CAPÍTULO 6

ESTUDIO IN VITRO DEL EFECTO ANTIFÚNGICO EN PRÓTESIS REMOVIBLES ACRÍLICAS COLONIZADAS POR CÁNDIDA ALBICANS DE 3 DESINFECTANTES COMERCIALESY 3 PLANTAS MEDICINALES

IN VITRO STUDY OF THE ANTIFUNGAL EFFECT OF 3 COMMERCIAL DISINFECTANTS AND 3 MEDICINAL PLANTS ON REMOVABLE ACRYLIC PROSTHESES COLONIZED BY CANDIDA ALBICANS

San Martin Andrade Daniela Fernanda
Universidad Católica De Cuenca
Daniela.sanmartin@ucacue.edu.ec
https://orcid.org/0000-0002-9449-7008
Azogues, Ecuador

Guallpa Ochoa Kevin Michael
Universidad Católica De Cuenca
kevin.guallpa.49@est.ucacue.edu.ec
https://orcid.org/0009-0004-8864-0041
Azogues, Ecuador

Calderón Alemán Doris Eliana
Universidad Católica De Cuenca
decalderona@ucacue.edu.ec
https://orcid.org/0000-0002-5982-0738
Azogues, Ecuador

Pichazaca Chimborazo Ezequiel Jonnathan
Universidad Católica De Cuenca
ezequiel.pichazaca.79@est.ucacue.edu.ec
https://orcid.org/0009-0009-0453-1187
Azogues, Ecuador

Destruge Vanegas Michael Mateo
Universidad Católica De Cuenca
michael.destruge.91@est.ucacue.edu.ec
https://orcid.org/0009-0001-4395-9962
Azogues, Ecuador

Gonzales Andrade Fernanda Nataly
Universidad Católica De Cuenca
fngonzaleza@ucacue.edu.ec
https://orcid.org/0000-0002-3675-0933
Azogues, Ecuador

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1. INTRODUCTION

Edentulism is the partial or total loss or absence of dental organs, affecting individuals regardless of gender, age, skin color, or ethnicity. It is characterized by alterations in masticatory function, nutritional status, general health, and quality of life (1-3). Among the rehabilitation methods for edentulous patients is the removable dental prosthesis, designed to restore essential functions of the stomatognathic system, thereby improving the life conditions of individuals. To maintain these benefits, proper hygiene and usage are essential to prevent the accumulation of biofilms and the colonization of pathogenic microorganisms (4).

The presence of microorganisms on dental prostheses is attributed to the inherent characteristics of heat-cured acrylic prostheses, whose surfaces have microporosities and roughness that facilitate the adhesion and accumulation of biofilm. This can lead to changes in the oral soft tissues and, in more severe cases, systemic alterations. One of the most prevalent diseases caused by this is denture stomatitis, with the etiological agent being the fungus Candida albicans (5).

Additionally, one of the most prevalent pathologies in prosthesis wearers is denture stomatitis, an infectious and inflammatory condition of the oral mucosa, typically located on the hard palate and sometimes on the mandible, causing redness in the prosthetic support area (6-8). The prosthesis itself can become a medium for physical aggression to the tissues of the oral cavity due to its acrylic structure and porous surface, creating an ideal environment for the adhesion and proliferation of microorganisms that

settle on the prosthesis and the lesion (8). Being a multifactorial pathology, direct causes include chronic infections by Candida albicans, and various predisposing factors such as poor oral hygiene, altered salivary flow, pH variations, constant use of prostheses, HIV infection, and systemic factors like diabetes, among others (9,10).

Oral prosthetic devices have various mechanisms for hygiene; manual methods emphasize the use of toothbrushes to dislodge microorganisms that may adhere to the prosthesis, while chemical methods utilize oral disinfectants. Another disinfection means includes natural methods, such as the use of plant infusions like mint, lemon verbena, and oregano. However, not all are sufficiently effective, and arbitrary application can alter the properties of the prosthesis material, such as changes in coloration or a reduction in its lifespan (4,9).

Therefore, there is a clear need to understand the appropriate use of each disinfectant to prevent the proliferation of infectious microorganisms and ensure the preservation of the prosthesis. This study aims to determine the antifungal effect on acrylic removable prostheses colonized by Candida Albicans using three commercial disinfectants and three medicinal plants.

2. MATERIALS AND METHODS

This is an in vitro experimental study using 180 thermocured acrylic resin samples, each measuring 15mm x 15mm x 3mm in thickness, which simulate the characteristics of a removable acrylic partial denture. The polymerization and polishing times were adhered to according to the

The following substances were used:

- Acetic acid (commercial vinegar)
- Disinfectant soap (Dettol brand)
- Disinfectant soap (Lifebuoy brand)
- Medicinal plants: mint at 50%, mint at 100%, mint and lemon verbena at 50%, mint and lemon verbena at 100%, mint and oregano at 50%, and mint and oregano at 100%.
- The samples were classified as follows:
- 9 samples for treatment with acetic acid
- 9 samples for treatment with Dettol disinfectant soap
- 9 samples for treatment with Lifebuoy disinfectant soap
- 9 samples for treatment with mint at 50%
- 9 samples for treatment with mint at 100%
- 9 samples for treatment with a mint and lemon verbena mix at 50%
- 9 samples for treatment with a mint and lemon verbena mix at 100%
- 9 samples for treatment with a mint and oregano mix at 50%
- 9 samples for treatment with a mint and oregano mix at 100%
- Positive control: 3 samples inoculated with Candida albicans and treated with sterile distilled water.
- Negative control: 3 samples without inoculation and treated with sterile distilled water.

To obtain Candida albicans, the strain was prepared according to the supplier's characteristics. It was initially cultured on Sabouraud agar with 40mg/ml gentamicin to prevent bacterial contamination and incubated at 36.5°C for 48 hours. After this, Candida albicans colonies were taken and diluted in sterile water, using the McFarland standard (0.5) to achieve the appropriate turbidity.

The acrylic pieces were immersed in thioglycolate broth with gentamicin (40mg/ml) and one milliliter of Candida albicans elution was added, followed by incubation at 36.5°C for 48 hours. Negative controls were not inoculated.

Once the Candida albicans strains were ready, the disinfectants were prepared at the corresponding concentrations. The 27 acrylic pieces were submerged in each of the disinfectants to be tested, according to the indicator explained in Table 1. The controls were submerged in sterile distilled water.

After the exposure time to the disinfectants, the resins were immersed in thioglycolate broth for 30 minutes, swabbed, and the swabs were placed in 10 ml of sterile water, mixed, and 1 ml of the sample was cultured on Sabouraud agar with gentamicin. Subsequently, they were incubated at 36.5°C for 48 hours. The growth of the microorganism was observed by visualizing the presence of colonies in the culture media.

3. RESULTS

The effectiveness of three commercial disinfectants (acetic acid, Dettol, and Lifebuoy) and three medicinal plants (mint, oregano, and lemon verbena) was evaluated through the observation of the growth or inhibition of Candida albicans.

In the samples treated with acetic acid, no fungal growth was detected starting from 2 hours after application. For the disinfectant soaps, it was observed that the commercial disinfectant "Dettol" showed suppression of fungal colony growth starting from 7 hours of exposure, while no inhibition of colony growth was observed in the samples treated with the commercial disinfectant "Lifebuoy" at any of the evaluated time intervals (Table 1).

Regarding the medicinal plants, the effectiveness of 50% mint was evaluated, showing an inhibitory effect starting from 10 minutes of exposure, while the 100% mint extract achieved inhibition starting from 5 minutes (Table 2). The 50% mint and lemon verbena mix showed fungal elimination starting from 10 minutes, and increasing the concentration of the mint and lemon verbena mix to 100% resulted in effective elimination starting from 5 minutes (Table 2). The 50% mint and oregano mix was effective starting from 10 minutes, with the same result observed when the concentration of the mix was increased to 100%.

The most effective solutions for the elimination of Candida albicans from acrylic dentures were the 100% mint extract and the 100% mint combined with lemon verbena extract (Table 2).

Table 1: Evaluation of Commercial Disinfectants Against Candida albicans

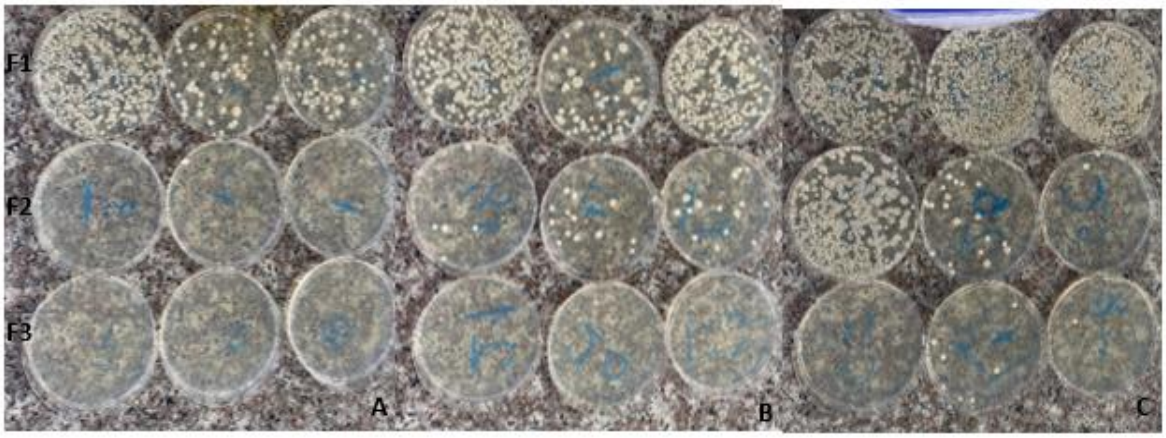
Disinfectant Group	Samples	1 Hour	2 Hours	7 Hours
Acetic Acid	9	+	-	-
Dettol	9	+	+	-
Lifebuoy	9	+	+	+

Table 2: Evaluation of Medicinal Plants Against Candida albicans

Natural Extract Group	Samples	5 minutes	10 minutes	15 minutes
Mint 50%	9	+	-	-
Mint 100%	9	-	-	-
Mint and Lemon Verbena (50%)	9	+	-	-
Mint and Lemon Verbena (100%)	9	-	-	-
Mint and Oregano (50%)	9	+	-	-
Mint and Oregano (100%)	9	+	-	-

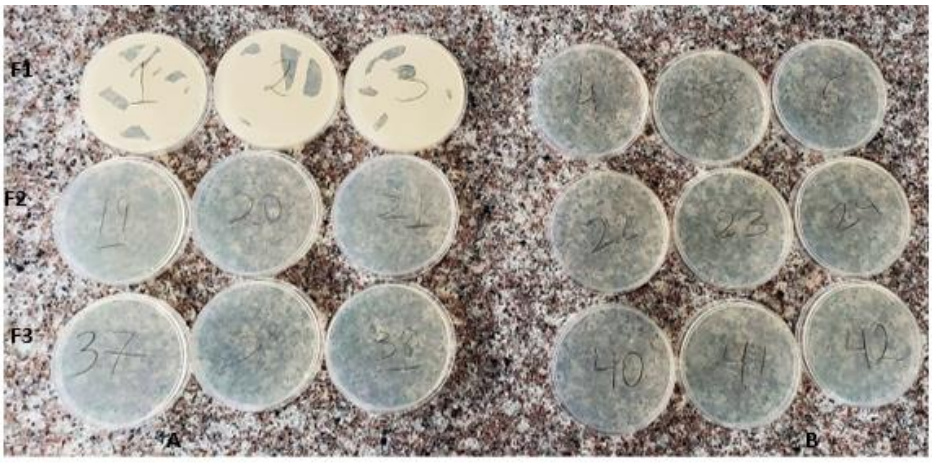
Nota. Abbreviations (+): growth of fungal strains confirmed by colony counts. Abbreviations (−): absence of fungal growth confirmed by colony counts.

Figure 1: Colony growth of *Candida albicans* in commercial disinfectants according to exposure time.



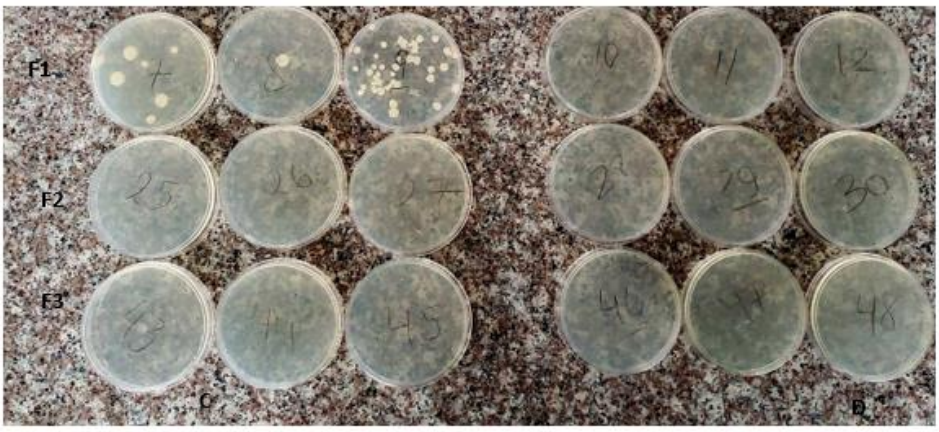
Inhibition of *Candida* is observed according to the exposure times of 1, 2, and 7 hours.

Figure 2: Growth of *Candida albicans* in natural disinfectants according to exposure time and disinfectant concentration.



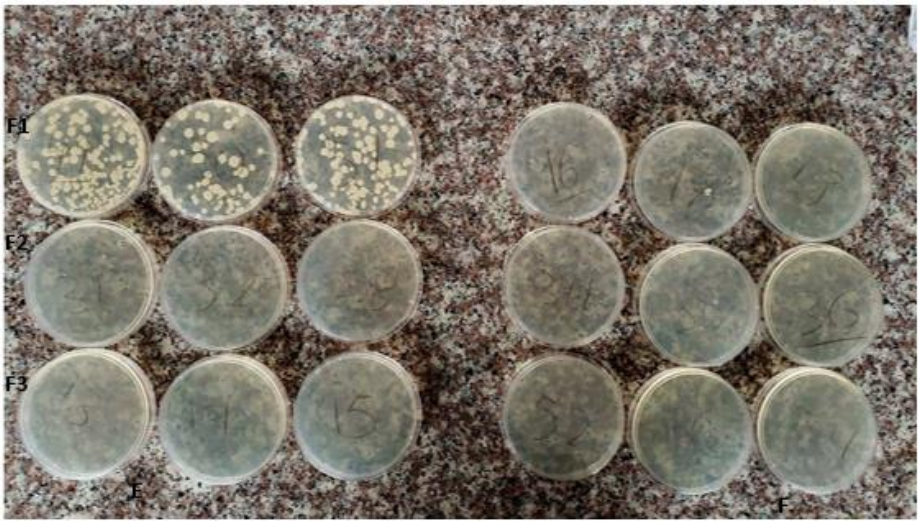
Inhibition of *Candida albicans* is observed in mint at 50% and 100% concentrations with exposure times of 5, 10, and 15 minutes.

Figure 3: Growth of *Candida albicans* in natural disinfectants according to exposure time and disinfectant concentration.



Inhibition of *Candida albicans* is observed in combinations of mint and lemongrass at 50% and 100% concentrations with exposure times of 5, 10, and 15 minutes.

Figure 4: Growth of *Candida albicans* in natural disinfectants according to exposure time and disinfectant concentration.



Inhibition of *Candida* is observed in combinations of mint and oregano at 50% and 100% concentrations with exposure times of 5, 10, and 15 minutes.

4. DISCUSSION

The results obtained from commercial disinfectants, such as vinegar, indicate the inhibition of *Candida albicans* growth at different concentrations and exposure times, with no fungal growth detected after 2 hours of application. In this regard, Budagara et al. (11) analyzed the effectiveness of vinegar against *Candida albicans* concerning concentration, which ranged from 6% to 8%, and reported inhibition zones between 6 and 6.12 mm. Similarly, Haggard et al. (12) referred to the use of homemade vinegar for a total prosthesis disinfection protocol, noting a lower inhibition effect with a prolonged exposure of 2 hours at 100% concentration, showing variation in concentration and exposure times used in the present study.

San Martin et al. (13) indicated that the effectiveness of Dettol and Lifebuoy is 95% when exposing the prosthesis for an 8-hour immersion period, with results improving when combined with mechanical cleaning methods such as brushing. A 10% concentration of Dettol serves as an effective antimicrobial agent against *Candida albicans* on any inanimate object; however, continuous use could contribute to the development of resistance in these pathogenic microorganisms. Likewise, Olorodea et al. (14) reported that acrylic plates submerged in 50% Dettol for 7 hours achieved a

notable reduction in *Candida albicans* colonies, making it a viable option for pathogen control.

Soares et al. (15) highlighted characteristics of Lifebuoy, such as the absence of cytotoxicity and the ability to maintain the structural properties of acrylic prostheses. Brushing the prosthesis with Lifebuoy or immersing it in 0.78% concentrations inhibits *Candida albicans*; however, it is recommended to combine it with sodium hypochlorite or alkaline peroxide to significantly reduce the metabolism of this pathogenic microorganism. Optimal prosthesis cleaning is best achieved through a combination of chemical and physical methods.

Recent studies on medicinal plants suggest they are a primary need in healthcare systems. Saharkhiz et al. (16) found that peppermint essential oil effectively stopped the growth of various yeasts, including *Candida albicans*, at concentrations ranging from 0.12 to 4 µL/ml. The oil also demonstrated fungicidal properties for all these yeasts at concentrations between 1 and 8 µL/ml, with no significant differences in concentration needed to inhibit resistant strains.

Rajkowska et al. (17) observed *Candida albicans* growth on solid media supplemented with peppermint and clove essential oils at concentrations between 0.0075% and 2.5% v/v, approximately half of their minimum inhibitory concentrations. The results suggest that the inhibition of microorganism colonization depends on the type and concentration of the essential oil to achieve significant changes in the fungus's colony morphology.

Agarwal et al. (18) analyzed the effect of 30 plant oils, including peppermint oil, on *Candida albicans*. Their results demonstrated that peppermint oil has notable antimicrobial activity, evidenced by an inhibition zone of 22.2 millimeters. They determined that the minimum inhibitory concentration (MIC) of peppermint oil required to completely inhibit *C. albicans* growth is 0.08%. They also classified oils based on their minimum fungicidal concentration (MFC), placing peppermint oil among the most effective with an MFC value of 0.15%.

Lekshmy et al. (19) showed that herbal extracts such as peppermint and lemongrass have antifungal properties equal to or greater than fluconazole against *Candida albicans* due to the presence of phytochemical compounds in the plant leaves. Similarly, Azuero et al. (20) analyzed 12 medicinal plants from Ecuador, including lemongrass, using the Candida albicans agar diffusion technique. Most plants showed a degree of inhibition against this fungal strain, presenting promising results for effective pharmacological development against these microorganisms.

On the other hand, the experimental study by Villavicencio Gatelú et al. (22) analyzed the antifungal effect of different types of oregano (*Origanum vulgare*) on *Candida albicans* strains through sensitivity tests using the disk diffusion method on agar cultures, showing positive results for all types of oils at concentrations of 12%.

Guallpa et al. (21) noted that the effectiveness of peppermint, oregano, and lemongrass against *Candida albicans* may be due to the similarity of phenolic components such as thymol and carvacrol found in these plants'

leaves, as well as the release of free radicals, cytochrome C, calcium, potassium, and magnesium ions that induce oxidative stress. They also emphasized that the antifungal properties of these plants are influenced by factors such as the region of origin, plant species variant, exposure time, and concentration.

5. CONCLUSION

When selecting a disinfectant for acrylic prostheses, it is crucial to choose one that has bactericidal or fungicidal properties. Additionally, the disinfectant should meet biocompatibility standards with the prosthetic material to preserve its longevity and ensure user health, while being easily accessible, practical to use, and cost-effective.

In this context, natural disinfectants such as mint, oregano, and lemongrass at concentrations above 50% are effective in reducing *Candida albicans* colonies. The effectiveness varies with immersion time; however, all natural disinfectants show successful results within 10 minutes.

On the other hand, disinfectants like vinegar and Dettol proved effective within a 2-hour exposure period, whereas Lifebuoy soap showed no inhibitory effect against *Candida albicans*. It is noteworthy that a significant reduction in the fungus on acrylic prostheses can be achieved by combining compatible disinfectants with physical cleaning mechanisms and increasing disinfectant concentrations. However, the final outcome will depend on user care and the properties of the prostheses, such as porosity, which may predispose them to microorganism retention.

6. DISCLOSURE

The authors declare no conflicts of interest

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