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ORIGINAL ARTICLE

IN VITRO ACTIVITY OF ETHANOLIC AND WATER EXTRACT OF GUAVA LEAVES AT VARIOUS CONCENTRATIONS AGAINST STAPHYLOCOCCUS AUREUS AND CANDIDA ALBICANS

Naveen Kumar P[1], Moonish Baabu S[2], Neathra M[3], R. Ganesh[4], M. Sasikala[5], B. Selvamani[6].

CRI[1,2,3], Professor and Head[4], Senior lecturer [5,6], Department of Public Health Dentistry, Priyadarshini Dental College and Hospitals, Thiruvallur, Tamilnadu, India.

ABSTRACT

AIM: To evaluate the antibacterial and antifungal efficacy of guava (Psidium guajava) leaf extracts (aqueous and ethanol) at 15% at 25% against Staphylococcus aureus and Candida albicans.

METHODOLOGY: Staphylococcus aureus and Candida albicans were used in the study. The strains were passaged in the nutrient agar environment and incubated for 24 h to have live and fresh strains for the test. Ethanolic and water extracts of guava leaves were prepared using a Soxhlet extractor. Two concentrations of 15% and 25% weight/volume of both extracts were prepared. Antimicrobial testing of extracts was done using the Agar well-diffusion method. Two plates each were prepared for both extracts. Chlorhexidine (0.2%) served as a positive control and distilled water as a negative control.

RESULT: Mean zone of inhibition produced by 15% and 25% ethanolic extract was 14 mm and 17.3 mm respectively against S. aureus and 14 mm and 17.6 mm respectively against C. albicans. Similarly, 15% and 25% water extract mean zone of inhibition was 12.6 mm and 14.6 mm respectively against S. aureus and 12.6 mm and 15.6 mm respectively against C.albicans. Statistical analysis of results using one-way ANOVA and post-hoc Tukey's test revealed that antifungal activity of 15% ethanolic extract and 15% water extract was significantly less than that of 0.2% chlorhexidine. But 15% ethanolic extract has similar antibacterial activity as that of 0.2% chlorhexidine. There was no statistical difference in efficacy of 25% ethanolic, 25% water extract of guava and 0.2% chlorhexidine in both organisms.

CONCLUSION: The ethanolic and water extract of guava leaves possess antibacterial and antifungal activity against S. aureus and C. albicans with 25% ethanolic and water extract being as efficacious as 0.2% chlorhexidine in both organisms. 15% ethanolic extract is as efficacious as 0.2% chlorhexidine only against S. aureus.

KEYWORDS: Antibiotic resistance, guava leaves, Staphylococcus aureus, Candida albicans

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 drug-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 drugs. 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 drugs, 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]

Corresponding Author: Dr. R. Ganesh, Professor and Head, Department of Public Health Dentistry, Priyadarshini Dental College and Hospital, Tiruvallur.

Email ID: ganesh.hodphd@indiraeducational.org

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 drugs. [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 dried in an oven at 60°C for 5 hours. The dried 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 dried in a rotary evaporator for 5-6 hours at 60°C. The dried 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

This study was conducted to assess the efficacy of Guava leaves on S. aureus and C. albicans using agar well-diffusion method. Mean zone of inhibition shown by 15% and 25% ethanolic extract, 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 efficacy (against S. aureus) of ethanolic extract of guava leaves at 15%, 25% concentration and 0.2% chlorhexidine was compared using one-way ANOVA ($F = 4.136$, $P = 0.074$). Results that showed a significant difference were further analyzed for statistical significance between specific groups using Tukey post-hoc analysis. There was no significant difference between the efficacy of 15% (14 ± 1 mm) and 25% (17.3 ± 1.527 mm) ethanolic extract and 0.2% chlorhexidine (17 ± 2 mm) ($F =$

4.136, P = 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]. Post-hoc analysis revealed that the 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 efficacy (against C. albicans) of ethanolic extract of guava leaves at 15%, 25% concentration and 0.2% chlorhexidine was compared using one-way ANOVA (F = 25.75, P = 0.001). Results that showed a significant difference were further analyzed for statistical significance between specific groups using Tukey post-hoc analysis [Table 3]. There was a significant difference between the efficacy of 15% (14 ± 0 mm) and 25% (17.6 ± 0.577 mm) ethanolic extract and 0.2% chlorhexidine (17 ± 1 mm) (F = 25.75, P = 0.001). On post-hoc analysis, it was revealed that the efficacy of 25% ethanolic extract (17.6 ± 0.577 mm) was not significantly different than 0.2% chlorhexidine (17 ± 1 mm) (P = 0.483). However, the efficacy of 15% ethanolic extract (14 ± 0 mm) was significantly lower than 0.2% chlorhexidine (17 ± 1 mm) (P =0.003) and 25% ethanolic extract (17.6 ± 0.577 mm) (P =0.001) [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]. Post- hoc analysis revealed that the 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

Leaves of Psidium guajava contain essential oil, Flavonoids, and saponins combined with oleanolic acid, Nerolidiol, β-sitosterol, and avicularin. Avicularin and its 3-l-4-pyranoside have been reported to have strong anti-bacterial action.[9] The flavonoid and tannic fractions from dried P. guajava leaves presented a relevant antifungal capacity. The study by Bezerra et al. reported that the combination between natural product and anti-fungal drug is efficient as it potentiated the action of the antifungal (Fluconazole), reducing its concentration and increasing its effectiveness. [10] Razak et al., reported that early plaque settlers were treated with 1 mg/ml of Psidium guajava leaf aqueous extract, which decreased the cell-surface hydrophobicity of Actinomyces sp., Staphylococcus mitis, and Staphylococcus sanguinis by correspondingly 54.1%, 49.9%, and 40.6%.[11] The antibacterial properties of guava leaf extracts were also documented by Vieira et al. in 2001, who reported that they prevented S. aureus from growing. [12] In 2012, Beatriz et al. in their research reported the activity of P. guajava leaf extracts (50 mg/mL) against various fungi. [13] This study aimed to assess the effectiveness of guava leaves, known for their high antibacterial and antifungal compounds, against S. aureus and C. albicans. The agar well- diffusion method, which has been shown to be more sensitive than other techniques like the disc diffusion method,[14] was used in this study for the microbiological assay. Staphylococcus aureus and Candida albicans are cultured in nutrient agar. Results of this study shown that 25% ethanolic and water extract is as efficient as 0.2% chlorhexidine in both organisms. 15% ethanolic extract has similar efficacy as 0.2% chlorhexidine only against S. aureus. It was discovered that the ethanolic extract at 15% and 25% was more effective than the water extract at 15% and 25%. The ethanolic and water extracts are not the same. While ethanolic extract has both flavonoids and tannins, water extract only has tannins or none. The varying solubility of different guava leaf components in water and organic solvents is the reason for the compositional discrepancy between the ethanolic and water extracts.[15] In this study, the antimicrobial activity of guava leaf extract against S. aureus and C. albicans was qualitatively assessed. However, more quantitative studies are required to determine the minimal inhibitory concentration and assess the safety and efficacy of guava extracts in vivo.

CONCLUSION

The ethanolic and water extract of guava leaves possess antibacterial and antifungal activity against S. aureus and C. albicans with 25% ethanolic and water extract being as efficacious as 0.2% chlorhexidine in both organisms. 15% ethanolic extract is as efficacious as 0.2% chlorhexidine only against S. aureus.

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