

Antibacterial Efficacy of Muringa Seed Extract and Potato Peel Extract Against *Enterococcus faecalis*

Abstract

Background: Elimination of infection and prevention of reinfection should be the main goal in the treatment of apical periodontitis. The most challenging part of endodontics is the complete disinfection of root canal system. Herbal alternatives have emerged as the more biofriendly approach in root canal irrigation and disinfection. Aim and Objective: The aim of this study was to evaluate the antimicrobial efficacy of natural extracts like muringa seed and potato peel extract against *Enterococcus faecalis*. **Materials and Methods:** The antimicrobial activity was determined using agar diffusion test. The solutions were divided into three groups: Group I- Muringa seed extract, Group II- potato peel extract, and Group III-5.25% sodium hypochlorite. The zones of inhibition of growth were recorded. The strain used for this study was *Enterococcus faecalis* ATCC 29212. **Results and Conclusion:** Muringa seed extract (Group I) demonstrated the best result among the tested solutions. Although there was no significant difference between potato peel extract (Group II) and 5.25% sodium hypochlorite (Group III). Within the limitations of this study, herbal extracts tested has shown significant antimicrobial action against *Enterococcus faecalis*.

Keywords: Antimicrobial efficacy, herbal irrigants, muringa seed extract, potato peel extract

Introduction

The primary focus of endodontic therapy should be the reduction and elimination of microbial flora from the root canal system, which will promote normal healing and reestablishment of health. Effective disinfection in endodontics can be achieved only by augmenting mechanical preparation with antimicrobial agents. Regardless of the use of stainless steel or nickel–titanium instruments, irrigating solutions and intracanal medicaments have a role in eradicating microorganisms effectively.^[1]

The role of irrigation in endodontic treatment cannot be overemphasized. Irrigants prevent the packing of soft and hard tissues in the apical part of the root canal and help in the reduction of bacterial load.^[2] A variety of chemicals have been promoted for this purpose. Sodium hypochlorite (NaOCl), with its unique capabilities, is the gold standard for endodontic irrigation, even though its optimum working concentration has not been universally agreed upon. It is a strong proteolytic and antimicrobial agent.

However, adverse effects of NaOCl have been reported, including unpleasant odor and taste, toxicity, possible paresthesia of the mandibular nerve, allergy, and an increase in coronal microleakage of adhesive restorations.^[3]

Toxicity and harmful side effects of the commonly used antibacterial irrigants have necessitated the need for alternative agents which are nontoxic, effective, and affordable. Recently, researches have shifted toward the use of herbal irrigants. Herbal alternatives have become popular mainly due to their easy availability, cost-effectiveness, increased shelf life, low toxicity, and environment friendliness.

Moringa oleifera is the most widely cultivated species of the genus *Moringa* in the family *Moringaceae*. It is a fast-growing, drought-resistant tree that is native to the southern foothills of the Himalayas in North-Western India but widely cultivated in tropical and subtropical areas.^[4] With its high nutritional value and an impressive range of medicinal uses, it is widely used for treating bacterial and fungal infections, inflammation, diarrhea, and malnutrition. Significant scientific

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researches have gone into the reported antibacterial activity of this tree.^[5]

Solanum tuberosum, known as potato, is a herbaceous, perennial shrub plant <1 m tall. The underground stem of this plant, which is a tuber, contains a large amount of starch. *Solanum tuberosum* peel contains ferritin, Vitamin C, riboflavin, alpha-tocopherol, flavone aglycones, quercetin, glutathione, etc., Furthermore, research has demonstrated analgesic, antioxidant, and antibacterial properties on some bacteria and blood pressure lowering, sputum collecting, muscle strengthening, and appetizing properties of potato.^[6]

Facultative organisms such as *Enterococcus faecalis* (Gram-positive bacteria) are the most resistant species and one among the possible causes of root canal treatment failure. These are commonly detected in asymptomatic and persistent endodontic infections. Compared with most other microbes, *E. faecalis* is considerably resistant to the common intracanal irrigants.^[7] The overly complex nature of the organism poses a great challenge for endodontists.

Aim

The aim of the study conducted was the comparative evaluation of the antimicrobial efficacies of the irrigants such as (1) Moringa oleifera seed extract (2) potato peel extract, and (3) 5.25% sodium hypochlorite, against *E. faecalis*.

Materials and Methods

This *in vitro* study was conducted at the Department of Microbiology, University campus, Palayad, Kannur. Moringa seed extract (Group I) and potato peel extract (Group II) were the experimental groups and 5.25% sodium hypochlorite (Group III) was the positive control group. ATCC 29212 strain of *E. faecalis* was used in this study to check the antimicrobial activity.

Collection, extraction, and preparation of the standard compounds

Preparation of moringa seed extract

Seeds of *M. oleifera* plant of pharmaceutical grade grown organically without the use of pesticides were collected. Authentication of seed was done at the Department of Microbiology at Palayad University campus.

The fleshy part of the moringa seeds was scraped out using a bp blade. These were then crushed with mortar and pestle and packed in an airtight plastic container. Aqueous extract was prepared by dissolving this paste in sterile distilled water in a ratio of 1:5, i.e., 40 g of plant paste material in 200 ml of water, in a sterile 250 ml flask. This was kept in a refrigerator at 4°C for 24 h and was then filtered using filter paper. The extract was then again kept in the refrigerator at 4°C, for further use.

Preparation of potato peel extract

The potatoes were washed and rinsed with distilled water and manually peeled to a depth of 1 mm. The peels were dried at 45°C for 24 h and then powdered. The aqueous extract was prepared by suspending 40 g of powder in 200 ml of distilled water. The resulting extract was decanted and filtered through a Whatman filter paper. The extract was again kept in the refrigerator at 4°C, for further use.

Agar-diffusion test

E. faecalis strains were inoculated in brain–heart infusion and incubated at 37°C for 24 h. Microbial cells were resuspended in saline to give a final concentration of 1.5×10^8 cells/ml, like that of tube #0.5 of the McFarland scale. For the agar diffusion test, Petri plates with 20 ml Mueller-Hinton Agar (Microexpress – A Division of Tulip Diagnostics [P] Ltd., Goa) were inoculated with 0.1 ml of the microbial suspensions. Five cultivated agar plates were taken and three holes (4 mm in depth, 6 mm in diameter) were punched. Moringa seed extract, potato peel extract, and 3% sodium hypochlorite were filled separately in these three holes of all the five plates. These plates were reincubated aerobically at 37°C for 24 h. Then, the diameter of microbial inhibition zones around each well was measured and recorded in millimeters.

Statistical analysis

The evaluation of antimicrobial properties was done by measuring the diameters of zones of inhibition using the agar well diffusion method against *E. faecalis*. One-way analysis of variance was performed to compare three groups followed by *post hoc* Tukey test.

Results

Inhibition zone obtained with the three samples is shown in figure 1.

Table 1 shows that there exists a significant difference between the diameters of zones of inhibition of bacterial growth obtained for Moringa seed potato peel extract and 5.25% sodium hypochlorite.

Table 2 shows post hoc tests – Tukey’s honestly significant difference for the inter comparison of the antimicrobial efficacy of three groups against *E. faecalis*.

Post hoc test revealed a statistically significant difference between moringa seed extract and two other groups but no significant difference between potato peel extract and sodium hypochlorite.

Graph 1 shows the mean of diameter of zone of inhibition formed which was recorded in millimeters.

Discussion

The success of endodontic treatment depends on the eradication of microbes from root canal systems and the

prevention of reinfection. Employment of mechanical instrumentation is the core method for bacterial reduction in the infected root canal, but achieving bacteria-free root canals still proves to be a difficult task. Effective disinfection in endodontics is achievable only with a combination of mechanical instrumentation and antimicrobial agents.

E. faecalis is the most commonly implicated microorganism in asymptomatic, persistent endodontic infections, with a prevalence of 24%–77%.^[8] Furthermore, *E. faecalis* is found in 4%–40% of primary endodontic infections. This organism can form a biofilm that helps it to resist destruction by enabling the bacteria to become 1000 times more resistant to phagocytosis, antibodies, and antimicrobials, than

nonbiofilm-producing organisms. *E. faecalis* in dentinal tubules has been shown to resist intracanal dressings of calcium hydroxide for over 10 days. This microorganism has overcome the challenges of root canal therapy and so its elimination from the dental apparatus may well define the future of the endodontic specialty.

This study has used 5.25% sodium hypochlorite as the positive control, which is considered as the gold standard endodontic irrigant because of its antimicrobial activity and tissue dissolving capacity. Tissue-dissolving action of sodium hypochlorite is directly proportional to its concentration.^[9] Major disadvantages of this irrigant are its cytotoxicity when injected into periradicular tissues, foul smell and taste, ability to bleach clothes, and cause corrosion of metallic instruments. Furthermore, it neither kills all bacteria nor removes the smear layer completely. It also alters the properties of dentin.^[10]

The discovery of novel antimicrobial agents is very important for the control of pathogenic microbes, especially for the treatment of infections caused by resistant microbes. Medicinal herbs with antimicrobial activities are considered a potent source of novel antimicrobial function. To the best of our knowledge, this is the first study in endodontics that has evaluated the efficacy of Muringa seeds and potato peel extract against *E faecalis*. This study entails the important antimicrobial activity of the Moringa seed extract in the inhibition of *E. faecalis* as a root canal pathogen.

The results of the study show that muringa seeds have superior antibacterial efficacy than 3% sodium hypochlorite. Muringa contain bioactive substances whose antibacterial potentials are highly comparable with that of antibiotics used in sensitivity tests.^[11] Phytochemical analysis of Moringa species reveals that this plant family is rich in compounds containing the simple sugar rhamnose, carotenoids, alkaloids, polyphenols, flavonoids, anthraquinones, coumarins, tannins, triterpenes, sterols, saponins, etc.^[12]

The seeds of muringa contain unique compounds such as N-4-(α -L-rhamnopyranosyloxy) benzyl carbamate, 4-(β -

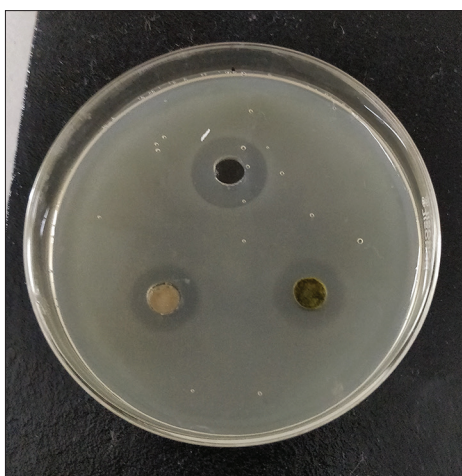


Figure 1: Inhibition Zone in three samples

Table 1: One-way analysis of variance of diameter of zone of inhibition

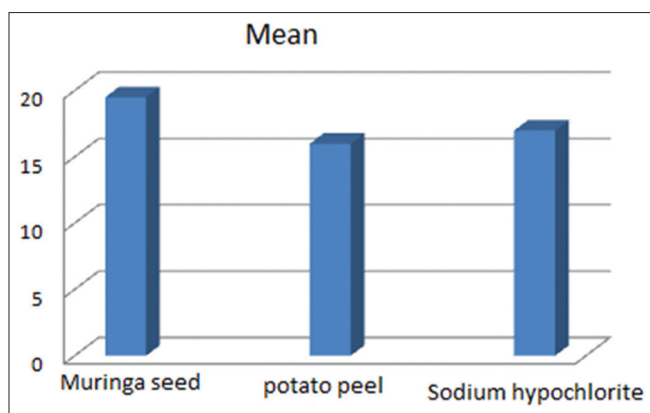
	Sum of squares	Df	MS	F	Significance
Between groups	31.633	2	15.817	13.000	0.001
Within groups	14.600	12	1.217		
Total	46.233	14			

ANOVA: Analysis of variance; SS: Sum of squares; Df: Degrees of Freedom; MS: Mean square

Table 2: Intergroup comparison by post hoc tukey’s test

VAR00001 (I)	VAR00001 (J)	Mean difference (I-J)	SE	Significant	95% CI	
					Lower bound	Upper bound
1.00	2.00	3.30000*	0.69761	0.001	1.3610	5.2390
	3.00	2.80000*	0.69761	0.005	0.8610	4.7390
2.00	1.00	-3.30000*	0.69761	0.001	-5.2390	-1.3610
	3.00	-0.50000	0.69761	1.000	-2.4390	1.4390
3.00	1.00	-2.80000*	0.69761	0.005	-4.7390	-0.8610
	2.00	0.50000	0.69761	1.000	-1.4390	2.4390

*The mean difference is significant at the 0.05 level. CI: Confidence interval; SE: Standard error



Graph 1: Represents the mean of diameter of zone of inhibition around each ingredient which was recorded in millimeters

D-glucopyranosyl-1→4- α -L-rhamnopyranosyloxy)-benzyl thiocarboxamid, 4-(α -L-rhamnopyranosyloxy) benzyl isothiocyanate, and Flo and Mo-CBP3. These alkaloids have demonstrated different antimicrobial activities.

Coelho *et al.* have identified the presence of antimicrobial peptides such as lectin in *M. oleifera* seed.^[13] The flocculation cationic polypeptide showed antibacterial activity by damaging bacterial cell membranes and causing fusion between the membranes. All of these could contribute to the superior antibacterial property of Muringa seed.

The present study has shown that potato peel extract exhibits antibacterial activity against *E. faecalis*, but the zone of inhibition formed was less when compared to other groups. There are several compounds in potato peel extract including alpha-tocopherol, flavone aglycones, chlorogenic acid, anthocyanins, and glutathione that have antioxidant properties.^[6] The antimicrobial action is because of the presence of phenolic compounds, flavones, and anthocyanin. In a study conducted by Bontempo *et al.*, anthocyanin compounds have shown a strong antibacterial effect against Gram-positive bacteria.^[14]

Kumar *et al.* could demonstrate the presence of steroidal alkaloids such as solanine and solasodine and all these alkaloids have antibacterial properties and cause growth inhibition of bacterial strains,^[15] which is consistent with the findings of the present study.

In the light of this study findings, potato peel extract exhibits good antibacterial properties against the Gram-positive bacteria *E. faecalis*.

For developing novel agents, it is important to promote the isolation and identification of certain molecules with antimicrobial activity, from these herbal agents. Evaluation of the safety and toxicity of antimicrobial agents is necessary before implementing the use of these compounds. Antibacterial effect of methanolic extract of herbal agents on *E. faecalis* was found to be most effective during the

first few hours. Hence, knowledge about the antimicrobial activity of compounds present in these agents and their interactions is required for the development and their use as irrigants in endodontics.

Conclusion

Within the limitations of this study, the herbal extracts tested have demonstrated significant antibacterial action. Muringa oleifera seed extract has better action against *E. faecalis* than 5.25% sodium hypochlorite. It appears that *M. oleifera* plant has a promising future in the control of infectious pathogenic bacteria and can be used in the discovery of novel antibacterial agents.

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Conflicts of interest

There are no conflicts of interest.

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