

# Antifungal Effects of Herbal Extracts and Fluconazole on Heat-polymerized Acrylic Denture Base Resin as Denture Cleanser: An *In Vitro* Study

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## ABSTRACT

**Aim:** The study aimed to investigate the antifungal effects of herbal extracts and fluconazole on heat-polymerized acrylic denture base resin as a denture cleanser.

**Materials and methods:** Several essential oils, such as origanum oil and grape seed oil and commercially available antifungal agent fluconazole were used as denture cleansers and their antifungal efficacy was evaluated using a spectrophotometer. Overall, 68 samples were obtained and were divided into four groups, each containing 17 samples. These samples were immersed in Sabouraud dextrose broth consisting of *Candida albicans* for 16 hours and later in these antifungal solutions for 8 hours and their antifungal efficacy was measured. Data were subjected to an ANOVA test.

**Results:** Among the study groups origanum oil showed the maximum antifungal activity with a mean optical density at  $0.072 \pm 0.014$  followed by fluconazole ( $0.094 \pm 0.155$ ), and least by grape seed oil ( $0.190 \pm 0.071$ ).

**Conclusion:** Results of this *in vitro* study showed that origanum oil was more effective than commercially available antifungal agents, and among the tested groups oregano oil was a potential agent in lowering the *C. albicans* colony.

**Clinical significance:** Origanum oil, being a herbal product, can be considered as a denture cleanser and also be used as an effective alternative to commercially available antifungal agents without any side effects.

**Keywords:** Antifungal efficacy, Denture cleanser, Essential oils, Fluconazole, Heat cure acrylic resin.

*The Journal of Contemporary Dental Practice* (2021): 10.5005/jp-journals-10024-3042

## INTRODUCTION

The increase in the use of complete dentures is seen in most of the elderly population, especially in the aging group of the world population, that is, above 60 years of age.<sup>1</sup> Most of these elderly have reduced resistance to infection and have difficulty in cleansing and maintaining the hygiene of their dentures due to the lack of proper motor coordination or skills.<sup>2</sup> Denture teeth with natural contours and the stippled surface of the denture provides more concave surfaces for the aggregation of debris, stains, and plaque and as a result, aggravate the patient's cleaning problems.<sup>3</sup>

Denture-induced stomatitis is the most common condition in chronic candidiasis and a common pathogenic reaction of denture-bearing mucosa. Although denture stomatitis is multifactorial, adherence of candida species, especially *Candida albicans* plays a major role.<sup>3</sup> *C. albicans* adhesion is seen more on the dentures intaglio surface than on the palate, signifying that the denture acts as a source of infection and that adhesion of yeasts to the surface of the denture is a normal prerequisite for the colonization of the palate.<sup>4</sup> The adhesion of *C. albicans* to fitting surface forms the initial step in colonization and the development of pathogenesis.<sup>4</sup>

Dentures are made of acrylic resins. Acrylic resins have inherent properties like hydrophobicity and high free surface energy. These properties aid in fast microbial colonization on the intaglio surface of the denture. Therefore, to reduce microbial colonization and to maintain oral health, well-fitting finished and polished denture and adequate denture cleaning are imperative.<sup>4</sup>

Proper denture cleaning can be accomplished with various methods that include mechanical methods, such as brushing

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**How to cite this article:** Volety S, Shetty PP, Kumar K, *et al.* Antifungal Effects of Herbal Extracts and Fluconazole on Heat-polymerized Acrylic Denture Base Resin as Denture Cleanser: An *In Vitro* Study. *J Contemp Dent Pract* 2021;22(2):162-165.

**Source of support:** Nil

**Conflict of interest:** None

or chemical methods, such as the use of commercially available denture cleansers and ultrasonic cleaning, or a combination of both methods.<sup>2</sup> Mechanical cleansing methods are effective but they can cause wearing and roughening of the denture surface, whereas chemical methods are simple and effective in reducing biofilm formation, especially in individuals who lack manual dexterity. Chemical methods will be an effective alternative to mechanical methods. Hence, chemical methods are commonly prescribed and are effective alternatives to mechanical cleaning.<sup>3</sup>

Natural products can be an alternative to synthetic chemical substances. Plant essential oils are known to contain anti-inflammatory and analgesic properties, provide relaxation, have aromatic properties and antimicrobial effects, and other benefits.<sup>2</sup> Origanum oil has carvacrol as a major constituent which has proven

effective against *C. albicans*<sup>5,6</sup>; grape seed oil has shown antifungal activity against *Candida glabrata* and *Candida krusei* species<sup>7</sup>. Hence, in this study, we attempted to check the efficacy against *C. albicans*. There is a limited number of studies that compared the antifungal activity of commonly used antifungal drugs, such as fluconazole and essential oils, such as origanum oil and grape seed oil. Thus, the objective of the study was to evaluate the antifungal activity of fluconazole, origanum oil, and grape seed oil against *C. albicans* on heat cure acrylic resins as denture cleansers.

## MATERIALS AND METHODS

### Sample Preparation

Specimens with the dimensions of  $10 \times 10 \times 2 \text{ mm}^3$  were obtained from the modeling wax (modeling wax, Hindustan Dental Products, Bengaluru, India); wax patterns were invested into the flask (Fig. 1) 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 (Fig. 2).



Fig. 1: Mold for fabrication of samples



Fig. 2: Finished and polished heat-polymerized acrylic resin samples

### Experimental Design

A total of 68 samples were divided into four groups with 17 samples in each group:

- Group 1: Grape seed oil (Falcon Essential Oils, Bengaluru, India),
- Group 2: Origanum oil (Falcon Essential Oils, Bengaluru, India),
- Group 3: Commercial antifungal agent (Fluconazole),
- Group 4: Control (Distilled water).

### In Vitro Analysis

#### Minimal Inhibitory Concentration (MIC) Test

This test was performed as per the Clinical and Laboratory Standards Institute guidelines.<sup>8</sup> Pre-cultured *C. albicans* (ATCC24433; Dextrose Technologies Pvt Ltd, Bengaluru, India) were standardized to have a concentration of  $1-2 \times 10^8$  cells/mL. The antifungal agents, such as fluconazole and both the essential oils were diluted in distilled water. This standardized inoculum was streaked on the Rose Bengal Agar culture plates on which wells were punched using a gel punching syringe and were later filled with diluted antifungal agents. The dilutions had concentrations of 50, 75, 100, and 150  $\mu\text{L}$ . 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  $\mu\text{L}/\text{mL}$ , grape seed oil with 150  $\mu\text{L}/\text{mL}$ , and fluconazole with 65  $\mu\text{L}/\text{mL}$  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 (Fig. 3). Finally, the specimens were washed and stained with crystal violet, the dentures were washed with 70% methanol to remove and the adhered *C. albicans* using a spectrophotometer at 595 nm (Figs 4 to 6) was analyzed. The results were subjected to statistical analysis using the ANOVA method.



Fig. 3: Samples immersed in Sabouraud broth containing *C. albicans*

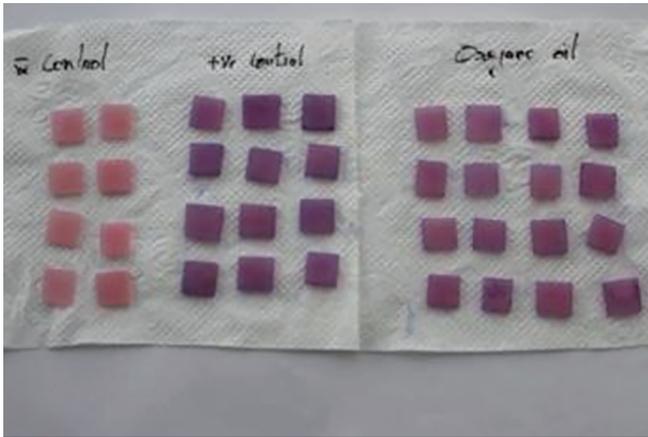


Fig. 4: Samples stained with crystal violet, control (distilled water), and oregano oil

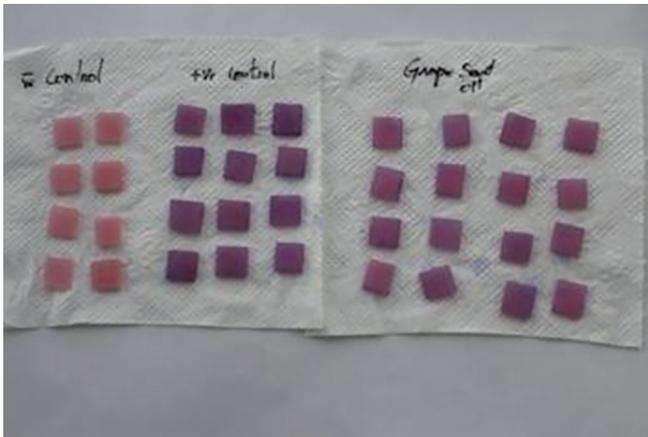


Fig. 5: Samples stained with crystal violet, control (distilled water), and grape seed oil

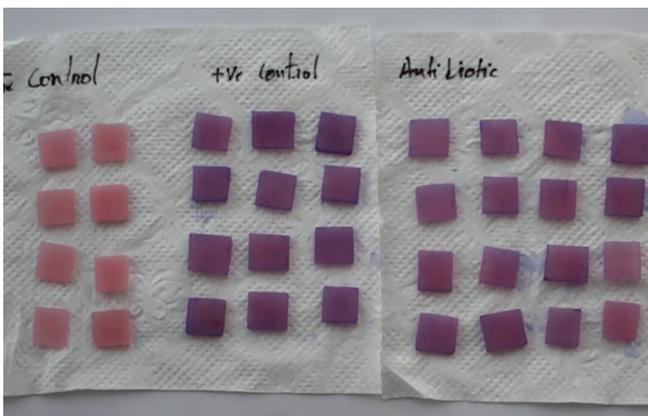


Fig. 6: Samples stained with crystal violet, control (distilled water), and fluconazole

## RESULTS

In this study, the effects of antifungal agents, such as origanum oil and grape seed oil used as denture cleansers on the heat-

Table 1: Comparison of the mean of the optical density at 595 nm using ANOVA

	N	Minimum	Maximum	Mean	Std. deviation	F-value	p-value
Oregano oil	17	0.046	0.094	0.07265	0.014465	8.42	0.00*
Grape seed oil	17	0.112	0.398	0.19053	0.071855		
Antifungal	17	0.015	0.690	0.09471	0.156327		
Positive control	17	0.119	0.312	0.19206	0.044913		

\*Significant

polymerized acrylic denture base resin were evaluated. Antifungal activity between the groups was compared using ANOVA.

The mean optical density observed with grape seed oil, oregano oil, and antifungal agent were  $0.190 \pm 0.071$ ,  $0.072 \pm 0.014$ , and  $0.094 \pm 0.155$ , respectively. Table 1 shows the comparison of the mean optical density between the four groups along with statistically significant results. It shows that among the four groups oregano oil showed the least optical density, which was statistically significant.

## DISCUSSION

Both *in vitro* and *in vivo* studies have shown that chemical agents used as denture cleansers have the benefit of being effortless to use and exceptional efficiency in lowering biofilm formation on the denture surface.<sup>3</sup>

Plant-derived products as disease control agents have low mammalian toxicity, higher public acceptance, and fewer environmental effects.<sup>9</sup> Origanum oil has shown its efficacy in several studies<sup>5,6</sup> and grape seed oil has shown antifungal activity against *C. glabrata* and *C. krusei* species<sup>7</sup>; hence, in this study, we have used oregano oil and grape seed oil to check its efficacy against *C. albicans*.

Oregano oil is an essential oil obtained from the dried leaves of the oregano plant. Its main constituents are 4-terpineol, g-terpinene, thymol, and carvacrol.<sup>6</sup> These phenolic compounds in the essential oils disturb membrane-embedded proteins, alter the ion transport processes of the cell membrane, modify the activity of calcium channel, and inhibit cellular respiration.<sup>6</sup> This can cause an increase in cell permeability and consequent release of vital intracellular constituents.<sup>6</sup> Hence, its role as a strong antifungal effect on the *C. albicans* has been established. This *in vitro* study showed that oregano oil had the highest antifungal activity than fluconazole, with the least activity shown by grape seed oil.

Immersion-type denture cleansers are the most commonly used methods for denture cleansing. The overall objective of immersing a denture sample in a cleansing solution was to obtain a hygienic, clean, and decontaminated prosthesis by eliminating microorganisms through the chemical action of the denture cleansing agents.<sup>4</sup> Several studies have shown that overnight immersion of dentures in denture cleanser solution may increase the antimicrobial function even more<sup>4, 5,10</sup>; therefore, an 8-hour immersion was used for testing these products.

Various studies<sup>5,6,11,12</sup> have shown that oregano oil is effective against *C. albicans* species. This finding corroborates our study results and the effect of oregano oil was most significant among the groups. The use of these essential oils as denture cleansers has

not been reported in previous studies. Fluconazole was not found as effective as origanum oil in hindering the growth of *C. albicans* in this *in vitro* study. Minguez et al. showed that the sensitivity of fluconazole is highly dependent on the test conditions.<sup>13</sup> It is therefore usual to obtain elevated *in vitro* MIC values for strains that are receptive to the drug *in vivo*. Therefore, further *in vivo* studies are required comparing these antifungal solutions to prove the efficacy of these products.

Antifungal properties of grapeseed oil have been proven in other studies<sup>14,15</sup> also where MIC value was obtained at 500 µg/mL.<sup>14</sup> However, in this study, mild antifungal activity of grape seed oil against *C. albicans* was observed due to lower concentration of oil that was used to test the efficacy, as the MIC of 500 µg/mL is very high.

Potent denture cleansers are the need of the hour for immunocompromised and physically handicapped individuals. They are especially helpful if they are derivatives of naturally occurring substances as chances of allergy and adverse reactions get minimized. The current study aimed at determining the antifungal activity of different antifungal agents as denture cleansers. Hence, oregano oil can be used as an effective alternative to commercially used drugs to prevent *C. albicans* adhesion on the intaglio and polished surface of heat-cure denture base resin.

### Limitations

We could have compared the test samples with commercially available denture cleansers to compare the ability of these essential oils to clean the denture and more number of samples could have been used so that the results could be generalized.

### CONCLUSION

Within the scope of this *in vitro* study it can be concluded that essential oil was more effective than a commercially available antifungal agent. Among the four groups tested, the oregano oil group was found a more potent agent in reducing the fungal colony. Origanum oil, being a herbal product, can be effectively used as a denture cleanser as an alternative to commercially available antifungal agents without any side effects. However, further studies are suggested to evaluate the impact of this oil on the physical and chemical properties, such as strength, structural durability, and color stability of the heat-cure denture base resin, and long term *in vitro* and *in vivo* studies are required to use this as a commercial product.

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