

# Comparative Evaluation of Antibacterial Efficacy of *Madhuca longifolia* Saponin Seed Extract against *Enterococcus faecalis*-An *In vitro* Study

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## Abstract

**Background:** The complexity of the root canal system, presence of numerous dentinal tubules and its invasion by microorganisms, formation of smear layer during instrumentation are the major obstacles in optimum canal disinfection. The adjunctive use of chemicals in the form of endodontic irrigants and medicaments are essential. **Aim:** To evaluate the antibacterial efficacy of novel irrigant from *Madhuca longifolia* saponin seed extract against *Enterococcus faecalis* and to compare its efficacy with Sodium Hypochlorite. **Materials & Method:** *Madhuca longifolia* saponin seed extract was prepared by Soxhlet extraction method. The antibacterial assay of the novel irrigant solution was checked against *Enterococcus faecalis*. The minimum inhibitory concentration assay was also done. **Results:** *Madhuca longifolia* saponin seed extract (Test group) produced a greater zone of inhibition than the the adjunctive use of chemical disinfection in the form of endodontic irrigants and medicaments are essential. The adjunctive use of chemical disinfection in the form of endodontic irrigants and medicaments are essential. 3% Sodium hypochlorite (Control group). The results were found to be statistically significant with a P value of less than 0.05. **Conclusion:** Within the limitations of this study, it can be concluded that *Madhuca longifolia* seed extract at higher concentrations was found to be superior in antibacterial efficacy than 3% Sodium hypochlorite.

**Keywords:** *Madhuca longifolia*; Saponin extract; Antibacterial efficacy; Intracanal irrigant

## Introduction

Bacteria have been recognized as the primary etiologic factors in the development of pulp and periapical lesions.<sup>[1,2]</sup> Successful root canal therapy depends on thorough chemomechanical debridement of pulpal tissue, dentin debris, and microorganisms.<sup>[3,4]</sup>

Usage of irrigants helps in flushing out debris, dissolving tissue, and disinfecting the root canal system.<sup>[5-14]</sup> Chemical debridement is especially needed for teeth with complex root canal anatomies such as fins or other irregularities that might be missed by instrumentation.<sup>[15,16]</sup>

Sodium hypochlorite has been the gold standard for root canal irrigants. It is an antiseptic and inexpensive lubricant that has been used in dilutions ranging from 0.5% to 5.25%.

Advantages of Sodium hypochlorite include its ability to dissolve organic substances present in the root canal system and its affordability. Limitations of this irrigants are its cytotoxicity

when injected into periradicular tissues, foul smell and taste and its ability to cause corrosion of metallic instruments. In addition, it does not remove the entire smear layer. Many alternative irrigating solutions have been tried but none of them had the same effectiveness as the gold standard.

*Madhuca longifolia* is a large evergreen tree widely distributed in tropical countries like India, Sri Lanka and Nepal.<sup>[17]</sup> *Madhuca* commonly known as Mahua or Butternut tree, 17 m high.<sup>[18]</sup>

It belongs to the family Sapotaceae. It has a significant place in tribal culture. So the aim of this study was to evaluate the antibacterial efficacy of *Madhuca longifolia* seed extract against *Enterococcus faecalis* and compare its efficacy with sodium hypochlorite 3%.

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## Discussion

The most common cause for the failure of an endodontic therapy is the persistence of microorganisms like *Enterococcus faecalis* and *Candida albicans*, which are highly resistant to mechanical and chemical cleaning irrespective of antimicrobial agents and mechanical methods used.<sup>[34-36]</sup> *Enterococcus faecalis* bacterium has the potential to survive even in obturated canals without any support from other microorganisms or with very small amounts of nutrients. *Enterococcus faecalis* synthesizes a wide variety of proteins when exposed to adverse environmental circumstances, such as environments with alkaline pH or exposure to sodium hypochlorite. Due to the dentin's buffering effect, it is unlikely that the high pH of calcium hydroxide would reach the deeper portions of dentinal tubules where *Enterococcus faecalis* is usually found. Although pH value of calcium hydroxide pastes used in endodontic treatments is 12.3, in the root dentin alkalinity achieved does not exceed 10.3 pH. After having the canals covered with calcium hydroxide, this value can even fall as low as pH 8.5-9.0 due to dentin plugging effect. However, this value is not sufficiently high to eradicate *Enterococcus faecalis*. Moreover, *Enterococcus faecalis* at high pH levels responds by sending (positively charged) potassium ions to the bacterial cytoplasm, thus achieving their acidification and preventing enzymatic inhibition.<sup>[37]</sup> Since this microorganism plays a major role in endodontic failures, this has been used in many studies for evaluating the antimicrobial properties of newer antimicrobial agents. For the same reasons, this study has also considered *Enterococcus faecalis* for evaluating the antibacterial property of the novel herbal irrigant.

Sodium hypochlorite is the most commonly used intracanal irrigant at various concentrations.<sup>[38]</sup> It has broader antimicrobial activity against endodontic microorganisms including those that are difficult to eliminate like *Enterococci*, *Actinomyces* and *Candida albicans*. It has been proved that 2.5% Sodium Hypochlorite can reduce the bacteria in the infected root canals by 90%.<sup>[39]</sup> According to Sharifian et al. Sodium Hypochlorite 5.25% showed the most effective antibacterial action and had considerably greater substantivity at various time intervals. However, Sodium Hypochlorite being a synthetic endodontic irrigant, it had its own limitations. There are studies which have evaluated the cytotoxicity of Sodium Hypochlorite.<sup>[40]</sup> The growing concerns about the chemical and toxic effects of the solution have led to the use of milder concentration of Sodium Hypochlorite (0.5%-1%) instead of 5.25%.<sup>[41,42]</sup> Antibiotics have also been used continuously to avoid and cure oral diseases. But, it often lead to overdosing and finally cause damage to the oral flora.<sup>[43,44]</sup>

Considering the limitations of the synthetic irrigants and antibiotics, researchers are looking for alternatives from natural products which are harmless and can perform as effectively as a standard irrigant. There are many herbs in tropical countries like India, which are abundantly available but inadvertently used.

*Madhuca longifolia* has been often referred to as universal panacea in tribal medicine, since all parts of this tree has the

potential to be used as a medicine. Literature has enough evidence to prove that it was used to treat various diseases owing to its analgesic, antimicrobial, and antidiabetic properties. Hence this study, aimed to evaluate its antimicrobial property against intracanal microorganism. Saponins extracted from *Madhuca longifolia* have shown to have a stronger antibacterial activity against *Streptococcus mutans*, *Streptococcus mitis*, *Staphylococcus aureus* and *Lactobacillus acidophilus*.<sup>[45-48]</sup> The strong antibacterial activity of *Madhuca longifolia* is attributed to the presence of more complex triterpenoid saponins or oleanane type triterpenoid glycosides present in the saponin extract. A pentacyclic triterpenoid saponin (madhushazone), an untypical isoflavone (madhusalmone) and bis (isoflavone) were also reported.<sup>[49]</sup> In another study, a new oleanane-type triterpene glycoside (madlongisides A-D) was isolated.<sup>[50]</sup> Our institution is passionate about high quality evidence based research and has excelled in various fields.<sup>[51-57]</sup>

Hassan et al. 2010 has also mentioned that pure saponin extracted from gaur meal showed hemolytic and antibacterial activity against *Staphylococcus aureus*, *Escheria coli*, *Lactobacillus* species and *Salmonella typhimurium*.<sup>[58]</sup> Hence, it is very much evident that the complex saponin molecules are potent antibacterial agents [Graph 1, Graph 2].

**Graph 1:** Comparison of zone of inhibition of different test samples. *Madhuca longifolia* seed extract and Sodium hypochlorite. *Madhuca longifolia* seed extract at 300 micrograms/ml had shown a greater zone of inhibition than the sodium hypochlorite 2.5%.

**Graph 2:** Box plot showing the comparison of zones of inhibition of different test samples. *Madhuca longifolia* seed extract and Sodium hypochlorite. *Madhuca longifolia* seed

extract at 300 mg/ml had shown a greater zone of inhibition than the sodium hypochlorite 2.5%.

## Conclusion

Within the limitations of this study, it can be concluded that *Madhuca longifolia* seed extract at higher concentrations was found to be superior in antibacterial efficacy than Sodium hypochlorite.

However, further clinical trials are required to evaluate their antimicrobial action against other microbes, biocompatibility before they can be recommended as an endodontic irrigant.

## Clinical significance

Owing to the adverse effects of Sodium hypochlorite, natural products with no or minimal cytotoxic effect are preferred. In this study, seed extract of *Madhuca longifolia* has been proved to have antibacterial effects. So this natural product can be used as an endodontic irrigant.

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