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ANTIBACTERIAL EFFECT OF METHANOLIC EXTRACT OF BETEL LEAF (*PIPER BETLE*) AGAINST *ENTEROCOCCUS FAECALIS*: IN-VITRO STUDY

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ABSTRACT

Background: The increasing interest in plant-derived medications has led to the exploration of various plants for their therapeutic properties. *Piper betle*, commonly known for its culinary and medicinal uses, possesses significant bioactive compounds that may exhibit antibacterial effects. **Objective:** This study aims to investigate the antibacterial properties of methanol extract of *Piper betle* against *Enterococcus faecalis.* **Materials and Methods:** Fresh *Piper betle* leaves were collected, cleaned, and air-dried. A methanolic extract was prepared using a Soxhlet apparatus. Different concentrations of the extract (1 mg/ml, 10 mg/ml, 100 mg/ml, and 1000 mg/ml) were tested for antibacterial activity using the well diffusion method on Mueller-Hinton Agar. The zone of inhibition was measured to assess antibacterial efficacy. **Conclusion:** The study demonstrated that the methanolic extract of betel leaves exhibited antibacterial effects against *Enterococcus faecalis.*

KEYWORDS: *Piper betle*, antibacterial properties, methanol extract, *Enterococcus faecalis*.

INTRODUCTION

The discovery of novel medicinal properties in various plant species has led scientists to focus on physiologically active compounds over the last few decades. This is due to the potent pharmacological activity and low to nonexistent toxicity of the bioactive compounds. The increased interest in plant-derived medications is mostly a result of the resistance brought on by the careless use of synthetic medicines and the persistent belief that green medications are less harmful than synthetic medications which may have serious side effects.[1]

The Piperaceae family includes *Piper betle L*. Chavicol, betal-phenol, and other phenolic chemicals are among the several chemical components found in betel leaves. It is well known that these ingredients have substantial antifungal and antibacterial qualities.[2] Recent research has shown that *P. betle* leaves possess antibacterial activity against a variety of Gram-negative and Gram-positive bacteria.[3] The leaves of the *Piper betle Linn*, or betel, have long been utilized in Indian traditional medicine. The leaves show antibacterial activity against oral bacteria such as *Streptococcus viridans, Staphylococcus aureus, and Streptococcus mutans.*[4]

Antibacterial property refers to the ability of a substance to inhibit the growth of bacteria or kill them, thus preventing infections. Plant leaves contain diverse compounds that exhibit antibacterial properties, serving as natural defences against pathogens. A crucial first step in oral disease is the alteration of the oral microbiota, whose composition is intimately linked to dental health. One of the bacteria that forms biofilms and is part of the oral microbiota, *S. mutans*, is regarded as the primary cause of dental decay.[5]

Failure of endodontic therapy can be caused by a variety of factors, including bacterial persistence, improperly cleaned and obturated root canals, faulty coronal seal (leakage), and untreated canals (missing canals). The presence of certain bacterial species, such as *Enterococcus (E.) faecalis*, within the root canal system is the primary cause of endodontic failure. A prolonged intra-radicular or extra-radicular infection results from those bacteria's increased resistance to disinfectants.[6]

Enterococcus faecalis is the major contributor to endodontic infections and failure of endodontic therapy. The increasing prevalence of antibiotic resistance necessitates the exploration of alternative treatments. Thus, the current study aims to investigate the antibacterial properties of methanol extract from betel leaf against *Enterococcus faecalis*.

MATERIALS AND METHODOLOGY

Betel Leaf Collection

Fresh betel leaves were harvested from a nearby garden and thoroughly cleaned under running water to remove any impurities. After washing, the leaves were allowed to air dry in shaded areas at room temperature. Once dried, the leaves were mechanically powdered to reduce them to fine particles for further use in the extraction process.

Methanolic Betel Leaf Extract

To prepare the methanolic extract, 100 grams of the grounded betel leaves were combined with 500 ml of 96% methanol in a Soxhlet apparatus. This mixture was then heated to 40°C and processed for eighteen hours to ensure efficient extraction of the bioactive compounds. Once the extraction was complete, the methanolic extract was dried in an incubator, resulting in a solid mass. Various concentrations of the methanolic betel leaf extract (1 mg/ml, 10 mg/ml, 100 mg/ml, and 1000 mg/ml) were then prepared and stored at 4°C for further experimentation.

Bacterial Strains

To evaluate the antibacterial properties of the betel leaf extract, microbial cultures of *Enterococcus faecalis* was prepared. These bacterial strains were obtained from the Microbial Type Culture Collection (MTCC) and grown in a nutrient-rich broth. A subculture of each microorganism was established by streaking the cultures onto Brain Heart Infusion (BHI) agar plates, which were then incubated at 37°C for 24 hours to allow for optimal growth.

Antibacterial Property

The antibacterial activity of the methanol extract of betel leaf was assessed using the well diffusion method on Mueller-Hinton Agar (MHA). Initially, 38 grams of the agar medium were suspended in 1000 ml of distilled water and heated with frequent agitation, followed by boiling to ensure complete dissolution. The medium was then autoclaved at 121°C for 15 minutes and cooled to room temperature before being poured into sterile Petri dishes to achieve a uniform depth. To prepare the bacterial isolates for testing, a 0.5 McFarland standard was prepared, and lawn cultures of *Enterococcus faecalis* was created on the MHA. Wells were punched into the agar, where 1 microliter of the various concentrations of the betel leaf extract was applied. Penicillin served as the control. The cultures were then incubated at 37°C for 16–18 hours, and the zones of inhibition around the wells were measured to evaluate antibacterial activity. Figure 1 shows a visual representation of *E.faecalis* on Mueller-Hinton Agar (MHA).

Figure 1: *E.faecalis* growth plate.

RESULTS

In the present study, the methanolic extract of betel leaves exhibited a zone of inhibition against *Enterococcus faecalis*. As shown in Figure 2, the zones of inhibition for the different concentrations are visible, with the largest zone observed at the highest concentration.

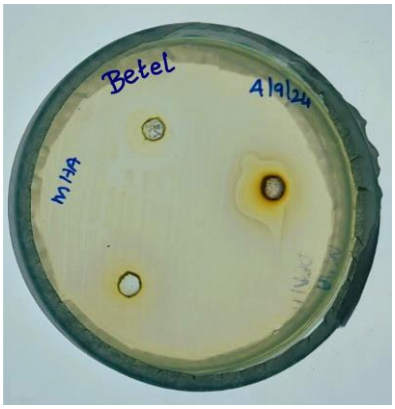

Figure 2: Zone of inhibition against *E. faecalis.*

DISCUSSION

The findings of this study underscore the significant antibacterial properties of methanol extract from betel leaves (*Piper betle*) against *Enterococcus faecalis*, which are critical pathogens associated with endodontic infections. The increasing zones of inhibition with higher concentrations of the extract suggest a dose-dependent relationship, which is consistent with the pharmacological principles of many natural products.

These findings have important ramifications, particularly in light of the growing prevalence of antibiotic resistance. The increasing ineffectiveness of synthetic antibiotics against certain bacterial strains necessitates the exploration of alternative treatments.

Enterococcus faecalis is a gram-positive bacterium that has been identified as a major pathogen in endodontic infections. Siqueira and Rocas (2005) highlighted that this organism is often resistant to conventional endodontic treatments, leading to treatment failures and persistent infections. The ability of *E. faecalis* to survive in the root canal system, even after treatment,

underscores the need for effective antimicrobial agents that can target this pathogen. Their review emphasizes the importance of developing alternative therapeutic strategies to manage infections caused by *E. faecalis*, which aligns with the objectives of our study.[7]

Nayaka and Sanjaya (2021) provided insights into the antibacterial properties of *Piper betle*, emphasizing its safety profiles and commercial applications in dental care.[8] The low toxicity of betel leaf extract and its significant antibacterial activity position it as a promising candidate for further research and development in clinical settings.

Nair and Chanda (2008) reported that betel leaf exhibits significant antimicrobial activity against several oral pathogens, including *E. faecalis*.[9] Research by Dwivedi and Mehta (2011) has identified a range of bioactive compounds in *Piper betel*, including aliphatic compounds that contribute to its antimicrobial properties. These compounds may disrupt bacterial cell membranes or interfere with metabolic processes, thereby inhibiting bacterial growth.[10] Additionally, Amonkar et al. (1991) reported the presence of hydroxychavicol, a phenolic compound in betel leaves, which has demonstrated antimutagenic properties and may also play a role in the antibacterial activity observed in this study.[11]

The traditional use of betel leaves in various cultures for oral health maintenance is supported by scientific evidence. The antimicrobial properties of betel leaves have been recognized in traditional medicine practices, where they are used to promote oral hygiene and prevent dental issues. This aligns with the findings of Pin et al. (2010), who demonstrated the antioxidant and anti-inflammatory activities of betel leaf extracts, further supporting their role in oral health.[12] This study is limited to in vitro evaluations. Future research should focus on in vivo studies to assess the clinical efficacy of betel leaf extract.

The results of this study are consistent with findings from other investigations that have documented the antimicrobial effects of *Piper betle* extracts. For instance, Agarwal and Singh (2012) evaluated the antimicrobial activity of various *Piper betle* cultivars and found significant inhibition of bacterial growth, reinforcing the potential of this plant as a natural antibacterial agent.[13] Furthermore, Chakraborty et al. (2011) reported the antioxidative and antihemolytic activities of betel leaf extracts, suggesting a multifaceted mechanism of action that could enhance its therapeutic efficacy.[14]

This study highlights the potential of betel leaf extract as a natural antibacterial agent, particularly in the field of dentistry. Further research is warranted to isolate and characterize the specific bioactive compounds responsible for the antibacterial activity and to evaluate their effectiveness in clinical applications. The development of formulations incorporating betel leaf extract could pave the way for innovative approaches to oral health care, addressing both therapeutic and preventive needs.

CONCLUSION

In conclusion, the methanol extract of betel leaf (*Piper betle L.)* demonstrated significant antibacterial activity against *Enterococcus faecalis*, indicating its potential as a natural remedy for oral health issues. The results support the traditional use of betel leaves in various cultures and highlight the need for further research to isolate and characterize the specific bioactive compounds responsible for this activity. Future studies should focus on in vivo evaluations and the development of formulations that can be effectively used in clinical settings, particularly in dental care. The findings of this study contribute to the growing body of evidence supporting the use of plant-derived medicines in modern healthcare practices.

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