

# Evaluation of Antimicrobial Activity of Root Canal Irrigants against *Enterococcus Faecalis*; An in Vitro Study

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## Abstract

The ultimate goal of endodontic treatment is either to prevent the development of apical periodontitis or to create adequate conditions for periapical healing in the presence of disease. Commiphora molmol has also been investigated for its antibacterial activity. It is another type of naturally occurring plant extract that has shown a significant effect on reducing bacterial infection. *Enterococcus faecalis* is a gram-positive facultative anaerobe. It is found in 4–40% of primary endodontic infections. The frequency of *E. faecalis* found in persistent periradicular lesions is much higher. In fact, failed root canal treatment cases are nine times more likely to contain *E. faecalis* than primary endodontic infections. Thus, the rationale of endodontic treatment is to eradicate the infection present and/or prevent the microorganisms from infecting or re-infecting the root canal system. A (Green tea, Zingiber Officinale, Commiphora Molmol (Myrrh) were prepared using the agar plates. Cultures of *E. faecalis* were grown in agar. We compared 5% NaOCl (sodium hypochlorite irrigation agent) as a control group with three sample groups.

There was no statistically significant difference between the zone of inhibition of sodium hypochlorite and green tea (p-value: 0.855), Commiphora Molmol (p-value: 0.639). However, there was significant difference between the NaOCl and Ginger group (p-value: 0.000). Overall, no significant comparison could be achieved when comparing the herbal extracts with sodium hypochlorite. Future studies can be much more useful if the combination of these herbal extracts can be tested for their efficacy.

**Keywords:** Root canal irrigation, Antibacterial agent, Antimicrobial activity, Root canal irrigants, *Enterococcus faecalis*, Endodontic treatment

## INTRODUCTION

Root canal treatment requires proper debridement of dead tissue and microbes, which play an important role in the failure to achieve success. One of the major consequences of improper debridement is the peri-apical infection. To avoid the above-mentioned problems related to root canal treatment, the use of irrigation agents has been proven to be effective against the bacteria that cause infection [1]. Irrigation of the root canal is considered to be an equally important step alongside instrumentation and obturation. Failure to achieve any of these procedures may not fulfill the eventual goal of the treatment. There have been various types of root canal irrigants developed and successfully used by dentists to accomplish the desired results [2].

Several materials have been utilized in the attempt at root canal debridement by dentists. They include sodium hypochlorite and chlorhexidine gluconate, which act efficiently against the bacteria infecting the root canal (mainly *Enterococcus faecalis*). When comparing the actions of these above-mentioned intra-canal agents, chlorhexidine gluconate was found to be significantly more effective against the bacteria [3]. However, the efficacy of root canal irrigation material mainly depends on the concentration and susceptibility of microorganisms [4].

Root canal irrigation materials may be associated with irritation and other side effects. To tackle this issue, the use of naturally available methods has been introduced to achieve the desired results without any concern about possible complications. One of these natural compositions is Zingiber Officinale, which is an effective anti-bacterial agent used in the form of extract. It has also been utilized as an ingredient of several dentifrices, with its action focused on the killing of the bacteria [5]. Studies have been conducted to assess the affectivity of Zingiber Officinale in root canal treatment, with it being successful but when compared to chlorhexidine, it was found to be less effective [6, 7].

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In continuation of discussing the use of natural extracts and materials in the root canal irrigation process, green tea is also known for its effectiveness against invading bacteria. When compared with the actions of sodium hypochlorite, green tea did not show superior activity against the bacteria. However, there was a significant effect of green tea as an antimicrobial agent, which can be useful when the dentist is not looking for any undesirable effects of sodium hypochlorite [8-10].

Additionally, *Commiphora molmol* has also been investigated for its antibacterial activity. It is another type of naturally occurring plant extract that has shown a significant effect on reducing bacterial infection. Once again, the affectivity depends on the dosage and concentration of these materials [11, 12]. These oils have been linked with their anti-inflammatory effects in gingiva as well. This property of *Commiphora molmol* is encouraging as this could be used in other areas of dentistry as well [13]. Furthermore, these natural oils have also been linked with their activity against *Candida albicans* [14].

The ultimate goal of endodontic treatment is either to prevent the development of apical periodontitis or to create adequate conditions for periapical healing in the presence of disease. Thus, the rationale of endodontic treatment is to eradicate the infection present and/or prevent the microorganisms from infecting or re-infecting the root canal system. *Enterococcus faecalis* is a gram-positive facultative anaerobe. It is found in 4–40% of primary endodontic infections. The frequency of *E. faecalis* found in persistent periradicular lesions is much higher. In fact, failed root canal treatment cases are nine times more likely to contain *E. faecalis* than primary endodontic infections [15-17].

### Aim and Objective

The aim of this study is to:

1. Assess the antimicrobial efficacy of herbal irrigating against *Enterococcus faecalis* which would probably be as effective or more and at the same time less irritating to the tissues than sodium hypochlorite.
2. Evaluate and compare the antimicrobial effect of alternative irrigants, (Green tea, Zingiber Officinale, *Commiphora Molmol* (Myrrh) and compare with the traditional endodontic irrigants 5% sodium hypochlorite (NaOCl) against the *Enterococcus faecalis*.

## MATERIALS AND METHODS

This in vitro study collected and processed samples between April 2022 and June 2022 in the College of Dentistry, King Saud University, Riyadh, Saudi Arabia. Ethical approval was taken from the Department of Research, King Saud University.

A (Green tea, Zingiber Officinale, and *Commiphora Molmol* (Myrrh) were prepared using the agar plates. Cultures of *E. faecalis* were grown in agar. We compared 5% NaOCl

(sodium hypochlorite irrigation agent) as the control group with three sample groups:

- Group A: Green tea solution
- Group B: Zingiber Officinale solution
- Group C: *Commiphora Molmol* (Myrrh) solution

The agar well-diffusion method was followed to determine the antimicrobial. Plates were incubated for 24 hrs at 37 deg C in an incubator.

### Preparation of Materials

1. Green tea water extract preparation
  - a. Grinding green tea plant (dry).
  - b. Soak 800 grams of green tea in 5 liters of water (cold) for 3 days with continuous stirring morning and evening
  - c. The extract was saved in the fridge at 2-4 C<sup>0</sup>
  - d. The extract is filtered by standard filter paper.
  - e. Finally, the solvent (water) evaporated on the rotary evaporator at low pressure (130mm Hg) and at 80 ° C
2. *Commiphora Molmol* (Myrrh) extract preparation
  - a. Grinding *Commiphora Molmol* plant (dry)
  - b. Soak 1000 grams of *Commiphora Molmol* in 5 liters of water (cold) for three days with continuous stirring morning and evening
  - c. The extract was saved in the fridge at C<sup>0</sup>
  - d. The extract is filtered by standard filter paper.
  - e. Finally, the solvent (water) evaporated on the rotary evaporator machine at low pressure (130 mm Hg) and at 80 ° C
3. Zingiber Officinale solution preparation:
  - a. Grinding Zingiber Officinale plant (dry)
  - b. Soak 900 grams of Zingiber Officinale in 5 liters of water (cold) for seven days with continuous stirring morning and evening
  - c. The extract was saved in the fridge at 2-4 C<sup>0</sup>
  - d. The extract is filtered by standard filter paper.
  - e. Finally, the solvent (water) evaporated on the rotary evaporator at low pressure (130mm Hg) and at 80 ° C

## RESULTS AND DISCUSSION

The results of the experimental lab trial concluded that the inhibition zone around green tea was 1 cm, *Commiphora Molmol* (Myrrh) was 0.75 cm, Zingiber Officinale (ginger) was 0 cm, whereas sodium hypochlorite was 0.95 cm (**Table 1**). It is worth noting here that sodium hypochlorite was the control group.

It can be noted from **Table 2** that there was no statistically significant difference between the zone of inhibition of sodium hypochlorite and green tea (p-value: 0.855), and *Commiphora Molmol* (p-value: 0.639). However, there was a significant difference between NaOCl and Ginger groups (p-value: 0.000).

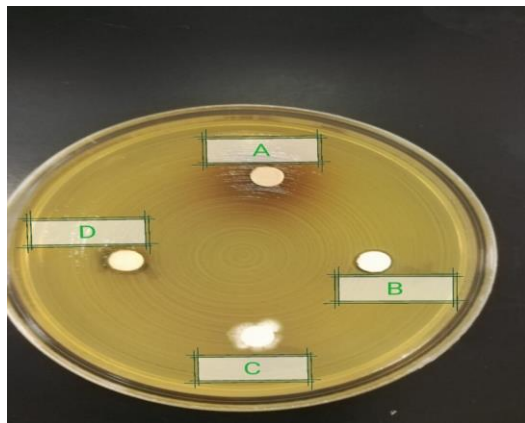
**Figure 1** shows the agents used in this study. A: Green tea, B: Sodium hypochlorite, C: Zingiber Officinale, and D: Commiphora molmol. When finding the answers to our study aims, it can be noted from the results that green tea and Myrrh showed better resistance towards *E. faecalis* as compared to Ginger extract. The antimicrobial effect of green tea was found to be better than Commiphora molmol (myrrh). On the other hand, when comparing the antimicrobial activity of these herbal extracts with NaOCl, green tea showed slightly better activity as compared to Commiphora Molmol. However, Zingiber Officinale (Ginger) did not show any antimicrobial activity.

**Table 1.** Descriptive findings of the experimental lab trial

Root Canal Irrigation Materials	Zone of Inhibition (in cms)
Green tea	1 cm
Commiphora Molmol (Myrrh)	0.75 cm
Zingiber Officinale (Ginger)	0 cm
Sodium Hypochlorite (NaOCl)	0.95 cm

**Table 2.** Comparison of case and control groups with their p-values

Root Canal Irrigation Materials	Zone of Inhibition (in cms)	Sodium Hypochlorite (Control)	P-value
Green tea	1 cm		0.855
Commiphora Molmol (Myrrh)	0.75 cm	0.95 cm	0.639
Zingiber Officinale (Ginger)	0 cm		0.000



**Figure 1.** All four agents placed in the agar dish with *Enterococcus faecalis*.

This study aimed to assess and evaluate the antimicrobial activity of natural herbs and their extracts towards *Enterococcus faecalis*, which are present in the root canal causing the peri-apical infection. Irrigation agents are used to kill these bacteria and several materials have been used to achieve this objective. We used three naturally occurring herbal extracts, which are found to be active against bacterial attack. Previously, sodium hypochlorite, chlorhexidine gluconate, and therasol have been commonly used to serve this purpose. However, they have been associated with a few toxic effects in and around the tooth structure when performing root canal treatment [18].

Several natural herbs and their extracts have been linked with antibacterial activity and are suggested to be used in dentistry. One of our materials of interest was Commiphora molmol, which has shown an efficient antimicrobial effect in humans [19]. Our results suggested similar information, which shows that Commiphora molmol is capable of resisting the *E. faecalis* bacteria to 0.75 cm in laboratory settings. However, this action did not match the affectivity exhibited by sodium hypochlorite.

Positive effects of green tea against bacterial activity have been reported by multiple studies [20]. Our study revealed that there is a strong effect of green tea on the repelling action of *Enterococcus faecalis* bacteria. In fact, it was found to be slightly better than the strong effect of sodium hypochlorite. Lastly, Zingiber Officinale has been strongly associated with anti anti-inflammatory effect [21]. Our findings suggest that the anti-bacterial action of this herb was not found to be effective. Moreover, the combination of these herbal extracts may be useful in providing the required protection against the bacteria with no side effects.

Overall, no significant comparison could be achieved when comparing the herbal extracts with sodium hypochlorite. Future studies can be much more useful if the combination of these herbal extracts can be tested for their efficacy. Patients' comfort comes first, which gives dentists opportunities to improve and use materials that are patient-friendly.

## CONCLUSION

It can be concluded from the findings that green tea and Commiphora molmol can be used as root canal irrigation materials. However, Zingiber Officinale showed no antibacterial activity, so it may not be advisable to use it in root canal treatment.

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**ETHICS STATEMENT:** This study fulfilled the ethical requirement.

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