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Antifungal Efficacy of Different Herbal Extracts As Irrigants Against Candida Albicans: An Invitro Study

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ABSTRACT

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: 30 extracted human mandibular premolars will be biomechanically prepared, vertically sectioned, and placed in tissue culture wells exposing the root canal surface to C. albicans grown on Sabouraud Dextrose Agar to form a biofilm. At the end of 2 days, 3 groups of 10 extracted teeth will be treated with test solutions (Psidium guajava leaf extract, Allium sativum extract) and control (saline) for 10 min and evaluated for Candida growth and number of colonies forming units. The data will be collected and analyzed using the ANOVA test and independent student t-test.

Results: Microbial count was maximum in the saline group and minimum in the garlic extract group. Guava leaf extract group also showed a higher antifungal efficacy as compared with saline group.

Clinical relevance – This study helps in evaluating different irrigants for the removal/reduction of the most commonly found fungi in root canals i.e. candida albicans

KEYWORDS: Candida albicans, Psidium guajava leaf extract, Allium sativum extract

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.[1]

This article 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.

This study 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.

So, fasten your seatbelts as we embark on a captivating exploration of nature's pharmacy and its potential to bolster our defences against Candida albicans – a menace that demands innovative solutions and a return to the healing embrace of the natural world.

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. [2] (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. [3] (Figure 1 - D)

Preparation of Garlic extract -

Using a sterile motar 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.[4] (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 cementoenamel 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.[2]

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.

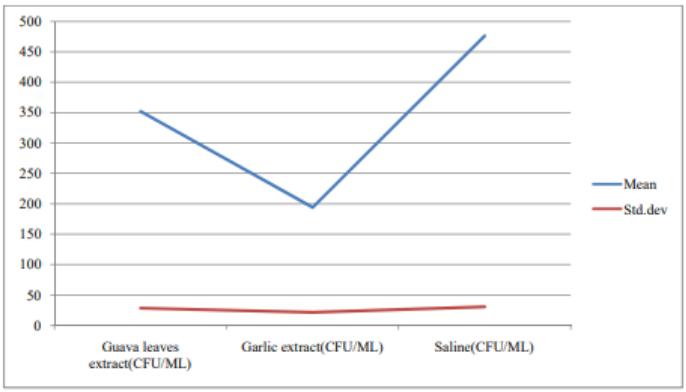
The mean and standard deviations obtained were as follows.

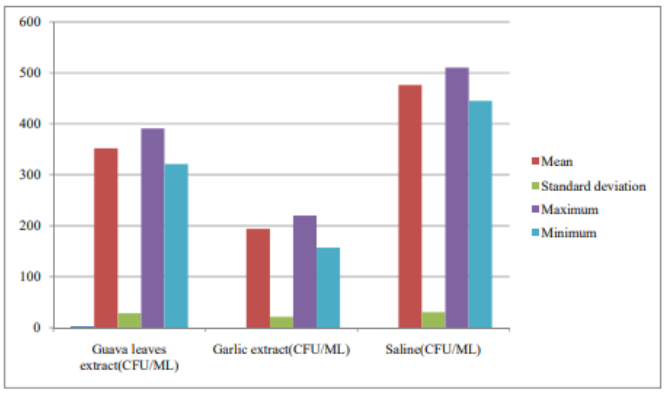
GROUPS	N	MEAN	STANDARD DEVIATION	MAXIMUM	MINIMUM	P VALUE
Guava leaves extract	10	351.9	28.32	391	321	<0.01*
Garlic extract	10	194	21.34	220	157	<0.01*

Saline	10	476.1	30.66	510	445	<0.01*

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

DESCRIPTIVE STATISTICS AMONG THE STUDIED GROUPS





DISCUSSION

C. albicans was the subject of this study since it is frequently found in recalcitrant endodontic infections.[2,5] 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.[2,6] 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. [2,7,8]

Although gram negative bacteria demonstrated decreased susceptibility to the antimicrobial function of Guava leaves (P. guajava leaf), guava leaves do possess strong antimicrobial (antibacterial and antifungal) properties. Guava leaves are abundant in a variety of polyphenolic chemicals (Phenol, flavonoids, and tannin), according to phytochemical study. Due to their antimicrobial properties, polyphenolic chemicals may be the most likely source of the antifungal and antibacterial properties of guava leaves. Guava leaves can also be employed as a bio preservative in the food and pharmaceutical industries in addition to their antibacterial properties.[9]

Due to its antibacterial, antifungal, and antiviral qualities, garlic (Allium sativum) has been used as a medicinal plant since ancient times. A wide range of Gram-positive and Gram-negative bacteria are inhibited from growing by garlic extract.[4,10] Diallyl sulphide, diallyl disulfide, and allyl methyl sulphide are among the thiosulfinates that give it its antibacterial and reducing properties. Bacterial inhibition has been linked to their disruption of cell components and their capacity to obstruct enzyme processes.[4, 11,12,13]

Garlic extract's ability to dissolve tissue is linked to its distinctive organosulfur components, such as allicin, which is the main active ingredient in fresh garlic extract and may be one of the causes of this activity. When garlic is crushed, the enzyme alliinase produces allicin, which metabolises to vinyldithiines. Allicin has the potential to damage the epidermal junction and result in coagulative necrosis of the tissues because it contributes in the metabolism of cysteine in proteins.[4,14] 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.[4,15]

According to a study by Khan L et al., the concentration of garlic extract used in this investigation was diluted to 64 mg/ml because it had the strongest antibacterial effects.[4,16] The extract can be kept at room temperature for no more than seven days, while it can be kept at 20°C for up to ninety days.[4,14] 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.[4,17,18]

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.

CLINICAL SIGNIFICANCE

This study's purpose is to find alternative endodontic irrigants which are effective against biofilm associated infections specifically candida albicans. They are also biocompatible and safe, reducing the systemic antifungal dependence. They are cost effective, easily accessible and have a potential for broader applications.

Overall, the findings of this study could contribute to the development of novel, plant-based antifungal agents for improved management of *Candida albicans* biofilms in endodontic infections, enhancing treatment success and patient outcomes.

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