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Fabrication and Evaluation of Anti-microbial Efficacy of Root Canal Sealer Using Moringa Oleifera Extract

Bakhtawar Yaqoob[1], Faisal Moeen[2], Maria Khalid[3], Sadaf Humayoun[1], Nousheen Ashraf[4], Qurat ul Ain[2]

[1]Rawal Institute of Health Sciences, Rawalpindi; [2]Islamic International Dental Hospital, Islamabad; [3]Health Services Academy, Islamabad; [4]HITECH Dental College, Taxila

ABSTRACT

Objective: To fabricate a root canal sealer by using extract of Moringa Oleifera, evaluate and compare the antimicrobial activity with Endomethasone N.

Methodology: A new Moringa Oleifera extract (ME) mixture was made and characterization was done using SEM, FT-IR and UV-vis spectrophotometry. In order to evaluate antimicrobial activity, agar well diffusion method was used. The antimicrobial activity was measured by their zone of inhibition against Staphylococcus aureus (ATCC 6538) and Escherichia coli.

Results: The ME mixture exhibited a non-homogenous mixture with a range of particle size distribution. Further, it showed highest zone of inhibition (36.36 + 3.74 mm) against S.aureus in comparison to Endomethasone N.

Conclusion: The results of ME mixture were better than Endomethasone N in terms antimicrobial efficacy. It can be concluded that ME mixture would serve as a better replacement in place of other root canal sealers.

Keywords: Endodontics, Moringa Oleifera, Root canal sealer, Antimicrobial efficacy.

Authors' Contribution:
[1,2]Conception; Literature research; manuscript design and drafting; [3,4]Critical analysis and manuscript review; [5,6]Data analysis; Manuscript Editing.

Correspondence:
Bakhtawar Yaqoob
Email: bakhtt.awan@gmail.com

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Introduction

Root canal treatment (RCT) is one of the most commonly practiced and significant treatments in clinical dentistry. RCT involves a series of procedures aimed to treat the underlying infection within the root canal(s) of the tooth.[1] RCT, also known as endodontics has been defined as "the field of dentistry associated with the diagnosis, prevention and treatment of pathological diseases and trauma of dental pulp and periapical tissues".[2] It aims to eradicate infections with bacterial origin within the root canal(s) system.[3] In a RCT procedure, contents of a root canal are completely removed and replaced by core material. The ending of the root canal is sealed by a material that is referred to as the 'root canal sealer'. The objective of the root canal sealer is to completely seal the canals in three dimensions. This is done in order to prevent the entry of any microbes or fluids from the oral cavity from apical and coronal directions. The clinician's choice of a good endodontic sealer is an important prognostic factor for endodontic success.[4]

Root canal sealers are categorized differently according to their composition and setting reaction. These include eugenol, calcium hydroxide, resin based and glass ionomer sealers.[5] An endodontic sealer tends to lubricate the canal space during the process of obturation, while also acting as a luting

agent. Gutta percha points act as core obturation materials and any sealer allows it to slide in the canal; and fill in the voids, irregularities, lateral and secondary canals. An ideal sealer is supposed to kill the bacteria left after debridement of the canal. During the RCT, attempts are being made to contain the sealer within the root canal space. However, some sealer material may extrude during the obturation.[4] This can lead to slow wound healing and persistent inflammation of periradicular tissues. Therefore, root canal sealers should establish a good seal while having adequate antimicrobial efficacy. These medicaments may come in contact with periapical tissue. Therefore, an ideal sealer material must be biocompatible while having minimal cytotoxic effects.[6]

Over the course of the last century, antibiotics have been overused leading to the formation of multi drug resistant strains of bacteria.[7] The occurrence of multi drug resistant bacteria demands for finding other substitutes to fight infections.[8,9] The antibiotics available in the market are usually quite expensive and in developing countries, medicinal plants are widely used as a treatment modality for infections. These offer a natural and affordable mode of treatment.[10,11] In most of the developing countries, these traditional herbal medicines play an important role in providing basic healthcare.[12] Evidence suggests that almost 61% of the drugs developed from 1981 to 2002 have originated from herbal extracts which have proven efficient in combating several infectious diseases and even cancer to some extent. Numerous plant extracts are widely used as antimicrobial agents.[13] Drugs derived from these extracts have been used to treat and prevent the spread of various general as well as oral diseases.[12] Currently, there is a lack of evidence regarding the antimicrobial efficacy of herbal medicines against oral infections. Moringa Oleifera is a common plant primarily indigenous to the Indian sub-continent. Interestingly, it is used by the natives both as a food source as well as a cure for various diseases.[14,15] This plant has been classified into Moringaceae, an onogeneric class of trees. Being a perennial plant, it has a fast-growing potential. It can grow up to a height of 7-12m with a diameter of 20-60cm. Different names have been given to this plant such as “drumstick tree” or “horseradish tree”. It has the ability to withstand harsh weather changes such as severe drought and frost conditions. The potential to survive in extreme conditions makes it widely cultivable across the world.[16] A substantial amount of work has been done in the past to investigate the biologically active components of different parts of the plant.[17,18] Moringa has been reported to have good for antimicrobial efficacy due to which it has been widely incorporated in various health care formulations being available in the market.[19] There is insufficient evidence to suggest the efficacy of Moringa oleifera extract as a dental material.[20]

Methodology

This study was conducted from February 2020 to March 2021.The study was approved by the ethical committee of Islamic International Dental College. All material preparation and testing were conducted at the Riphah Institute of Pharmaceutical Sciences, Islamabad, except for characterization using scanning electron microscope. This was done at Institute of Space and Technology, Islamabad. This study involved the synthesis and characterization of a mixture using M.oleifera extract. It includes investigation of its antimicrobial properties and comparison with a known sealer (Endomethasone N). Moringa Oleifera plant was collected from Islamabad Nursery farms and later identified at Herbarium of Pakistan, Quaid-e-Azam University, Islamabad.

The following six chemicals were used for the study:

1) Zinc Oxide (DAEJUNG)
2) Barium Sulphate (VWR Chemicals)

3) Hydrocortisone Acetate (AK Scientific)
4) Thymol Iodide (DAEJUNG)
5) Eugenol (DAEJUNG)
6) Magnesium stearate (DAEJUNG)

The study was conducted on two groups of samples: M.oleifera extract mixture and Endomethasone N (Spécialités Septodont, Saint-Maur-des-Fossés, Cedex, France).

Preparation of M.oleifera extract:

The fresh and healthy leaves of MO were cleaned, shade dried and powdered. The extraction of Moringa oleifera was done by using the standardized procedure of Maceration with mixing. The dried leaves were powdered in a mechanical grinder. The dried powder was mixed with methanol and was put on a magnetic stirrer and continuously stirred for two days. A filter paper was used to filter the extract. The rotary flask evaporator (EYELA, Japan) was used to evaporate the solvent. The methanolic extract yielded a dark greenish residue which was kept in a sterile beaker. Lyophilization or freeze drying of the extract was done using ShinBioBase TFD5503 to extend its shelf life for stability of the extract.

Preparation of ME mixture:

Moringa extract mixture was prepared by using basic sealer composition. Different concentrations of MO extract were added to the sealer and mixed thoroughly. Eugenol was added at the end followed by mixing of sealer until a homogenous mixture was obtained.

Characterization of ME mixture:

SEM Analysis:

The newly made ME mixture was filled in standard polyethylene tubes for its characterization. The surfaces of samples were sputtered with gold in 95% relative humidity after 48 hours at 37°C. These samples were then analyzed using 25000X magnification of SEM. The results were evaluated qualitatively.

UV-vis spectrophotometry: Moringa oleifera extract, chemical components of the sealer UV-vis absorption spectrums were evaluated by using Jasco V-530.

FT-IR Analysis:

The Fourier transform infrared spectroscopy (FT-IR) was recorded for Moringa oleifera extract, chemical components of the sealer and ME mixture.

Antimicrobial Activity:

In order to evaluate the antimicrobial activity, Agar well diffusion method was used to check the prepared samples against Staphylococcus aureus (ATCC 6538) and Escherichia coli. For sample preparation the sealers prepared by authors composition ME mixture and the control group; Septodont Endomethasone N were manipulated as mentioned on the manufacturer's instructions.

The cultures of Staphylococcus aureus (ATCC 6538) and Escherichia coli strain were collected from the Department of Microbiology, Riphah Institute of Pharmaceutical Sciences and were confirmed by sub-culturing in appropriate selective media.

For inoculation of S.aureus and E.coli, 0.65gms of nutrient broth (Oxoid, England) was dissolved in 50ml water in a beaker, made separately for both strains. It was poured in a test tube and autoclaved. A sterile wire loop of 4mm diameter was used to scrape each bacterial strain and dipped in the respective test tubes with nutrient broth. They were incubated at 37°C for 24 hours in an incubator (Model B-53 Rmeco). The nutrient broth turned turbid the next day indicating that the strain had been produced.

For preparation of Petri dishes a total of 2.8gms of nutrient agar (Oxoid, England) was dissolved in 100ml water in a flask and then autoclaved. Nutrient agar plate was prepared by pouring it in a sterilized petri dish (94mm via delle, Italy). The incubation period was for 24 hours at 37°C (Model B-53 Rmeco) to confirm its sterility. The inoculation was done by using a sterile cotton swab was dipped in a test tube containing S.aureus (ATCC 6538) and E.coli. Lawn technique was used to spread it uniformly on freshly

made agar plates. Equal sections (four) were made in the plate (for each bacterial strain). In each section of plate, a sterilized borer was used to create 6mm diameter wells. The four wells in each plate were then filled with the ME mixture, Endomethasone N, Eugenol (positive control) and distilled water (negative control).

Afterwards, for two hours the inoculated plates with the tested medicaments were kept at room temperature. This method allows the diffusion of the tested groups in the agar. The agar plates were incubated at 37°C. The procedure was repeated six times for each isolate and in the end; the mean zone of inhibition was assessed. All incubated plates were placed for 72 hours at 37°C in an incubator (Model B-53 Rmeco) and zones of inhibition were measured at 24, 48, and 72 hours.

By the end, SPSS version 25.0 was used to enter and analyze the data. In order to assess the antimicrobial efficacy comparison of mean zone of inhibition of ME mixture and Endomethasone N. For this analysis independent sample T-test was conducted. An arbitrary value of 0.05 was significant for analysis.

Results

In Figure 1, Scanning Electron Microscope analysis showed hierarchically megascopic structures were observed in the ME mixture. There was a range of particle size distribution which is of different shapes and sizes probably associated with different compositions. Fourier Transform Infrared Spectroscopy was recorded for M.oleifera extract, chemical components of the sealer and ME mixture. The resulting analysis showed a blend of chemical components of sealer and M.oleifera extract in the ME mixture as shown in Figure.2. A comparative UV Visible analysis was performed for M.oleifera extract, chemical constituents and ME mixture which showed different absorption spectrum for all listed components i.e. λmax at 278nm and 397nm for chemical components of sealer and M.oleifera extract respectively as shown in Figure 3.

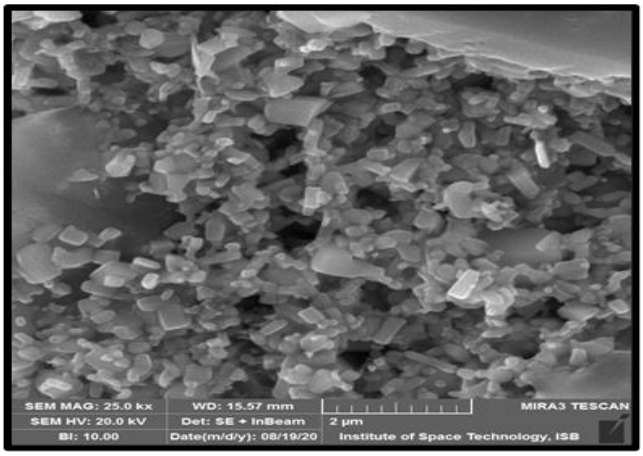

Figure 1: SEM image of ME mixture

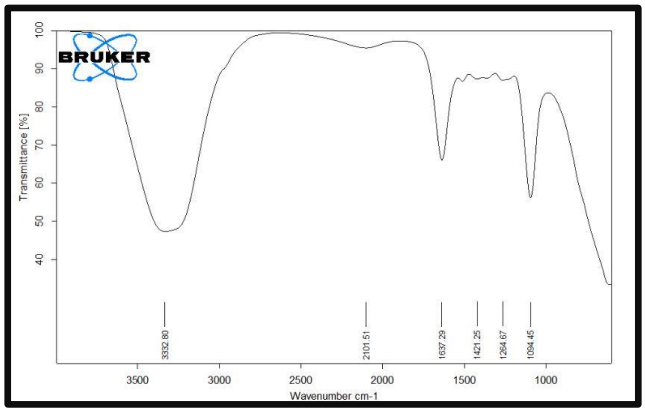

Figure 2: FTIR Spectrum of ME mixture

Table I: Mean diameter of zones of inhibition produced by Endomethasone N and ME mixture expressed as mean ±SD (n=5)

Microorganism	Time interval	Zone of inhibition Endomethasone N(mm)	Zone of inhibition ME mixture(mm)
S. ureus	24 hours	12.22 ± 1.59	36.36± 3.74
	48 hours	12.82 ± 0.90	34.91± 3.33
	72 hours	13.56 ± 1.21	33.90± 3.45
E. coli	24 hours	27.64± 1.91	28.57 ± 1.35
	48 hours	28.28 ± 1.47	29.78 ± 0.33
	72 hours	27.60 ± 1.46	29.47 ± 1.15

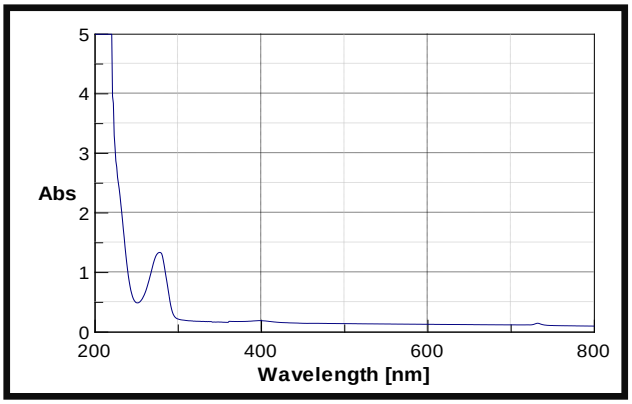

Figure 3: UV-Vis spectrum of ME mixture

In terms of its Antimicrobial properties, it was observed that the largest zone of inhibition produced by ME mixture was 36.36± 3.74 mm, against S. aureus at 24 hours intervals and minimum antimicrobial activity was observed for Endomethasone N against S.aureus (12.22 ± 1.59 mm) at 24 hours interval. The largest zone of inhibition against S.aureus was shown by ME mixture which was significantly higher in comparison to that of Endomethasone N (p value <0.05).

Discussion

In this study, the antimicrobial activity of ME mixture and Endomethasone N was tested against *Staphylococcus aureus* and *Escherichia coli*. These microorganisms are associated with secondary root canal infections, dental caries and respiratory infections.[21]

The findings observed by measuring the diameters of inhibitory zones showed a statistically significant difference between the ME mixture and Endomethasone N (p<0.05). The highest antibacterial activity was observed in ME mixture while Endomethasone N showed minimum antibacterial activity at all time intervals.

Saha et al. mixed the extracts of three different herbal plants – *Glycirrhiza glabra (Liqorice); Mimusops elengi (Bakul)* and *Tinospora cordifolia (Guduchi)* – with three different commercial RCT sealers: Endomethasone (zinc oxide eugenol based), AH Plus (epoxy resin-based) and Apexit Plus (calcium hydroxide-based). The authors compared the antimicrobial efficacy of the sealers against seven different microorganisms. The results showed statistically significant zones of bacterial growth inhibition against *S.aureus* and *E.coli*, when liquorice extract was added in Zinc oxide eugenol-based sealer.[22]

Elgamily et al. conducted a study similar to our study. The authors used samples from different parts of the *M.oleifera* plant (leaves, root and seeds) and tested the antimicrobial efficacy of these extracts against *S.mutans* and *S.aureus.* Further to taking the extracts from the different parts of the plant, the authors also used three different extraction methods (ethanolic, acetone and ethyl acetate). Similar to the results of our study, the greatest mean ZOI against *S.mutans* and *S.aureus* was exhibited by the leaves extract (Mean ZOI by Acetone method = 11.25 $\pm$ 0.96 mm).[23]

This indicates antimicrobial potential of M.oleifera leaf extract. Rahman et al. in 2013 and Devedra et al. in 2011 showed that its leaf extract exhibited highest antimicrobial activity as compared to roots and seeds of the plant because of certain bioactive components.[23] These bioactive ingredients include phytochemicals such as saponins, tannins, flavonoids, and other phenolic compounds that contribute towards the antimicrobial activity of the M.oleifera. The antibacterial potential of the plant has been demonstrated against a wide variety of microorganisms. Moreover, El-Gindy et al. in 2017 also explained that M.oleifera leaves contain phytochemical components such as flavanoids, phenols, cartenoids, taninis and saponins that have antimicrobial activities.[24] The partitioning effect on plasma membrane of microbes and hydrophobicity caused by essential oils present in M.oleifera plays a significant role in antimicrobial activity.[25]

It can be concluded that ME mixture would serve as a better replacement in place of other root canal sealers. Due to limitations of resources, a formulation was not made by using Moringa extract. The benefit of making a formulation is that the quantity of active component is uniform. It is easy to handle and administer and has better shelf life. Various formulations can be made by using different ratios of Moringa extract and characterized by applying various tests such as content uniformity test. Antimicrobial efficacy can be investigated by collecting the flora from root canal or by direct contact.

Conclusion

In this research, we used Moringa Oleifera extract to synthesize a root canal sealer. The need for using a natural compound to make a potential root canal sealer is due to the increasing antimicrobial resistance and cytotoxicity of commercially available root canal sealers. Endodontic treatment is the most widely practiced dental treatment and root canal sealer ensures the good long-term prognosis. The use of natural compound imparts good biocompatibility and lower side effects. The benefit of using a native natural compound in a product makes it easily available and cost effective. Our study focused on making a potential sealer by using Moringa extract and evaluating its antimicrobial activity. The antimicrobial activity was checked against S.aureus and E.coli. The ME mixture showed the highest zone of inhibition (36.36 + 3.74 mm) against S.aureus. The results of ME mixture were better than Endomethasone N in terms of antimicrobial efficacy.

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