

## RESEARCH ARTICLE

# Antimicrobial Efficacy of *Simarouba glauca* (Lakshmi Taru) Plant Extract against *Enterococcus faecalis* Biofilm: An *in vitro* Study

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## ABSTRACT

**Aim:** The aim of this study was to assess the antimicrobial activity of *Simarouba glauca* plant extract as intracanal medicament against *Enterococcus faecalis* and to compare its effect with that of metapex (calcium hydroxide with iodoform paste).

**Materials and methods:** In the study, 30 human premolar teeth infected with *E. faecalis* were used. Contaminated teeth were randomly separated into three groups (n = 10) and treated as follows: Group I, calcium hydroxide-based medication; group II, *S. glauca*-based medication; and group III, contaminated teeth without medication. After 7 days of treatment, teeth from each group were assessed for the level of microbial growth after a period of treatment.

**Results:** Metapex and *S. glauca* were associated with a reduction in *E. faecalis* after removal of the dressing; however, saline is associated with an increase in colony-forming units (CFU) per milliliter.

**Conclusion:** In this laboratory study using extracted teeth, *S. glauca* (Lakshmi Taru) was able to significantly reduce the CFU per milliliter values of *E. faecalis* in the root canal system similar to metapex.

**Keywords:** *Enterococcus faecalis*, Metapex, Phytotherapy, *Simarouba glauca*.

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## INTRODUCTION

The goal of endodontic treatment is complete disinfection of complex root canal system, thereby preventing

treatment failures and apical periodontitis.<sup>1</sup> Bacterial colonization in root canal is difficult to eradicate by routine cleaning and shaping procedures.

*Enterococcus faecalis* is a facultative anaerobic Gram-positive bacterium that has been isolated in high proportions in endodontic failures. They can invade the dentinal tubules to a depth of 300 to 1500  $\mu\text{m}$  and can resist antimicrobial agents used during treatment and endure potential starvation in cleaned and obturated canal.<sup>2</sup>

Intracanal medicaments help to eliminate or reduce microorganisms that remain after mechanical preparation so as to improve predictability and prognosis of treatment.<sup>3</sup> Calcium hydroxide introduced by Herman in 1920 is routinely used as an intracanal medicament. The mechanism involves diffusion of hydroxyl ions in concentrations to reach adequate pH levels.<sup>4</sup> Due to limited antibacterial efficiency of calcium hydroxide, several works have studied the mixture of other substances to calcium hydroxide.<sup>3</sup>

Metapex is silicon oil-based calcium hydroxide paste containing 38% iodoform.<sup>5</sup> It has a pH below that which is effective to kill *E. faecalis*. The superior antimicrobial effects of metapex may be due to the combination with iodoform and to the viscous and oily vehicle, which may prolong the action of the medicament.<sup>6</sup>

The oily vehicles increase the antimicrobial effects of calcium hydroxide against *E. faecalis* and other bacteria. The use of iodoform and calcium hydroxide combination has been proved to be highly microbicidal due to synergistic effect.<sup>1</sup>

The superior antibacterial effects were demonstrated at dentinal tubule depths of up to 250  $\mu\text{m}$ .<sup>5</sup> The strong bactericidal properties of iodoform paste have been demonstrated in previous studies.<sup>7,8</sup> Iodoform is effective as a disinfectant; it has been shown to be biocompatible with human periapical tissues and is often used as a resorbable dressing in pulpectomies of infected primary teeth.<sup>9,10</sup> In the treatment or retreatment of persisting apical periodontitis, metapex may be the medicament of choice for the elimination of *E. faecalis* from the dentinal tubules.<sup>5</sup>

*Simarouba glauca* is commonly known as "Lakshmi Taru" or "paradise tree" belonging to the family of

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*Simaroubaceae*. It has a long history in herbal medicine in many countries. The bark and leaf extract of Simarouba is well known for its different types of pharmacological properties, such as hemostatic, antihelminthic, antiparasitic, antidyenteric, antipyretic, and anticancerous.<sup>4</sup>

The aim of this study was to assess the antimicrobial activity of *S. glauca* plant extract as intracanal medicament against *E. faecalis* and to compare its effect with that of metapex (calcium hydroxide and iodoform paste).

## MATERIALS AND METHODS

### Preparation of Plant Extract

The leaves of *S. glauca* plant were dried in shade and powdered well. The powder was mixed with 200 mL of ethyl acetate and shaken well in a conical flask for 48 hours. Then, 100 mg of extract is added to 400 mg of polyethylene glycol in 5 mL of distilled water and stirred for 2 hours; 500 mg of carbopol in 5 mL of distilled water was prepared and to which the extract was added simultaneously while stirring (Fig. 1A).

### Tooth Selection and Biomechanical Preparation

Thirty freshly extracted single-rooted premolars were selected. The tooth crowns were sectioned using a high-speed motor and cylindrical diamond drill under cooling to standardize the length of each specimen at 14 mm. The root canals were minimally instrumented using Gates Glidden drill sizes 1 and 2, K files 10 and 15, and with ProTaper F2 rotary system up to 1 mm short of the root apex. Root canal irrigation with 2 mL of 1% NaOCl was carried out at each file change. Following this, canals were irrigated with 17% ethylenediaminetetraacetic acid (EDTA) for 3 minutes and then with 5 mL of saline solution. Afterward, apical foramen was sealed with light-cure composite resin, and the canals were dried with absorbent paper cones and autoclaved at 121°C for 20 minutes.

### Inoculation of *E. faecalis*

The procedures were carried out in a laminar flow chamber (clean air systems CAH600). Test bacteria included *E. faecalis* American Type Culture Collection (29212). The culture bacterial suspension contained  $1 \times 10^7$  CFU/mL of *E. faecalis*.

Root canals were contaminated with this mixture. A sterile cotton pellet was placed at the root canal entrance. The microplates containing specimens were kept in microaerophilic environment at 37°C for 14 days (Fig. 1B). Brain heart infusion broth was applied to the root canals on alternate days. The root canal samples were harvested using sterile paper points to confirm contamination and transferred to tubes containing sterile saline. The contents of each canal were serially diluted and plated on anaerobic blood agar. The CFUs were counted after 24 hours of incubation (Fig. 2A).

### Division of the Experimental Groups

The microplates containing the roots were randomly divided according to the medication used:

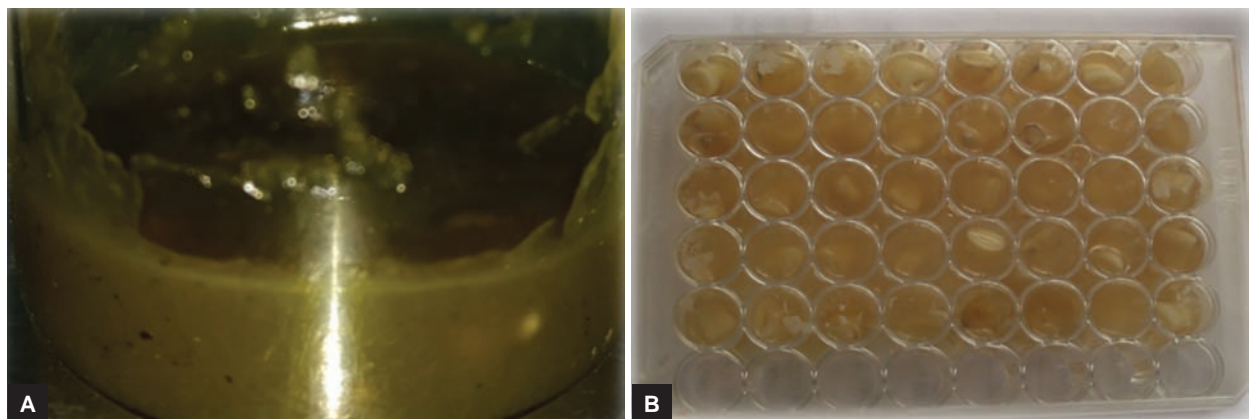
- Group I: Metapex group
- Group II: *S. glauca* group
- Group III: Without intracanal medication

After confirmation of bacterial contamination in the experimental groups, root canals were reprepared and irrigated with 5 mL of sterile saline. Following this, canals were irrigated with 17% EDTA for 3 minutes and then with 5 mL of saline solution. After that root canals were dried with sterile paper points and filled with intracanal medicaments.

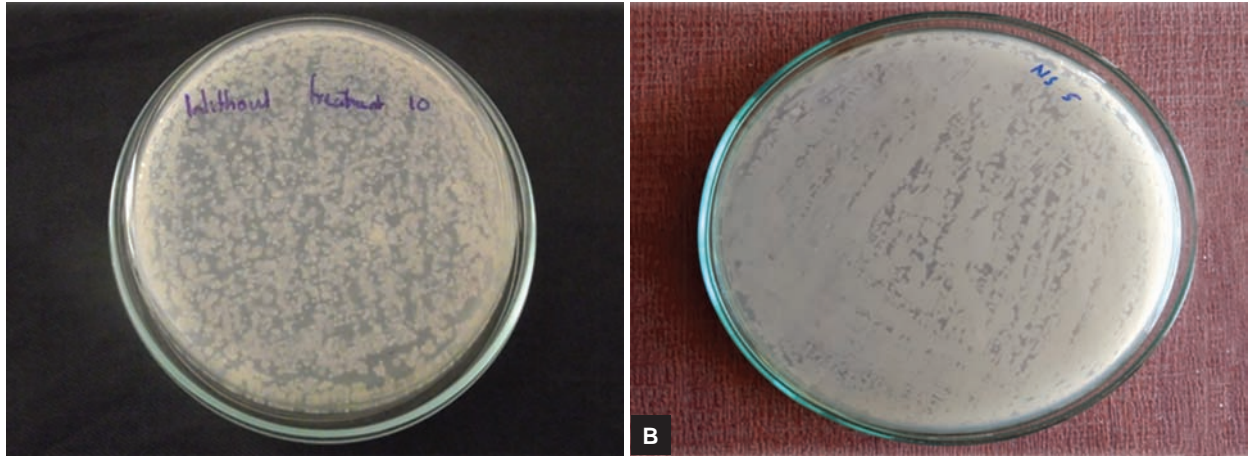
Sterile cotton pellets were placed at the root canal access cavities and specimens were incubated in microaerophilic environment for 7 days.

### Microbiological Analysis after Treatment

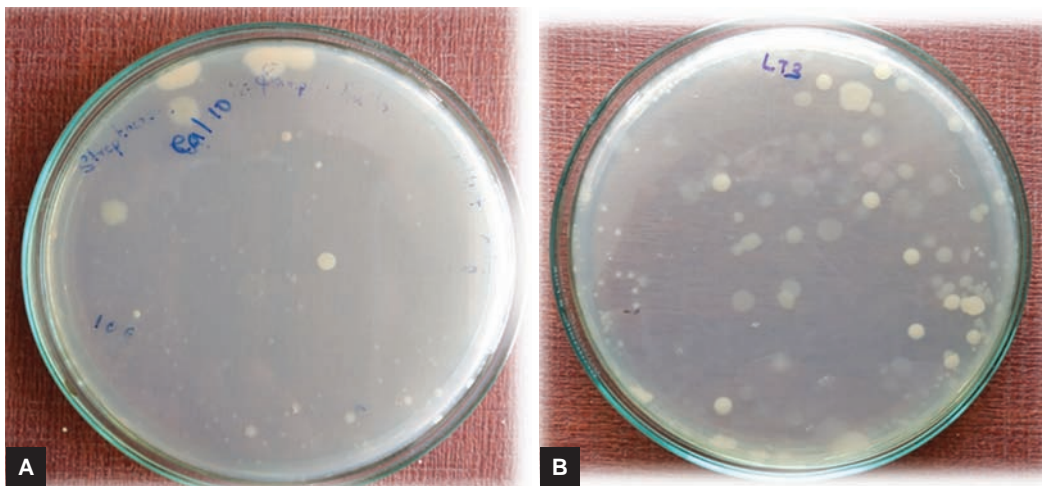
After the dressings had been in place for 7 days, teeth from each group were irrigated with 5 mL of the



**Figs 1A and B:** (A) Prepared gel of Lakshmi Taru; and (B) inoculation with *E. faecalis*



**Figs 2A and B:** (A) Premedication harvest; and (B) postmedication harvest without any medicament (saline)



**Figs 3A and B:** (A) Postmedication harvest with metapex (A); and (B) *S. glauca*

sterilized saline solution, and a sterilized absorbent paper point was inserted into each root canal. The paper point was transferred to a test tube with sterile saline solution. The contents of each canal were serially diluted and plated on anaerobic blood agar. The CFUs were counted after 24 hours of incubation.

### Statistical Analysis

Comparison between the experimental groups was carried out by Scheffe multiple comparison method. Comparison was between the control group and experimental groups using paired t-test. Significance level was set at  $p < 0.05$ .

### RESULTS

The bacterial contamination was detected by recovering pure *E. faecalis* strains from all teeth at the initial harvest performed 14 days after incubation, which showed similar CFU/mL counts for all groups (Fig. 2B). After treating with intracanal medicaments, such as Lakshmi Taru, metapex postmedication harvest was taken, which showed reduction in the CFUs (Fig. 3). Specimens without

intracanal medicaments (saline) confirmed the viability of *E. faecalis* throughout the study.

Groups I and II had lowest CFUs/mL ( $p < 0.05$ ) at the final harvest. Group III showed highest CFUs/mL ( $p < 0.05$ ) with no statistically significant difference between groups I and II, as shown in Table 1.

### DISCUSSION

The prognosis of endodontic treatment is based on the disinfection of root canal system.<sup>2</sup> The methodology used in this study simulated the clinical conditions in teeth with infected root canals, in which microorganisms may harbor in the areas of difficult access for the root canal preparation<sup>2</sup> and medicaments with excellent coronal and apical seal.<sup>2</sup> This study investigated the disinfection potential of *S. glauca* as an intracanal medicament.

*Enterococcus faecalis* was chosen because it is a facultative anaerobic microorganism present in root canal system and it has resistance to calcium hydroxide medicament-based intracanal dressings.<sup>11</sup> *Enterococcus faecalis* is associated with persistent apical periodontitis and resist

**Table 1:** Comparison of pre- and posttest values in different groups

Group (CFU/mL)	Stage	Mean	SD	n	Mean difference	Paired t	p-value
<i>Simarouba glauca</i>	Pretest	98,960.0	19,092.5	10	92,640.0	14.58**	0
	Posttest	6320.0	2782.8	10			
Calcium hydroxide	Pretest	92,620.0	17,180.5	10	88,884.0	15.83**	0
	Posttest	3736.0	2182.8	10			
Saline (control)	Pretest	96,480.0	18,250.6	10	31,360.0	3.92**	0.004
	Posttest	65,120.0	13,762.2	10			

\*\*Significant at 0.01 level; SD: Standard deviation

**Table 2:** *Simarouba glauca* composition

Contents	Active constituents
Alkaloids	Ailanthone
Flavonoids	Benzoquinone
Cardenolides	Vacanthin
Glycosides	Dehydro-glaucarubinone
Phenolic compounds	Glaucarubin
Saponins	Glaucarubolone
Fixed oils	Holacanthone
	Melianone
	Simaroubidin
	Simarolide
	Simarubin

elimination from root canals.<sup>11</sup> This microorganism has been used in several studies.<sup>4</sup> The incubation period of 14 days allowed the suspension of microorganism to diffuse throughout the root canal system.<sup>5</sup> The results revealed that medicaments tested reduced *E. faecalis* from the root canal system after removal of the dressing.

Metapex was used in this study because of its superior properties when compared with other calcium hydroxide-containing intracanal medicaments. Iodoform is incorporated to increase the antimicrobial properties of the material. Silicone oil acts as a vehicle. The mechanism of action can be described by the influence of pH on growth, metabolism, and bacterial cell division. The superior antimicrobial effects of metapex may be due to the combination with iodoform and to the viscous and oily vehicle which may prolong the action of the medicament.<sup>6</sup>

*Simarouba glauca* (Lakshmi Taru) is a tree with various medicinal values for the past few years. All the parts of this plant is used in some way or another. It is widely used as a medicine for the treatment of various diseases, like cancer, amoebiasis, ulcer, and diarrhea.<sup>12</sup>

**Table 3:** Effectiveness of medicaments in reducing bacterial CFU/mL

Group (CFU/mL)	Mean	SD	n	f-value	p-value
<i>Simarouba glauca</i>	98,960.0	19,092.5	10	0.31	0.737
Metapex	92,620.0	17,180.5	10		
Saline	96,480.0	18,250.6	10		

SD: Standard deviation

The alkaloids present in plant extract have alkaline pH (Table 2). They could affect the growth and metabolism of *E. faecalis*. So their pharmacological actions may be the reason for the antibacterial property of *S. glauca*.<sup>12</sup>

A study was done using *S. glauca* plant extract as intracanal irrigants. It was concluded that the irrigants could reduce the number of CFUs of *E. faecalis* per milliliter. But the effectiveness was not as good as chlorhexidine. In this study, plant extract was used as medicaments and it was found that the medicament could reduce the amount of microorganism more than when it was used as intracanal irrigant.

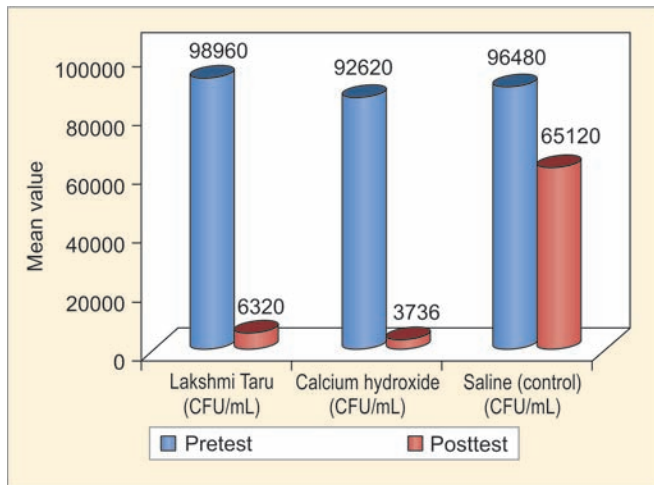
In this study, a gel was prepared from the extract of *S. glauca* plant; here vehicle is carbopol. It was used as a medicament in biomechanically prepared extracted teeth, and the effectiveness in the removal of *E. faecalis* was determined along with metapex. An incubation period of 14 days within the root canal system effectively increased its action. Colony forming units per milliliter of *E. faecalis* were calculated before and after placing medicaments.

The CFU of *E. faecalis* per milliliter was calculated without using medicaments (Table 3). There was no significant difference in the amount of colonies in experimental groups and control group. The CFUs of *E. faecalis* per milliliter was calculated after the use of *S. glauca*, metapex in groups I and II respectively. The CFUs/mL of *E. faecalis* in *S. glauca* group was compared with metapex group and saline group. Similarly, metapex group was compared with saline group (Table 4). There was no

**Table 4:** Comparison of posttest value based on group

Group (CFU/mL)	Mean	SD	n	f-value	Sig.	Scheffe multiple comparison pair	f-value	p-value
<i>Simarouba glauca</i> (A)	6320.0	2782.8	10	179.1**	0	A and B	0.2	0.782
Metapex (B)	3736.0	2182.8	10			A and C	128.4**	0
Saline (control) (C)	65,120.0	13,762.2	10			B and C	140**	0

\*\*Significant at 0.01 level; SD: Standard deviation



**Graph 1:** Comparison of effectiveness of medicaments in reducing bacterial CFU unit/mL

significant difference in the reduction of CFUs. Here it was found that *E. faecalis* colonies were effectively reduced with the use of metapex.

When comparing pretest and posttest values in *S. glauca*, paired t-test was used for the comparison and it was found that the values are significant at 0.01 level. Comparing pretest and posttest values of CFU/mL of saline, there was no significant reduction in the amount of *E. faecalis*. This clearly indicated that *S. glauca* was effective in reducing the CFU of microorganisms per milliliter and was as effective as metapex.

The pretest and posttest values of CFUs/mL were compared (Graph 1), and it was found that there was a good reduction in the amount of *E. faecalis* in both metapex and *S. glauca* group without any statistically significant difference. In saline group, there was no reduction in the amount of CFUs in the pretest and posttest groups.

The alkaline nature of *S. glauca* extract could be the reason for its antimicrobial activities. The future of herbal intracanal medicaments hold a promising effect in the reduction of bacterial colonies within the root canal system.

## CONCLUSION

Based on the findings obtained, it is possible to conclude that *S. glauca* leaf extract is efficient in reducing endodontic microorganisms as comparable to metapex. The alkaloids present in Lakshmi Taru could be able to enhance the antimicrobial property, by inhibiting the growth and metabolism of *E. faecalis*. Further investigations are required to

know about the efficacy of *S. glauca* against multispecies biofilm and mechanisms inside the root canal system as well as the need to associate it with other medicaments. So phytotherapy is a viable option for endodontic management.

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