti- virulent activity of Origanum majorana L. essential oil on Candida albicans. Dalwai et al. [28], in his study found similar results wherein he compared antifungal action of tea tree oil, chlorhexidine gluconate and fluconazole on heat polymerized acrylic denture base resin. Similar findings were reported by the study done Srivatstava [11] who experimented Origanum oil at varying concentrations by incorporating into a poly (methyl methacrylate) based tissue conditioner, and its antifungal activity against Candida albicans. Origanum oil contains carvacrol, 4-terpineol, and thymol, with wide variations in their relative percentages, depending on geologic and climatic conditions as seen in a study done by Giordani, Kaloustian et al.[33] Plant-derived products as disease control agents have low toxicity, higher public acceptance, and fewer environmental effects. Origanum oil has shown potent efficacy as a herbal denture cleanser in the present study, Tambekar, Dahikar[9] in their study found similar results a n d suggested that ayurvedic herbal preparations extracts have great potential as antimicrobial activity against enteric bacterial pathogens and they can be used in the treatment of infectious diseases. The present study showed optical density of fittydent group is significantly higher than that of origanum oil group that comes in agreement with the study done by Manohar, Ingram et al.[7], and Muttagi [4] who have shown that origanum oil is effective against C. albicans species with least optical densities. Volety, Shetty [35] examined the antifungal properties of Origanum oil during an in vitro and in vivo study and concluded the complete growth inhibition of C. albicans in culture along with the germination and the growth of mycelial C. albicans. Srivatstava[11] experimented Origanum oil at varying concentrations by incorporating into a poly (methyl methacrylate) based tissue conditioner and concluded that Origanum oil imparted a significant antifungal quality to the tissue conditioner. Puškárová[14] Studied Six essential oils (from oregano, thyme, clove, lavender, clary sage, and arborvitae) that exhibited different antibacterial and antifungal properties provided novel approaches for assessing the antimicrobial potential as well as antifungal properties of essential oils in both direct contact and the vapor phase and also demonstrated the valuable properties of the phenol-free arborvitae oil. The clinical significance of this study lies in its potential to revolutionize denture hygiene practices, improved oral health for Geriatric Population, Reduced risk of oral infections by evaluating the efficacy of herbal denture cleansers like Origanum oil, the research offers a promising avenue for improving oral hygiene among older adults who rely on complete dentures, Alternative to chemical cleansers which may have adverse effects and are not economically feasible for all patients, Origanum oil offers a natural alternative that is potentially safer, more accessible, and cost-effective. Minimal Risk of resistance development, biocompatibility and safety profile, preventive oral care approach that promotes autonomy and self-care among geriatric individuals.

Limitation of the present study

Firstly, the study primarily relies on an in vitro analysis to evaluate the antifungal effects of Origanum oil. While in vitro studies offer controlled experimental conditions, they may not fully replicate the complex oral environment and interactions that occur in vivo.

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Secondly, the study primarily focuses on short- term evaluations of Origanum oil's antifungal activity on denture surfaces. Thirdly, clinical relevance and effectiveness in preventing or managing denture stomatitis in actual patient populations remain to be determined. Lastly, the sample size in the study may be limited, larger sample sizes and inclusion of diverse patient populations are needed to enhance the robustness and external validity of the results.

Conclusion

The present study was conducted to evaluate and compare the antifungal effects of herbal extracts and commercially available denture cleanser on heat-polymerized acrylic denture base resin. Within the limitations of the study the following conclusions were drawn:

1. The antifungal effects of Origanum oil exhibits comparable efficacy to the commercial denture cleanser Fittydent tablet on heat-polymerized acrylic denture base resin.

2. The potential of Origanum oil as a viable alternative to conventional denture cleansers for managing denture stomatitis devoid of adverse effects.

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