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Comparative Evaluation Of Antimicrobial Efficacy Of Moringa Oleifera Extract and Calcium Hydroxide Against E Faecalis

Research Article

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Abstract

Introduction: Cleaning and shaping of the canal is an important step in endodontic treatment for achieving a sterile environment. The most persistent organism that usually leads to endodontic failure is Enterococcus faecalis. Moringa Oleifera is an herbal product whose extracts have shown considerable antimicrobial property. Thus, the present study was conducted to evaluate the antimicrobial efficacy of Moringa Oleifera leaf extract against E. faecalis and compare it with Calcium Hydroxide which is currently the gold standard.

Materials and Method: The leaf extracts of Moringa Oleifera were obtained using the Soxhlet extraction method and its antimicrobial efficacy was tested using the Disc Diffusion test. Statistical analysis of the result was performed using ANOVA test.

Results: The results of the present study have revealed that 100µg/ml concentration of Moringa Oleifera leaf extract (11.89 ± 0.43mm) has higher antimicrobial efficacy against E faecalis as compared to calcium hydroxide (8.56 ± 0.73mm) while a concentration of 25µg/ml (1.05 ± 0.01mm) was not as effective as calcium hydroxide in inhibiting the growth of E faecalis.

Conclusion: The antimicrobial effect of Moringa Oleifera leaf extract against E. faecalis makes it a potential herb that could be further investigated for its use as an intracanal medicament.

Keywords: Moringa Oleifera; Medicinal Herbs; Root Canal Medicaments; Calcium Hydroxide; Enterococcus Faecalis.

Introduction

Root canal infections are poly microbial, mainly dominated by obligatory anaerobes. The most frequently isolated microorganisms from an intraradicular endodontic infection includes, gram negative anaerobic rods, spirochetes, Fusobacterium, Tannerella forsythia, Dialister species, gram positive anaerobic rods and gram positive cocci. The extraradicular endodontic infection is mainly dominated by anaerobic bacteria [1].

Cleaning and shaping of the canal is imperative to obtain a sterile environment during an endodontic treatment. However, some microorganisms may survive even after adequate cleaning of the canal [2]. Intracanal medicaments are placed in the root canals between appointments to eliminate persistent microorganisms that remain even after instrumentation and to reduce inflammation of the periapical tissue. Calcium Hydroxide as an intracanal medicament has been the gold standard for years [3, 4]. However,

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literature states that it is less effective against E. faecalis and C. albicans [5, 6] E. faecalis and C. albicans are the most commonly observed pathogens in a retreatment endodontic case [7]. The use of chemical medicaments is associated with certain disadvantages such as side effects and cytotoxic reactions. Owing to the low toxicity and side effects of herbal products, they have gained popularity amongst researchers who are trying to study their benefits over chemical compounds in the field of medicine [8].

For centuries, herbal products have been used to treat and cure diseases [9] Medicinal plants are a source for natural products that contain biologically active compounds such as alkaloids, flavonoids, coumarins, triterpens, phytoesters, tannins and vitamins. Neem, Tulsi, Triphala, Green tea, Turmeric are a few amongst the many herbs whose extracts have been tested for their antibacterial activity. In dentistry, Phytomedicine finds its application in dentifrices, mouth wash, irrigants and intra canal medicament [10, 11].

Moringa Oleifera also known as drumstick plant, is once such herb that is native to India. Its extracts have revealed promising results when studied for anti-inflammatory, antifungal and antibacterial properties. These properties have been attributed to different parts of the plant such as leaves, roots, seeds, fruit, flower and unripe pods [12].

Previously our team has a rich experience in working on various research projects across multiple disciplines [13-27] Now the growing trend in this area motivated us to pursue this project. In the present study, the extract from the leaves of Moringa Oleifera have been tested for its antimicrobial activity against E. faecalis and its efficacy has been compared against the gold standard calcium hydroxide medicament.

Materials and Method

Extraction Method

The leaves of the Moringa Oleifera plant were washed with distilled water, dried in shade and then grinded to powder. About 200g of the powder were separately soaked in 400ml of 95% Ethanol and allowed to stand for 7 days. The ethanol was then filtered using What man No1 filter paper. The filtrate was placed in to the thimble of the Soxhlet extraction apparatus chamber. The extraction of the sample was carried out for 12 hours at 4 cycles per hour. The solvent was removed by the means of a rotary evaporator after extraction, yielding the extracted compound. The final concentration was maintained as 100µg/ml by re-dissolving the crude extracts in 10% dimethylsulfoxide for bioassay analysis and fractionated in to 100µg/ml, 50µg/ml and 25µg/ml concentrations needed for the bioassay.

Sterility Test Of The Plant Extract

The ethanolic extract of the plant was tested for growth or contamination. This was carried out by inoculating 1ml each of them on nutrient agar and incubated at 37°C for 24hours. The plates were observed for growth. The sterility of the extract was indicated by no growth in the extract after incubation. The extracts were then accessed for antimicrobial activity.

Table 1. Values of zone of inhibition shown as mean and standard deviation NI means no inhibition zone.

Extract Conc (µg/ml)	E. faecalis (Zone of inhibition)
25	1.05 ± 0.01
50	4.01 ± 0.31
100	11.89 ± 0.43
Calcium Hydroxide	8.56 ± 0.73
Negative Control	NI

Figure 1. Petri Plates before Incubation.

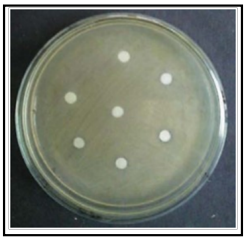
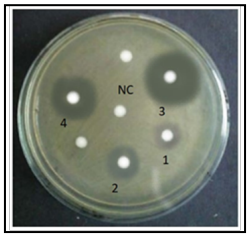


Figure 2. Petri Plates after Incubation Indicating Zone of Inhibition. NI - No inhibition zone; NC - Negative control; 1 - MOE (25µg); 2 - MOE (50µg); 3 - MOE (100µg); 4 - Ca(OH)2.



E. Faecalis Broth

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

Antimicrobial Susceptibility Testing

Disc diffusion test was the method adopted for studying the antimicrobial susceptibility. For the preparation of impregnated discs, a stock solution of plant extract was prepared by dissolving 100µg of extract with one ml of their respective solvents and 6mm of blank discs were then impregnated with the extract. Distilled water and dimethyl sulfoxide-loaded discs were used as negative controls for ethanolic extract respectively. All impregnated discs were ensured to be fully dried in 45^0C incubator for 18 to 24 hours prior to the application of bacteria. The standard 10% Calcium Hydroxide was used as positive control. Using sterile forceps, the discs which had been impregnated with plant extracts were applied on the inoculated Mueller Hinton agar once it had completely dried. To ensure uniform contact with the agar surface, the disks were pressed gently against it. Further more, each one of the test plates was composed of seven discs which placed about equidistant to each other to avoid the overlapping of the inhibition zone. Then, for 24 hrs, the plates were inverted and incubated at 37°C. To assess the antibacterial activity, the diameter of the inhibition zone around the treated discs and around the control discs were measured. If the inhibition zone was present, their diameters were measured with a ruler to the nearest whole millimetre. All tests were carried out three times to ensure reliability, and the average of the three replicates for each extract, and standard were calculated.

Statistical Analysis

The statistical analysis of data was performed using one-way analysis of variance (ANOVA) for comparisons followed by the post hoc analysis. Level of significance was set if $p < 0.05$. Data were analyzed using SPSS.

Results

The results of the disc diffusion test revealed that the leaf extract of Moringa Oleifera at a concentration of 25 (µg/ml) gave the lowest mean zone of inhibition measuring 1.05 ± 0.01mm, while a concentration of 100 (µg/ml) gave the highest mean zone of inhibition measuring 11.89 ± 0.43mm. The mean zone of inhibition obtained from Calcium hydroxide measured 8.56 ± 0.73mm (Fig 1 and 2) (Table 1). Negative control gave no zone of inhibition. The antimicrobial activity of Moringa Oleifera leaf extract at three different concentrations was statistically significant when compared with the antimicrobial activity of Calcium Hydroxide. (Table1).

Discussion

Our institution is passionate about high quality evidence based research and has excelled in various fields [17, 28-37].

With the introduction of calcium hydroxide by Hermann in 1920, it has widely been used as an intracanal medicament for treatment of cases with apical periodontitis [38]. There have been several studies to assess the effectiveness of calcium hydroxide as an intracanal medicament. However, it has also been proven that calcium hydroxide is less effective against E. faecalis, which is commonly observed gram positive cocci in cases of endodontic failure and retreatment [39, 40]. The prevalence of E faecalis in such cases ranges from 24% to 77% due to its ability to survive harsh environmental conditions and a high alkaline pH [41]. This demands the need of an alternate medicament. Although Chlorhexidine is highly effective in eliminating E. faecalis, it exhibits neurotoxicity as well as cytotoxicity on endothelial cells. Further, chlorhexidine is not capable of staying in canal for longer periods as it does not act as a physical barrier thus eventually losing its antibacterial property over longer periods [42].

The need to look for herbal alternatives in the modern era of dentistry is due to the fact that they have better patient tolerance, fewer side effects and they are renewable as well as less expensive. Further, due to the development of drug resistance by human pathogens, there is a need to develop new antimicrobial agents from other sources [43]. Herbal extracts are an effective way to treat many diseases due to their medicinal properties. Moringa Oleifera is one such herbal alternative that is native to India. It is a species that belongs to the monogeneric family moringaceae and possesses a broad spectrum of pharmacological activities. Almost every part of the Moringa Oleifera tree (leaves, roots, bark, fruit flowers, immature pods and seeds) is used as food with a high nutritional quotient, thus making it a plant that is highly valued [44]. It has been shown that its leaves, roots, fruits and flowers possess anti-inflammatory and analgesic activity. The ethanolic extracts of Moringa Oleifera has previously shown antimicrobial activity against species such as Escherichia Coli, Pseudomonas aeruginosa, Staphylococcus aureus and Salmonella typhi. Its antibacterial activity has been attributed to the presence of flavonoids, tannins, glycosides and terpenoids [45-47]. Another study revealed that the chloroform and aqueous crude extracts of Moringa Oleifera leaves was active against Escherichia coli, Pseudomonas aeruginosa and Salmonella typhi [48]. According to a study conducted by Elgamily H.et.al, the ethanolic extracts of Moringa Oleifera has shown the highest zone of inhibition against Staphylococcus aureus and Streptococcus mutans and has shown significant antibacterial activity when used in an experimental dentifrice [49].

In the present study E. faecalis has been chosen as the organism for testing the antibacterial efficacy of Moringa Oleifera due to its relevance in Endodontic failure cases. It has been observed that a concentration of 100µg/ml of Moringa Oleifera leaf extract had a higher antimicrobial activity when compared to Calcium Hydroxide, while a concentration of 25µg/ml and 50µg/ml had lower antimicrobial activity in comparison to Calcium hydroxide.

The susceptibility of E. faecalis to ethanolic extracts of Moringa Oleifera leaf as observed in the present study is in accordance to the results obtain in a study conducted by Peixoto JR.et.al [50]. The ability of the ethanolic leaf extracts of Moringa Oleifera to inhibit the growth of E. faecalis is an indication of its antibacterial potential which could be employed in the management of endodontic infections.

Conclusion

With in the limitations of the present study it was concluded that leaf extracts of Moringa Oleifera has a significant antimicrobial effect against E. faecalis. This further opens perspectives for its use as an intra canal medicament. Although the in vitro observations of the effectiveness of Moringa Oleifera extracts seem promising, further researches are required to test its safety and biocompatibility before conclusively recommending it as an intra canal medicament.

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