Research Article

Antibacterial Efficacy of *Psidium Guajava* Leaf Extract on *E. feacalis – In Vitro* Study

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Abstract

Background: Debridement and disinfection of the root canal system is the most important and critical step in endodontic treatment. Most of the irrigants presently used in the endodontic treatment can have an impact on the microbes surviving in the bio ilm but none of them are able to do all of the required tasks and extrusion of few irrigant causes side effects. Research is going on its full swing in order to produce an endodontic irrigant having ideal properties. **Aim:** The aim of this study is to evaluate the antibacterial efficacy of *Psidium guajava* against *Enterococcus faecalis*. **Materials & Methods:** The dried and powdered *Psidium guajava* leaves were taken. This powder was used for preparing ethanolic and water extracts at 5% and 20%. And the extracts were subjected to test for their antibacterial efficacy against *Enterococcus faecalis*. **Results:** Results showed that 20% ethanolic guava leaf extract was better than other ehanalolic and water extracts, and with the control groups. **Conclusion:** This study concluded that this 20% ethanolic extract can be used as an endodontic irrigant as this possess antibacterial efficacy similar to that of chlorhexidine.

Keywords: Antibacterial; Endodontic irrigant; Microorganism; Root canal disinfection

Introduction

The infected root canal is a source of aerobic, anaerobic, gram negative and gram positive organisms; hence it is polymicrobial in nature.

The main objectives of root canal therapy are cleaning, shaping and obturating of the root canal system in a three dimensional manner and to prevent the reinfection. [1,2]

The root canal therapy aims at removal of diseased tissue, elimination of microorganisms present in the canals and dentinal tubules and prevention of recontamination after the treatment.

Root canal debridement may leave many areas of the root canal untouched by the instruments thus a root canal irrigant is needed to aid in the debridement of the canals.

This untouching of the instruments during cleaning and shaping occurs mainly due to the variations and complexities in the root canal. [3–5]

Endodontic infections are caused by oral microorganisms, which are mostly opportunistic pathogens that may invade the root canal containing necrotic tissue and establish an infectious process. [6,7]

The number of facultative anaerobic bacteria increases when the root canal remains infected for long periods. [8] *Enterococcus faecalis*, a facultative anaerobic gram positive coccus, is the most common Enterococcus species that is cultured from non-healing endodontic cases. [9–11]

Successful root canal therapy relies on the combination of proper instrumentation, disinfection and obturation of the root canal. Of all these essential steps, disinfection of the root canal is the major determinant in the healing of periapical tissues. [12,13] Infection of the root canal at the time of obturation has a negative influence on the prognosis of endodontic therapy. [14]

Currently, endodontic infections are treated by mechanical debridement followed by chemical disinfection. Irrigants are used in endodontic treatment to flush out the loose debris, lubricate the dentinal walls and dissolve organic matter in the canal and to provide antimicrobial activity. [15,16]

The current methods of root canal cleaning and shaping produce a smear layer [17,18], containing inorganic and organic

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substances, microorganisms and necrotic material. [19] One of the most widely used endodontic irrigants is sodium hypochlorite.

In spite of its advantages like broad antimicrobial spectrum, strong and fast oxidizing ability, easy to use, cheap etc, it suffers from several drawbacks like unpleasant odour and taste and the most of all aggressiveness versus host soft tissues, irritant to periapical tissues, stains instruments, inability to remove smear layer, burning of surrounding tissues and reduction in elastic modulus and flexural strength of dentin. [20–23]

Chlorhexidine is a broad spectrum of antimicrobial agent that has a substantive antimicrobial activity and relatively low toxic effects; it does not dissolve organic tissues. [24,25] *In vitro* studies have shown that there will be sustained antimicrobial activity in the root canal for some time even after using chlorhexidine as irrigant. The advantages of chlorhexidine are it is unique to bind to dentin and effectiveness of antimicrobial agents and substantivity in root canal. [26]

The *Psidium guajava* is a phytotherapic plant commonly known as guava. The leaves of *P. guajava* Linn are reported to have anti-allergy, antioxidant, hepatoprotective, antimicrobial, antigenotoxic, antiplasmodial, cytotoxic, antispasmodic, cardioactive, anti-cough, antidiabetic, antinociceptive and anti-inflammatory activities. [27–30] Leaves of guava tree are rich in source of flavonoids, especially quercetin which is mainly responsible for antibacterial activity. It contains tannins which are found to be effective against many bacteria like *Escherichia coli*, *Staphylococcus aureus*, etc. [31,32] Previously our team has a rich experience in working on various research projects across multiple disciplines. [33–47] Now the growing trend in this area motivated us to pursue this project. We have numerous highly cited publications on well-designed clinical trials and lab studies. [48–62] The present study was carried out with the hypothesis that the guava leaves possess antibacterial efficacy against *Enterococcus faecalis*. The aim of this study was to compare the antibacterial efficacy of ethanolic and water extracts of guava leaves at two different concentrations, 5% and 20% against *E. faecalis*.

Materials and Methods

The *Psidium guajava* leaves were obtained, cleaned, dried and powdered for making the ethanolic extract of 5% and 20% and water extracts of 5% and 20%.

Preparation of extracts

Aqueous extract: For preparing extract, 10 gm of the powdered sample was mixed with 120 mL of sterile distilled water and was added, and agitated (130 rpm) overnight at 20°C in a temperature-controlled bioshaker.

The aqueous fraction was separated with the use of sterile cheesecloth and filtered through sterile Whatman filter paper (no. 2). All the extracts were then concentrated with a rotary vacuum evaporator at 40°C and the concentrated extracts were diluted to 10 mg/mL, sterilized, and kept at 20°C until use.

Ethanolic extract: About 100 gm of the powdered sample was mixed with 1000 ml of ethanol and kept for 48 hours at room temperature to ensure maximum metabolite extraction. The extract obtained was filtered and concentrated.

The extraction method is based on the solubility of the constituents of the sample in ethanol.

The filtrate is placed into the thimble of the Soxhlet extraction apparatus chamber. The samples were extracted at 4 cycles per hour for 12 hours.

After extraction, the solvent was removed by the means of a rotary evaporator, to yield the extracted compound. The final concentration was maintained as 100 mg/ml by redissolving the crude extracts in 10% dimethyl sulfoxide for bioassay analysis.

Sterility test of the plant extract: The extracts were tested for growth or contamination. This was carried out by inoculating 100 µl each of them on nutrient agar and incubated at 37ºC for 24 hours.

The plates were observed for growth. No growth in the extract treated plates after incubation indicates that the extracts were sterile. The extracts were then accessed for antimicrobial activity.

Microorganism

A pure culture of test strain of *E. faecalis* ATCC 29212 was inoculated in sterile nutrient broth. The presence of *E. faecalis* was confirmed in the nutrient broth by pipetting 10 microliter of the broth and observing its presence under microscope. Nutrient broth inoculated with *E. faecalis* was transferred in a sterile container and used for the experiments.

Antimicrobial testing

Well diffusion test: Testing samples were classified into 6 groups. Group I: Ethanol (50 µl); Group II: 5% Guava leaf Ethanolic Extract (GEE); Group III: 20% GEE; Group IV: 5% guava leaf aqueous extract (GE); Group V: 20% GE; Group VI: CHX.

Application of test samples: The wells were created on the E. faecalis inoculated Mueller Hinton agar plate and then filled with 50 µl of the test samples.

The wells were created about equidistant to each other to avoid the overlapping of the inhibition zone. Then, the plates were inverted and incubated for 24 hours at 37°C.

The diameter of the inhibition zone around the treated wells or around the control wells were measured for the antibacterial activity assessment.

If present, their diameters were measured to the nearest whole millimetre with a ruler. All tests were carried out three times to ensure reliability, and the average of the three replicates for each test samples were calculated [Figure 1].

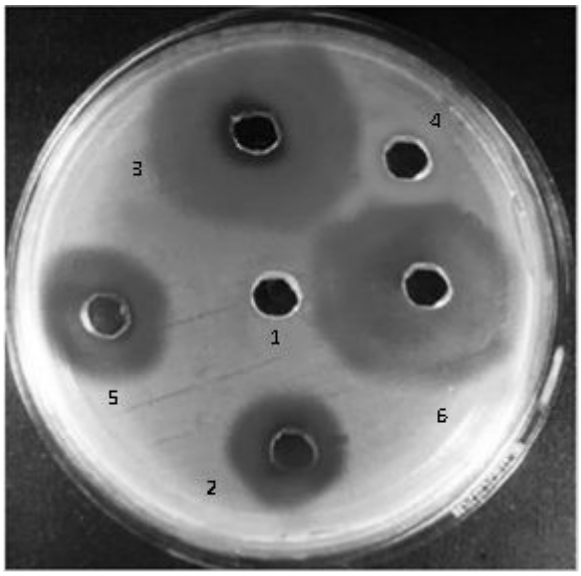

Figure 1: Zone of inhibition against *E. faecalis.* 1: Ethanol, 2: GEE 5%, 3: GEE 20%, 4: GE 5%, 5: GE 20%, 6: CHX.

Statistical analysis

For statistical analysis of data, multiple comparisons were performed using one-way analysis of variance (ANOVA) followed by the LSD test for post hoc analysis. Statistical significance level was accepted at P<0.05. Data were analyzed using SPSS (version 22.0).

Results

The present study was conducted to evaluate the efficacy of guava leaves against *E. faecalis* using agar well diffusion method. This study results showed that there is a significant difference in all groups when compared with a negative control. The GE 5% (water extract) showed no effect against *E. faecalis*. The CHX 2% showed the zone of inhibition about 19.8+1.4 mm. whereas the GEE 20% showed zone of inhibition about 17.4+0.56 mm [Table 1].

Table 1: Inhibitory zone of the microorganism. NI means no inhibition zone. Each value is expressed as mean+SD (n=3). *: p<0.05 as compared with negative control.

Samples	E. faecalis Zone of inhibition (mm)
Ethanol	NI
GEE 5%	7.8+0.48*
GEE 20%	17.4+0.56*
GE 5%	NI
GE 20%	8.6+0.47*
CHX 2%	19.8+1.4*

Discussion

In this study extracts of *P. guajava* leaves were tested against *E. faecalis*. These *P. guajava* leaves had been reported to contain essential oils, flavonoids, nerolidiol, saponins, β-sitosterol, ursolic, crategolic and guayavolic acid, which are reported to have strong antibacterial action.

Phenolic compounds protect plants, fruits and vegetables from oxidative damage and they have been used as antioxidants by humans. The most suitable solvents are aqueous mixtures containing ethanol, methanol, acetone, and ethyl acetate. The presence of various antioxidant compounds with different chemical characteristics and polarities may or may not be soluble in a particular solvent. [63]

Ethanol has been known as a good solvent for polyphenol extraction which is also safe for human consumption. Generally methanol has been found to be more efficient in extraction of lower molecular weight polyphenols, whereas aqueous acetone is good for extraction of higher molecular weight flavanols. Thus in this study both ethanolic and aqueous extracts have been used to evaluate the antioxidant property.

Prabu et al, demonstrated a flavonoid in a methanolic extract of guava leaves which showed antibacterial effect against caries causing *S. mutans*. Thus this study is attempted to evaluate the antibacterial against *E. faecalis*. [64]

Deepika et al, demonstrated the antibacterial activity of *P. guajava* leaves against *Lactobacillus acidophilus*, where there is no statistical difference between chlorhexidine and 20% ethanol extract against *L. acidophilus*. [65]

Dutta et al, demonstrated the anti-inflammatory effect of the leaves of *P. guajava* Linn on experimental animal models, where they conclude that the ethanolic extract of guava leaves has an anti-inflammatory activity.

Jang et al, also demonstrated the anti-inflammatory effect of *P. guajava* leaf extract both *in vitro* and *in vivo*, which showed that these extracts inhibit the secretion of inflammatory mediators. [66] Our institution is passionate about high quality evidence based research and has excelled in various fields. [67–73] We hope this study adds to this rich legacy.

In this present study, the group 1 (ethanol) and group 4 (5% GE) showed no effect on *E. faecalis*. There is a significant difference between chlorhexidine 2% and GEE 5%, GEE 20% and GE 20%. Though both the ethanolic extracts showed the antibacterial activity against *E. faecalis*, GEE 20% seemed to have antibacterial efficacy significant to that of chlorhexidine. However, furthermore studies are needed to know about the minimum inhibitory concentration, anti-inflammatory reaction and to evaluate safety and effectiveness of this extract *in vivo*.

Conclusion

The 20% water and both ethanolic extracts showed antibacterial activity against *E. faecalis*, with 20% ethanolic extract is being as effective as chlorhexidine. Further studies are required to enhance the use of this antibacterial potential of this extract in endodontic practice.

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