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ORIGINAL ARTICLE

ETHANOLIC AND WATER EXTRACT OF GUAVA LEAVES AT VARIOUS CONCENTRATIONS AGAINST STAPHYLOCOCCUS AUREUS AND CANDIDA ALBICANS – AN INVITRO STUDY

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ABSTRACT

AIM: The purpose of this study was to determine the antibacterial and antifungal activities of aqueous and ethanolic extracts of Psidium guajava (Guava) leaf at 15% and 25% concentrations against Staphylococcus aureus and Candida albicans.

METHODOLOGY: The standard strains of S. aureus and C. albicans were sub-cultured on nutrient agar for 24 hours to generate fresh cultures. Guava leaf extract was extracted using Soxhlet extraction and formulated in aqueous and ethanolic form at 15% and 25%. The antimicrobial activities were tested by agar well diffusion method. For each extract concentration, two plates of agar were prepared. Chlorhexidine at 0.2% as positive and distilled water as negative control.

RESULT: Inhibition zones of 14.0 mm and 17.3 mm of the 15% and 25% ethanolic extracts were noted against S. aureus and 14.0 mm and 17.6 mm against C. albicans. The corresponding inhibition zones for the aqueous extracts were found to be 12.6 mm and 14.6 mm against S. aureus and 12.6 mm and 15.6 mm against C. albicans. 15% extracts had lesser antifungal activity when compared with chlorhexidine; the 15% ethanolic extract possessed antibacterial activity similar to that of chlorhexidine. There were no significant differences between the 25% extracts and chlorhexidine.

CONCLUSION: Antimicrobial activity of "guava leaf extracts" was found to be equivalent to 0.2% chlorhexidine in case of both 15% and 25% extracts.

KEYWORDS: Psidium guajava, guava leaf extract, Staphylococcus aureus, Candida albicans, chlorhexidine, agar.

INTRODUCTION:

Antimicrobial resistance can be described as the ability of a microorganism to resist the action of antimicrobials, which regularly occurs through continuous exposure to them. The level of resistance of a mutant strain can vary widely depending on the mechanism of resistance resulting in its evolution, either by spreading between similar or dissimilar strains. [1] Overuse of these agents has caused the worldwide emergence of Dr. ug-resistant pathogens that pose serious life-threatening issues.[2] The improper use of antimicrobials stimulated the emergence of genetic modifications that contributed to circumventing the mechanism of action of Dr. ugs. Therefore, the expansion of resistant strains results in damage to public health as it leads to infectious conditions that require difficult treatment. [3] Bacterial pathogens such as S. aureus and fungal organisms like C. albicans are notorious for causing both superficial and systemic infections, with severe implications for immunocompromised individuals.[3] C. albicans is a commensal fungus that colonizes the oral cavity, vagina, and gastro-intestinal tract in most humans.[4] In immunocompromised individuals the infections due to bacteria and fungi may occur concurrently.[5] Conventional antimicrobial Dr. ugs, though effective, are increasingly losing their efficacy due to the rise in resistance, thereby necessitating the search for novel and sustainable antimicrobial agents. Chlorhexidine, a broad-spectrum antimicrobial agent, has been a cornerstone in infection control practices. Chlorhexidine is commonly used in oral rinses, skin disinfectants, and wound care products due to its efficacy in preventing biofilm formation and reducing microbial load. However, its prolonged use has been associated with side effects such as staining of teeth, taste alteration, and, in rare cases, hypersensitivity reactions. Additionally, the emergence of microbial resistance to chlorhexidine has prompted the exploration of alternative natural agents that can complement or replace its usage. [6]

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Medicinal plants have gained attention as potential sources of bioactive compounds with antimicrobial properties. Among these, Psidium guajava (guava) holds a prominent place in traditional medicine due to its broad spectrum of pharmacological activities. Guava leaves, in particular, are rich in secondary metabolites such as flavonoids, tannins, saponins, alkaloids, and phenolic compounds, which contribute to their antimicrobial, anti-inflammatory, and antioxidant effects. [7] The application of guava leaf extracts in combating microbial infections is a promising avenue, as they are not only natural and cost-effective but also associated with lower risks of adverse effects compared to synthetic Dr. ugs. [8] Furthermore, previous studies have demonstrated the potential of guava leaves in inhibiting the growth of pathogenic microorganisms. However, limited research has focused on comparing the activity of these extracts at varying concentrations against both bacterial and fungal pathogens, specifically S.aureus and C. albicans. [7] This study aims to bridge this gap by evaluating the in vitro antimicrobial efficacy of ethanolic and aqueous guava leaf extracts against these clinically relevant pathogens. The research seeks to identify and provide a scientific basis for the development of plant-based antimicrobial agents, which could serve as complementary or alternative therapies in the management of microbial infections.

MATERIALS AND METHODOLOGY

TEST ORGANISMS

The microorganisms used in the present study were standard C. albicans (ATCC No 10231) and S. aureus (ATCC No 23235), which were provided from Saveetha Dental College and Hospital, Chennai, India. Fungal strains were passed through a Sabouraud dextrose agar environment for 24 hours to obtain live and fresh strains for the test. The pure cultures of S.aureus were subcultured on nutrient agar slants. About 18-hour broth culture of the test bacteria isolate was suspended into sterile nutrient broth and kept at 4 degrees Celsius until ready for the study.

PREPARATION OF EXTRACT

This preliminary study considered only the qualitative analysis of activity of Guava leaves extract against S.aureus and C.albicans. Therefore, the two higher concentrations, that is, 15% and 25% w/v were tested for antimicrobial activity. The plant specimen (Leaves of P. guajava Linn.) for the proposed study was collected in the month of December 2024. Leaves of P. guajava L. (Myrteceaae) were cleaned and Dr. ied in an oven at 60°C for 5 hours. The Dr. ied leaves were then grounded to powdered form. Preparation of the extract was done using Soxhlet extractor. The extracts were filtered using Whatman no. 4 filter paper and then Dr. ied in a rotary evaporator for 5-6 hours at 60°C. The Dr. ied extract was converted into a powder form which was utilized for the preparation of desired concentrations of the extracts. The required concentrations of 15% and 25% ethanolic extract were prepared by adding 1.5 g and 2.5 g of powder respectively in 10 ml of ethanol. Similarly for the water extract the same amount was added in the distilled water. The extracts were stored at 4°C in sterile bottles.

ANTIMICROBIAL TEST

The antibacterial and antifungal activity of guava extracts was checked by agar well-diffusion method which was performed on the next day of preparation of the extract. Antibacterial and antifungal testing was carried out in laminar airflow to avoid contamination by other organisms. Two groups of plates were prepared: antibacterial test group (S.aureus) and antifungal test group (C.albicans). In each group, there were 3 plates. In all the plates, 6 wells were punctured in agar with the help of well borer. The 6 wells prepared in both groups were filled carefully with 0.08 ml of 15% ethanolic extract of guava, 25% ethanolic extract of guava, 15% water extract of guava, 25% water extract of guava, 0.2% chlorhexidine (positive control) and sterile distilled water (negative control). All the plates were kept in an incubator at 37°C for 48 hours. After 48 hours zones of inhibition were measured.

STATISTICAL ANALYSIS

Statistical analysis was conducted using IBM SPSS Statistics 20.0 (Chicago). An ANOVA test was used to determine differences between groups, including 15% and 25% ethanolic extract, 0.2% chlorhexidine, and 15% and 25% water extract. Post-hoc analysis was performed to identify which specific groups showed significant differences. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Agar well diffusion technique 15% and 25% water extract ,0.2% chlorhexidine and distilled water against S. aureus. [Table 1, Figure 1] and C.albicans [Table 2, Figure 2] are shown. The antibacterial activities against S. aureus of 15% and 25% ethanolic guava leaf extracts were compared with 0.2% chlorhexidine. There was no statistically association between the 15% extract group (14 ± 1 mm), 25% extract group (17.3 ± 1.5 mm), and 0.2% chlorhexidine group (17 ± 2 mm) ($F = 4.136$, $0P = 0.074$). There was a significant difference in the activity of 15% (12.6 ± 0.577 mm) and 25% (14.6 ± 0.577 mm) water extract and 0.2% chlorhexidine (17 ± 2 mm) ($F = 9.071$, $P = 0.015$) [Table 2]. Activity of 0.2% chlorhexidine (17 ± 2 mm) was significantly higher than 15% water extract (12.6 ± 0.577 mm) of guava leaves. There was no significant difference

between 0.2% chlorhexidine (17 ± 2 mm) and 25% water extract (14.6 ± 0.577 mm) of guava leaves [Table 2]. The antifungal activities against C. albicans of 15% and 25% ethanolic guava leaf extracts were compared with 0.2% chlorhexidine. There was a statistically significant difference between the groups (F = 25.75, P = 0.001). Post-hoc analysis revealed that the 25% extract group (17.6 ± 0.58 mm) had antifungal efficacy similar to 0.2% chlorhexidine group (17 ± 1 mm; P = 0.483). However, the 15% extract group (14 ± 0 mm) showed significantly less efficacy than 25% extract group (P = 0.001) and 0.2% chlorhexidine group (P =0.003) [Table 3]. There was a significant difference in the activity of 15% (12.6 ± 2.309 mm) and 25% (15.6 ± 0.577 mm) water extract and 0.2% chlorhexidine (17 ± 1 mm) (F = 6.65, P = 0.030) [Table 4]. Activity of 0.2% chlorhexidine (17 ± 1 mm) was significantly higher than 15% water extract (12.6 ± 2.309 mm) of guava leaves. There was no significant difference between 0.2% chlorhexidine (17 ± 1 mm) and 25% water extract (15.6 ± 0.577 mm) of guava leaves [Table 4].

DISCUSSION

The bioactive components of Psidium guajava leaves include essential oils, flavonoids, saponins, oleanolic acid, nerolidol, β-sitosterol, andavicularin. Out of these bioactive components, avicularin along with its 3-L-4-pyranoside derivative shows notable antibacterial activity. [9] Apart from this, it was noted that flavonoids and tannins in dried guava leaves possess significant antifungal activity. Further, Bezerra et al. noted that when the natural products derived from Psidium guajava were combined with fluconazole, they were effective in enhancing the antifungal efficacy of fluconazole at lower concentrations. [10] Razak et al. noted that the aqueous guava leaf extract (1 mg/mL) treatment reduces the cell surface hydrophobicity of Actinomyces spp., Streptococcus mitis, and Streptococcus sanguinis by 54.1%, 49.9%, and 40.6%, respectively. [11] Vieira et al. noted that the guava leaf extract is capable of inhibiting the growth of S. aureus. [12] Beatriz et al. noted that the P. guajava leaf extracts (50 mg/mL). possess significant antifungal activity against different types of fungi. [13] Agar well diffusion technique, known to have greater sensitivity compared to the disc diffusion technique, was employed in order to assess the antimicrobial effects of guava leaf extract on S. aureus and C. albicans. [14] It was observed that the efficacy of 25% ethanolic extract and the aqueous extract was similar to 0.2% chlorhexidine for both microorganisms. However, 15% ethanolic extract had the same efficacy as 0.2% chlorhexidine but only for S. aureus. It can be observed that the ethanolic extracts of 15% and 25% were more effective in inhibiting bacterial growth as compared to the aqueous extracts, and this might be due to the differences in phytochemical composition because ethanolic extracts are rich in flavonoids as well as tannins, while aqueous extracts contain tannins only. [15] It is concluded from the present study that guava leaf extracts possess antimicrobial properties when used against S. aureus and C. albicans, but further quantitative studies are required for determination of MIC and safety/effectiveness in vivo.

CONCLUSION

Both the ethanolic as well as aqueous extracts of guava leaves has anti-bacterial and anti-fungal properties against S. aureus and C. albicans. While 25% extracts show comparable anti-microbial activities to 0.2% chlorhexidine against both S. aureus and C. albicans, 15% ethanolic extracts possess activity similar to 0.2 chlorhexidine against S. aureus only.

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