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## Comparative Evaluation Of Antibacterial Efficacy Of Ocimum Tenuiflorum And Syzygium Aromaticum Formulations As An Intracanal Medicament Compared To With Calcium Hydroxide On E. Faecalis

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**Abstract: Background:** Intracanal medicament is a fundamental part of endodontic procedures that must have an antibacterial impact on the infected root canal bacteria for endodontic procedures to be effective. Calcium hydroxide is the current "gold standard" intracanal medication. The process of seeking for an effective and perfect intracanal medication is a constant and challenging effort. In recent years, there has been increasing interest in herbal products due to their antibacterial, antiseptic, and analgesic characteristics.

**Aim:** To assess the antibacterial activity of herbal extracts of tulsi and clove with calcium hydroxide as a control, in vitro assessment as an intracanal medicament on *Enterococcus faecalis*.

**Materials And Method:** This is an in vitro study using *E. Faecalis*, which is typically detected in infected root canals. *E. faecalis* was inoculated onto a Mueller Hinton agar plate. Wells with diameters of 6-8mm were punched and the herbal extract mixtures pipetted into these agar wells. After 48 hours of incubation, the diameter of the inhibition zones around all of the wells was measured manually. The inhibition zones of tulsi and clove combinations were then compared to the inhibition zone of calcium hydroxide.

**Results:** The herbal based intracanal medicament showed antimicrobial effect against test microbes, where the mean value of inhibition zone size (mm) was increased by increasing the concentration. When compared to tulsi and its mixtures, calcium hydroxide herbal formulations demonstrated superior antibacterial activity.

**Conclusion :** The herbal based intracanal medicament seems to be more effective against *E. Faecalis* compared to that of calcium hydroxide. Herbal based intracanal medicament performed better antibacterial efficacy as compared to conventional calcium hydroxide.

**Keywords:** Ayurveda, antibacterial , intracanal medicament , Zone of inhibition, Calcium hydroxide

### 1. Introduction

Caries is a global public health concern that is defined as a dynamic process of demineralization of inorganic and organic structures of teeth beginning with enamel and progressing to dentin and, if left untreated, pulp(1,2). Bacteria and their byproducts are primarily to blame for tooth pulp necrosis and periapical lesions that necessitate root canal treatment. Many bacteria are typically found in the oral cavity, including *S.mutans*, *S.salivarius*, *Lactobacilli*, *Actinomyces*, and *E.faecalis*. *E.faecalis* is the most resistant species in the oral cavity and may be the cause of root canal treatment failure.

Endodontic treatment's ultimate goal is the full eradication of bacteria, their byproducts, and pulpal remnants from diseased root canals, as well as the complete seal of disinfected root canals(3,4). Intracanal medications have been suggested to be a crucial step in eradicating bacteria in root canals. Because of its antibacterial action, calcium hydroxide has been used as a gold standard material for intracanal medicament, however it is still far from being an ideal intracanal medicament.(5)

Ayurveda is an ancient Indian health-care and longevity system. According to Ayurveda, there are over 1250 Indian medicinal plants that are utilised in the formulation of therapeutic concoctions, with many more still to be found. This 3000-year-old medical system recommends therapies using specific herbs and minerals to cure a variety of ailments(6). Herbal products have gained popularity in recent years due to their antibacterial, antiseptic, and analgesic characteristics. In our hunt for novel materials, we attempted to combine current technology and expertise with the old, but time-tested, benefits of Ayurveda. (7) Due to many concerns about disinfection, many recent studies have focused on alternate antibacterial agents, including plant compounds. Herbal formulations are critical in the development of novel antimicrobials against a diverse range of pathogenic microorganisms. Recently, the biological manufacture of herbal-based intracanal medicaments based on plant extracts was demonstrated to be highly efficacious(8). Tulsi and clove have been frequently utilised among the numerous options due to their potential anti-bacterial, anti-fungal, and anti-proliferative effect.(9) As a result, it is less harmful and not a hazardous material, and it is as simple, quick, and cost effective as solvents, uses less energy, performs under moderate operating conditions, and combines the efficacy of antibacterial activity and plant active components. Calcium hydroxide has numerous advantageous qualities, including a high pH (12.5-12.8), insolubility in alcohol, and limited solubility in water. Its low water solubility is especially advantageous because it allows us to wait longer before it dissolves in tissue fluids.(5) However, calcium hydroxide has been shown to be ineffective against *E. faecalis*. *E. faecalis* can colonise inside dentinal tubules and so avoids hydroxyl ions. Furthermore, ramifications, abnormalities, and isthmuses contain necrotic tissue that can protect bacteria from the effects of calcium hydroxide.(10)

The antibacterial efficiency of a green synthesised based intracanal medicament and its involvement in microbial load reduction were investigated in this study by analysing the bacterial colony count using the disc diffusion method against *E. Faecalis*. The study's goal was to compare the antibacterial activity of an intracanal medicament containing Tulsi and clove to that of commercially available calcium hydroxide.(11) The objective of this study is to investigate the antibacterial activity of calcium hydroxide in conjunction with tulsi and clove as an intracanal medication against *Enterococcus faecalis*. We paired tulsi with clove and Calcium hydroxide as control in this study to investigate if their combination has an antagonistic or synergistic impact against *E. faecalis* because, to the best of our knowledge, no studies have been done on the combinations of the aforementioned products.

## 2. Materials And Methods

It was an in vitro study conducted in the month of September 2022 – December 2022 in the city of Chennai, Tamil Nadu.

### Plant material and silver particle characterisation

Tulsi and clove leaves were collected from a university campus in Chennai, Tamil Nadu, India in May. To eliminate the dirt and dust on the surface of the leaves, they were carefully washed with running water. They were air dried for 10 days before being placed in a hot air oven at 600 degrees Celsius for 24-48 hours. After that, the leaves were pulverised into a fine powder. Separately, 1 mm silver nitrate was added to the plant extracts to form a final solution of 200 ml, which was centrifuged at 18,000 rpm for 25 minutes. The supernatants were heated from 50 to 950 degrees Celsius. During the heating procedure, the colour of the solution changed after 10-15 minutes.

### Antimicrobial analysis

The disc diffusion method was used to test antibiotic activity. Mueller Hinton agar plates were prepared and sterilised for 15 minutes before solidification. Following solidification, wells were cut with a 9mm sterile polystyrene tip and bacterial cultures (*E. Faecalis*) swabbed on these plates. Herbal prepared solutions of various concentrations (10 $\mu$ L, 25 $\mu$ L, 50 $\mu$ L, and 100 $\mu$ L) and the standard calcium hydroxide medicament were placed on four different wells of the agar plate and incubated for 24 hours at 37 degrees Celsius. Inhibition zones were measured. The studies were done three times, and the mean zone diameter measurements were provided. The antibacterial ability of materials was evaluated at the point where circular zones of bacterial inhibition (halo) developed around each hole. The size of these bacterial inhibition zones was measured in millimetres using a ruler (from the edge of the zone from one end to the next edge).

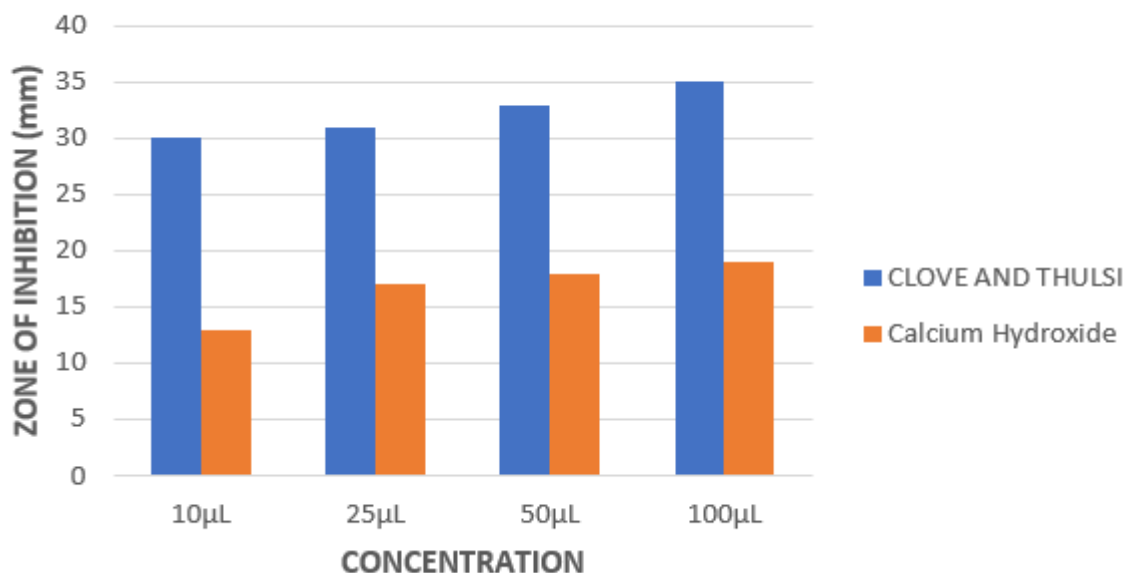


Figure 1: Showing microbial zone of inhibition of ( Calcium hydroxide ) after 24 hours plates swabbed with E.Faecalis.



Figure 2: Showing microbial zone of inhibition ( herbal extracts )after 24 hours plates swabbed with E.Faecalis.

### 3. Results



**Graph 1: Zone of inhibition of Tulsi and Clove mediated intracanal medicament compared with calcium hydroxide against E.Faecalis**

As observed in the aforementioned graph, the herbal-mediated intracanal medication at the 100µl dosage seems to be the most successful overall. All of the intracanal medications were more effective against bacterial colonies when compared to fungal colonies. With regard to *E. faecalis*, the traditional Calcium hydroxide seems to work better. At all concentrations, herbal-based intracanal medication outperformed traditional calcium hydroxide. Additionally, a clove and tulsi-based herbal intracanal medication solution performed better with increasing concentration. For *E. faecalis*, the maximum zone of inhibition was observed.

#### 4. Discussion

The primary objective of root canal therapy is to achieve maximum root canal disinfection, which is a difficult undertaking given the intricate structure of the root canals(4). By shaping the root canals and primarily using intracanal medications, the quantity of germs is decreased. To get rid of the remaining germs in the canals, an intracanal medicine with strong antibiotic action is required(12). As the most resilient species in the oral cavity and a potential cause of root canal failure, *Enterococcus faecalis*, a gram-positive facultative anaerobe, was selected as the test organism.(13)*E. faecalis* has been The most often isolated species from tooth canals causing post-treatment illnesses is *Enterococcus faecalis*. In 10–30% of root canal failures, inflammatory root canals, and untreated canals, found.(3,14) There is a critical need to research intracanal medications that can eradicate *E. faecalis* given its resistance.(15)

The intracanal medication that is currently most frequently utilised is calcium hydroxide paste. After a number of variations and combinations, the materials that were combined are examined as intracanal medications. Combinations, the proportion of liquid (distilled water) to powder (tulsi and clove) were chosen. It was created to resemble the consistency of calcium hydroxide, a medication administered into the root canals . This was done to make it easier to move the substance into the canals, much like calcium hydroxide does.(16),(17)

Currently, the majority of commercial products used in endodontics as intracanal medications are cytotoxic and unable to remove bacterial remnants from the dentinal tubules, prompting a rapid tendency towards the

application of biologic drugs made from organic plants.(18) The main benefits of using herbal substitutes are their affordability, low toxicity, ease of accessibility, lengthened shelf life, and reduced microbial resistance.(19) Tulsi is known as "The Queen of Herbs" in India due to its healing and spiritual effects.Tulsi (*Ocimum sanctum*) includes active ingredients such as eugenol and ursolic acid and carvacrol, which are responsible for its antibacterial properties.(20)(9,17)An aromatic plant from the Myrtaceae family, *Syzygium aromaticum* (Clove) is indigenous to Indonesia and is also known by that name. It has qualities that are anti-cancer, anti-ulcerogenic, anti-mutagenic, analgesic, antiplasmodial, antibacterial, antiviral, anti-fungal, anti-bacterial, antiviral, anti-inflammatory, antioxidant, and antigenotoxic. Eugenol, which is derived from the botanical name *Eugenia caryophyllata*, is the primary component in clove oil.

The zone of inhibition of the synthesised herbal-based intracanal medicament against *E.faecalis* at 10µl, 25µl, 50µl,100µl and the commercially available Calcium hydroxide medicament is 13mm,17mm,18mm,and19mm,respectively. Similarly, the intracanal medicaments based on clove and tulsi were determined to be 30mm, 31mm, and 35mm. Oleanolic acid, linalool, ursolic acid, and beta caryophyllene are phytochemical constituents of extracts that can infiltrate and precipitate in various lesions, producing enamel hardening. As a result, using herbal intracanal medicaments can lower microbial load while also inhibiting caries progression.

The majority of commercial products used in endodontics as intracanal medications at the moment are cytotoxic and unable to remove germs from the dentinal tubules, prompting a quick tendency towards the use of biologic drugs made from organic plants. The primary benefits of using herbal substitutes are their affordability, low toxicity, ease of availability, longer shelf life, and reduced microbial resistance.

## 5. Limitations

Within the limitations of the study , we have tested the antibacterial properties of clove and tulsi individually and in combination against one organism.In this study, we performed a preliminary screening of clove and tulsi herbal combinations using the agar diffusion method to evaluate if they had antibacterial effectiveness. The combinations we created have shown antimicrobial effectiveness. The combinations demonstrated a zone of inhibition; we further proceeded to the anti inflammatory ,minimum bactericidal concentration (MBC), and cytotoxicity tests, which were supposed to represent the second part of the investigation.

## 6. Conclusion

Within the limitations of this study, the following could be concluded:

- (1) The four concentrations of herbal ( Clove and Tulsi ) intracanal medicament had better antibacterial effects than conventional calcium hydroxide.
- (2) The antibacterial effect against *E.Faecalis* organisms gradually increases with the increase of the herbal based intracanal medicament concentration.
- (3) The synthesis of herbal mediated intracanal medicament showed better antibacterial activity than antifungal activity when compared against oral pathogens.

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## CONFLICT OF INTEREST

There are no conflicts of interest.

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