

**An Evaluation of Antifungal Efficacy of Different Herbal Extracts as Irrigants against Candida Albicans**

<sup>1</sup>Hehan A M, Associate Professor, Faculty of Dentistry, Egypt.

**Corresponding Author:** Hehan A M, Associate Professor, Faculty of Dentistry, Egypt.

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**Abstract**

Candida albicans, a fungal species, stands as a formidable foe, often causing a range of infections in humans, especially in immunocompromised individuals. Assessed the antifungal efficacy of Psidium guajava leaf extract and Allium sativum extract against Candida albicans biofilm. It was hypothesized that these herbal extracts would exhibit antifungal activity and have the potential to be used as natural alternatives for the treatment of Candida albicans infections.

**Keywords:** Candida Albicans, Guava Leaf Extract, Infections, Antimicrobial Properties

**Introduction**

In the realm of healthcare, the battle against microbial invaders is a constant and evolving challenge. Among these microbial adversaries, Candida albicans, a fungal species, stands as a formidable foe, often causing a range of infections in humans, especially in immunocompromised individuals. While conventional antifungal agents have been instrumental in combating Candida infections, the emergence of drug resistance and

concerns about their side effects have spurred the exploration of alternative solutions. Enter the world of herbal extracts – a treasure trove of nature's remedies, offering a promising avenue for combating these fungal threats.

This delves into a fascinating journey of discovery, where science meets the ancient wisdom of herbal medicine. We embark on an in vitro study that seeks to unearth the antifungal potential of various herbal extracts when employed as irrigants against Candida albicans. By doing so, we aim to shed light on the therapeutic possibilities that lie within these botanical wonders, potentially revolutionizing the way we approach fungal infections in clinical settings.

**Aim**

To check and compare for the antifungal efficacy of different herbal extracts as irrigants against Candida Albicans.

**Objectives**

To disinfect the root canal space, biomechanical preparation and irrigation with antimicrobial treatments

are required. The nature of endodontic infections is polymicrobial. The most frequent fungus isolated from unsuccessful endodontic cases is *Candida albicans*.

This in-vitro study focused on the antifungal activity of *Psidium guajava* leaf extract and *Allium sativum* extract on *Candida albicans* biofilm developed in root canals of teeth.

## Materials and Methods

### *C. albicans* culture preparation

On Sabouraud Dextrose Agar, a pure colony of *Candida albicans* was inoculation, and cultured at 37°C overnight.<sup>2</sup> (Figure 1 – B, C)

### Test solutions preparation

#### Preparation of Guava leaf extract

Guava leaves were collected and dried outdoors in the open while being shielded from the sun. A beaker with 20 ml of sterile distilled water was filled with 2 g of powdered leaves. This was heated in a water bath to create the hot water extract. The liquid that resulted from completely evaporating the extract's water content was filtered using filter paper.<sup>3</sup> (Figure 1 - D)

#### Preparation of Garlic extract

Using a sterile mortar and pestle, tablets containing 100% garlic extract were homogenised aseptically. Distilled water was then added, bringing the concentration to 64 mg/ml. 20 ml in total were made.<sup>4</sup> (Figure 1- E)

### Tooth samples preparation

Type 1 single rooted mandibular premolar teeth (Figure 1-A) from the Vertucci classification were cut into 8 mm sections below the cemento enamel junction. The teeth were cleared of calculus, tissue tags, and surface debris before being preserved in ordinary saline. The root canals were then instrumented using rotary instruments and the crown down procedure to an F3 apical size. During the cleaning and shaping process, a total of 2 cc of 5% sodium hypochlorite was used between each

instrument. Along the mid-sagittal plane, all teeth were divided vertically into two parts. In order to obtain a flat surface that would allow for insertion in tissue culture wells and expose the root canal surface to *C. albicans* for the formation of a biofilm, the concave tooth surface underwent minor grinding. The samples were then put in the wells of tissue culture plates after being sterilised by UV light in a biosafety cabinet. At 37°C for two days, the cultivated yeast was injected into the wells containing tooth samples.<sup>2</sup>

### Grouping and assessment protocol

The samples were divided into 3 experimental groups with 10 samples each and irrigated with 3 ml of each irrigant for 10 min.

Group 1 — Guava leaf extract

Group 2 — Garlic extract

Group 3 — Sterile saline.

Root canal samples were taken using the sterile paper point technique, inoculated in Sabouraud Dextrose Agar, and incubated at 37°C for 24 hours in a Petri dish. (Figure 1-G) The results were then measured using a manual colony counter, and the data were statistically analyzed using ANOVA test. (Figure 1- H)



Figure 1: A – 30 single rooted mandibular premolar teeth Type 1 Vertucci classification. B, C – Samples

were then put in the wells of tissue culture plates. D,E,F – Different irrigants groups prepared G – Samples irrigated with different groups and root canal samples were taken using the sterile paper point technique, inoculated in SDA. H – Number of colonies formed counted using a manual colony counter device.

**Result**

Readings of microbial count obtained from manual colony counter after irrigation with respective irrigant were as follows: Table 1.

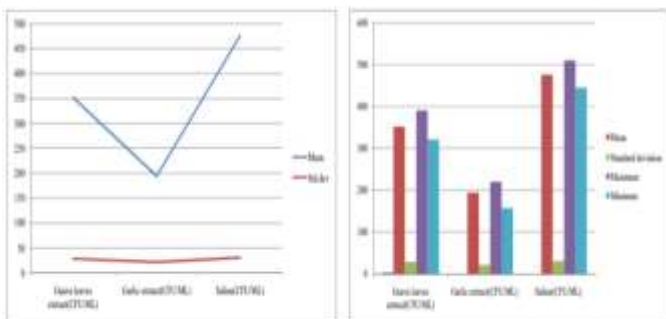
The mean and standard deviations obtained were as follows.

Table No.1: Descriptive statistics among the studied groups.

| Groups                       | N  | Mean  | Standard deviation | Maximum | Minimum | p value |
|------------------------------|----|-------|--------------------|---------|---------|---------|
| Guava leaves extract(CFU/ML) | 10 | 351.9 | 28.32              | 391     | 321     | <0.01*  |
| Garlic extract(CFU/ML)       | 10 | 194   | 21.34              | 220     | 157     |         |
| Saline(CFU/ML)               | 10 | 476.1 | 30.66              | 510     | 445     |         |

ANOVA (Post-hoc Tukeys) test. p value <0.01\* was considered statistically significant.

1. Microbial count was maximum in the saline group and minimum in the garlic extract group.
2. Guava leaf extract group also showed a higher antifungal efficacy as compared with saline group.
3. Saline (negative control) had least antifungal activity as expected.
4. All the between group differences were statistically significant.
5. The order of efficacy of different groups was as follows: Garlic extract > Guava leaf extract > Saline.



**Discussion**

This study since it is frequently found in recalcitrant endodontic infections.<sup>2,5</sup> Initially adhering for 0–2 hours, C. albicans then forms microcolonies for a further 2-4 hours. Following that, dimorphic switching took place with a change from filamentous pseudo- and true-hyphal forms to budding-yeast forms (4-6 h). The hyphal extensions then connect the microcolonies, resulting in the formation of a confluent monolayer (6–8 hours). The biofilm becomes more complicated as it ages (8–48 h), developing a 3D architecture and geographic heterogeneity. After 24 and 48 hours, a mixture of yeast cells, pseudo-hyphae, and genuine hyphae make up the biofilm. Yeast cells were found in the bottom layer, and filamentous forms played a major role in the 3D architecture.<sup>2,6</sup> Mutants of C. albicans that lack the capacity to produce hyphae have shown a lack of ability to build 3D biofilms. The 48 h biofilm model was chosen because the dimorphic switching seen in this species is crucial for the development of biofilms and the pathogenic potential of C. albicans.

It completely blocks RNA production while only partially inhibiting DNA and protein synthesis. Allicin also affects DNA transcription and other DNA-related processes. At least 33 sulphur compounds, numerous enzymes, 17 amino acids, and minerals like selenium are all present in garlic.

The therapeutic benefits of garlic as well as its strong odour are both caused by sulphur compounds. About 1% of alliin (S-allyl cysteine sulfoxide) is present in dried, powdered garlic.

**Conclusion**

Under the limitations of this study, it was concluded that:

1. Garlic extract performed better than guava leaves extract against *C. albicans* biofilm formed on extracted tooth surface.
2. Guava leaf extract also had a good antifungal action

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