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IP Indian Journal of Conservative and Endodontics

Journal homepage: https://www.ijce.in/

Original Research Article

Comparative evaluation of antimicrobial efficacy of natural extracts and sodium hypochlorite against persistent root canal pathogens: An in-vitro study

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ARTICLE INFO

Article history:
Received 28-02-2022
Accepted 02-03-2022
Available online 31-03-2022

Keywords:
E faecalis
Carica papaya
Moringa oleifera

ABSTRACT

Background: The purpose of the present study was to evaluate the antimicrobial efficacy of natural extracts and Sodium hypochlorite against E. faecalis.
Aim: The present study aimed to evaluate the antimicrobial efficacy of natural extracts (Moringa oleifera and Carica papaya) and Sodium hypochlorite against E. faecalis.
Materials and Methods: Carica papaya and Moringa oleifera leaf extracts were obtained. 30 extracted incisor teeth were taken and access cavities were prepared, canals were prepared using the crown-down technique. E. faecalis suspension was placed into root canals and was incubated. Teeth were divided into three groups based on irrigating solutions: 1. 3% NaOCl, 2. Moringa leaf extract, 3. Papaya leaf extract. Dentinal shavings were collected on BHI agar and were incubated. Colonies of E. faecalis were counted.
Results: Differences were found in colony count for different irrigating solutions. p-value < 0.05 is considered statistically significant.
Conclusions: Moringa oleifera has good antimicrobial properties compared to Sodium hypochlorite and Carica papaya.
Relevance for patients: The results should be considered for further research in minimizing the use of chemical substances and increasing the use of effective herbal extracts in root canal treatment.

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1. Introduction

The main objective of root canal treatment is to eliminate the microorganisms from the complex root canal system. The persistence of the microorganisms leads to failure of the root canal treatment.[1] Enterococcus faecalis is a gram-positive, facultative anaerobic coccus that can survive in various environments with different pH, and is a pathogen that is seen in persistent peri-radicular lesions even after root canal treatment.[2]

Sodium hypochlorite is the most commonly used endodontic irrigant, has excellent tissue dissolution and antimicrobial properties. It has undesirable effects like tissue toxicity, pain, swelling due to accidental injection beyond the apex and it leads to the development of secondary infection and paresthesia.[3]

Carica papaya commonly known as the Papaya tree has good medicinal properties in its fruits and leaves. The young Papaya leaves contain papain which is a proteolytic enzyme and is used to soften the meat. Leaves of the Papaya tree contain alkaloids, saponins, tannins, glycosides which have both antibacterial and antiviral properties. Flavonoids present in the leaves are known as 'biological response modifiers' as it protects against oxidative and free-radical damage.[4,5]

Moringa oleifera commonly known as Moringa has curative properties. The leaves contain alkaloids, flavonoids, saponins, triterpenoids that contribute to the antibacterial

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https://doi.org/10.18231/j.ijce.2022.003
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properties.[6,7]

The objective of the present study was to evaluate the antimicrobial efficacy of the Moringa oleifera and Carica papaya leaf extract against E. faecalis, a persistent root canal pathogen. The outcomes of the research may help in giving scope for further research on the herbal products uses in endodontics thus, eliminating the undesirable effects caused by chemical irrigants.

2. Materials and Methods

Moringa oleifera leaf extract, Carica papaya leaf extract, 3% Sodium hypochlorite, E. faecalis, BHI broth, and agar were used in this study.

2.1. Preparation of leaf extracts

Carica papaya and Moringa oleifera leaves were collected and were thoroughly washed in distilled water and were dried in daylight. The dried leaves were ground into fine powder. 40 grams of Papaya leaf powder was taken in a cloth pouch and 250 ml of Ethanol was taken (Figure 1). Both the ingredients were placed in the Soxhlet apparatus and the temperature was maintained at 60°C for four days. The resultant liquid was filtered using Whatman no.1 filter paper and was concentrated in an evaporator at 40ºC to obtain a concentration of 30 mg/ml. Similarly, Moringa leaf extract was prepared with the same concentration as mentioned above.

Fig. 1: Soxhelt apparatus

2.2. Preparation of the specimens

Thirty extracted human incisor teeth with a single canal were collected and stored in the normal saline solution until use (Figure 2). Access cavities were prepared on the lingual surfaces of the teeth with diamond points using a high-speed airotor handpiece (Figure 3). Canal patency was confirmed using the #15k file and working length was determined (Figure 4). The root canals were prepared using the crown-down technique with Protaper hand files. The canals were irrigated with 3% NaOCl in between instrumentation. Then the root apices of the teeth were sealed with nail varnish and then sterilized in an autoclave (Figure 5).

Fig. 2: Extracted incisor teeth

Fig. 3: Access cavity preparation in incisors

Table 1: Antimicrobial efficacy of the irrigants in terms of colony-forming units

Extracts	Mean ±SD	SE	95% CI for Mean	
			Lower Bound	Upper Bound
Sodium Hypochlorite	3.30 ± 1.70	0.54	2.08	4.52
Moringa oleifera	1.10 ± 0.74	0.23	0.57	1.63
Carica papaya	5.60 ± 2.07	0.65	4.12	7.08

Table 2: Pairwise comparisons of the three irrigants for antimicrobial efficacy in terms of CFU

Extracts	Mean ±SD	Mean ranks	U-value	Z-value	p-value
Sodium Hypochlorite	3.30 ±1.70	14.65			
Moringa oleifera	1.10 ± 0.74	6.35	8.50	-3.1371	0.0017*
Sodium Hypochlorite	3.30 ±1.70	7.15			
Carica papaya	5.60 ± 2.07	13.85	16.50	-2.5324	0.0113*
Moringa oleifera	1.10 ± 0.74	5.50			
Carica papaya	5.60 ± 2.07	15.50	0.00	-3.7796	0.0002*

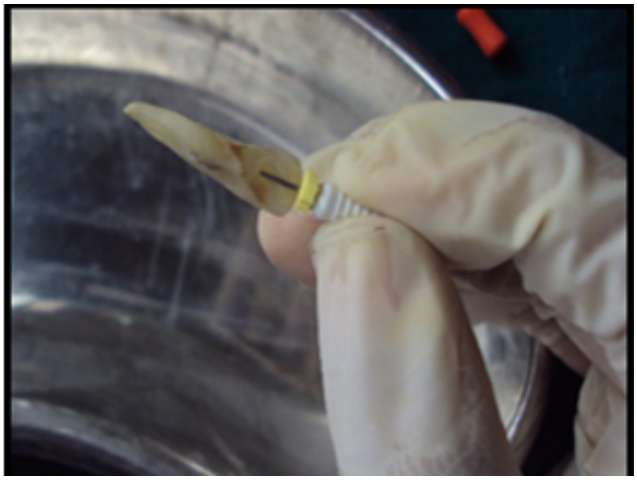

Fig. 4: Confirmation of canal patency

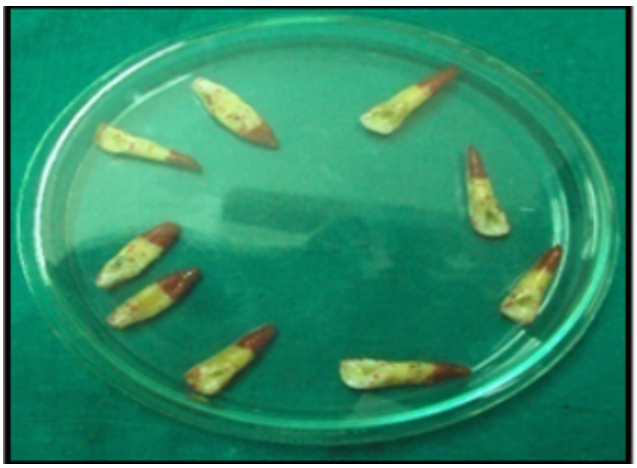

Fig. 5: Rootapices sealed with varnish

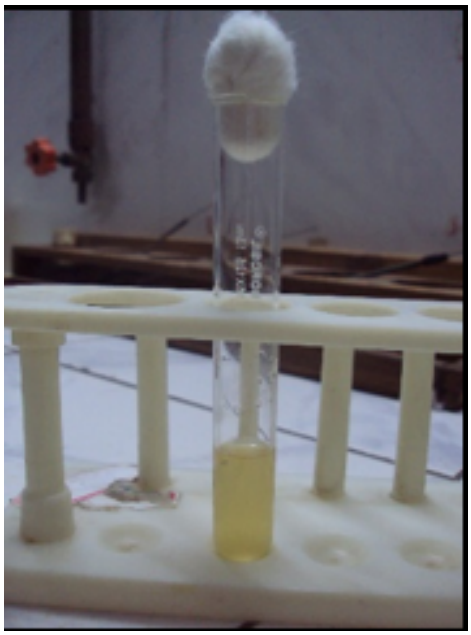

Fig. 6: Turbid suspension of E. Faecalis

2.3. Preparation of Enterococcus faecalis suspension and inoculation into the specimens

A turbid suspension of E. faecalis was obtained by growing E. faecalis cells in the BHI (Brain heart infusion) broth for 6 hours at 36.5ºC (Figure 6). This suspension was inoculated into the root canals and the teeth were incubated for 24 hours at 36.5ºC (Figure 7).

2.4. Grouping based on the irrigants and formation of E faecalis colonies

Teeth were divided into three groups (n=10).

Group 1: 3% NaOCl.

Group 2: 30 mg/ml Moringa leaf extract.

Fig. 7: Inoculation into teeth

Fig. 8: Collection of shavings by drilling

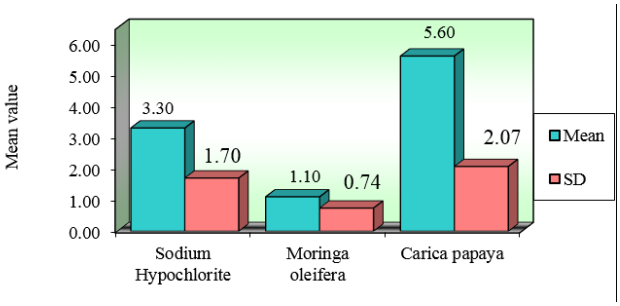

Fig. 9: Comparison of three extracts with antimicrobial efficacy in terms of CFU.

Group 3: 30 mg/ml Papaya leaf extract.

In all three groups, canals were irrigated with 2 ml of the respective irrigant solutions using a sterile needle for one minute. Then a cavity was made at the middle third of the proximal surface of the root into the root canal using a sterile round bur. The shavings were collected on the BHI agar plate and then incubated for 48 hours at 36.5ºC (Figure 8).

E. faecalis were expressed as colony-forming units (CFU). The obtained data were statistically analyzed using the Mann-Whitney U test. p-value < 0.05 is considered statistically significant.

3. Results

Table 1 shows the antimicrobial efficacy in terms of the colony-forming units for all three irrigants individually. It shows that the colony-forming units with Moringa oleifera leaf extract as irrigant is low compared to the other two irrigating solutions indicating its good antimicrobial potential against E. faecalis. The second lesser colony count is seen with 3% NaOCl as an irrigant, with the highest number of colonies for papaya leaf extract.

Table 2 Shows the pair-wise comparisons of the irrigants (NaOCl and Moringa, NaOCl and Papaya, Moringa and Papaya). It shows that the difference in antimicrobial efficacy of Moringa and Papaya is more statistically significant compared to other pairs.

4. Discussion

Sodium hypochlorite has been used as the most effective root canal irrigant due to its high antimicrobial efficacy and the ability to eliminate persistent endodontic microorganisms. However, it has few disadvantages like the inability to remove the smear layer, unpleasant taste, allergic potential, and toxicity.[5] According to Grigoratos D et al., there was a decrease in elastic and flexural strengths of dentin after immersion in NaOCl for 2 hours or when used for a longer duration.[8]

Moringa oleifera leaves contain phytochemicals that have anti-cancer, anti-inflammatory, and antibacterial properties. According to Lucio Arevalo Hijar et al, Moringa oleifera demonstrated antimicrobial effect against E. faecalis without any toxicity when used at low concentrations.[9]

Carica papaya contains papain, alkaloids that have toxic properties against microbes that can effectively kill bacteria and viruses. According to Snigdha Shubham et al, the antimicrobial efficacy of crude Papaya extract is comparable to Sodium hypochlorite and CHX.[5]

The antimicrobial properties of the plant extracts are due to the presence of components known as Bioactive compounds like alkaloids, flavonoids, saponins. Alkaloids are the main group of bioactive components present in the leaves of the Moringa and Papaya tree.[10,11] According

to Pedro et al, the leaf extracts of plants contain the components that have the highest antibacterial and antiviral properties.[5]

It has been demonstrated that the solvents used for the extraction also influence the antibacterial activity of herbs. Various solvents like distilled water, ethanol, phenol can be used for the extraction of bioactive components. Ethanolic extracts showed more antimicrobial potential compared to other extracts. According to Nwah NS et al., when aqueous and ethanolic extracts of papaya leaves were compared, the ethanolic extracts showed a significant antimicrobial effect whereas aqueous extract showed no antibacterial effect.[12]

In this study, the order of antimicrobial efficacy is Moringa oleifera > Sodium hypochlorite > Carica papaya. The clinicians must understand the relationship of a microorganism in the development of endodontic disease to plan for better treatment. The use of herbal alternatives as root canal irrigants is proved to be advantageous considering the several undesirable characteristics of chemical irrigants. Further studies are required to evaluate other desirable properties of herbal irrigants.

5. Conflict of Interest

The authors declare that there is no conflict of interest.

6. Source of Funding

None.

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Cite this article: Rao SA, Sindhuja A. Comparative evaluation of antimicrobial efficacy of natural extracts and sodium hypochlorite against persistent root canal pathogens: An in-vitro study. *IP Indian J Conserv Endod* 2022;7(1):11-15.