

Pulp Tissue Dissolving Ability of Ocimum Sanctum (Tulsi) Essential Oil Extract: A Pilot Ex Vivo Study

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Abstract

Background: Successful endodontic therapy relies on the effective elimination of microorganisms and necrotic pulp tissue from the root canal system. Sodium hypochlorite remains the gold standard irrigant due to its strong tissue-dissolving ability, but its cytotoxic effects limit its biocompatibility. Herbal alternatives have gained interest owing to their antimicrobial and anti-inflammatory properties. Ocimum sanctum (Tulsi), a medicinal plant widely used in traditional medicine, exhibits proven antimicrobial and anti-inflammatory effects. However, its pulp tissue dissolving efficacy has not been evaluated. This pilot ex vivo study aimed to investigate the pulp tissue dissolving potential of Ocimum sanctum essential oil extract. **Material and Methods:** Essential oil of Ocimum sanctum was extracted using a Clevenger apparatus. Pulp tissues were obtained from freshly extracted human third molars, sectioned into standardized fragments, and allocated into four groups: Group I (100% O. sanctum oil), Group II (50% O. sanctum oil), Group III (5.25% sodium hypochlorite, positive control), and Group IV (distilled water, negative control). Each pulp fragment was immersed in 4 mL of the respective solution and incubated at 37°C. Tissue dissolution was assessed at 24, 48, and 96 hours by an independent, blinded evaluator, and dissolution speed was calculated. **Results:** Neither 50% nor 100% concentrations of O. sanctum essential oil extract demonstrated pulp tissue dissolving activity at any evaluated time interval. Sodium hypochlorite showed complete tissue dissolution, while distilled water produced no effect. **Conclusion:** Ocimum sanctum essential oil extract lacks pulp tissue dissolving efficacy and therefore cannot replace sodium hypochlorite as a primary irrigant. Nonetheless, given its antimicrobial and anti-inflammatory properties, it may be valuable as a biocompatible intracanal medicament to support root canal disinfection and periapical healing.

Keywords: Ocimum Sanctum, Essential Oil Extract, Tulsi, Pulp Tissue, Intracanal Medicament.

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INTRODUCTION

The pivotal role of microorganisms in the initiation and progression of pulpal and periapical inflammation has been extensively established.^[1] Once the host defense mechanisms lose access to the necrotic pulp space, opportunistic microorganisms adapted to harsh ecological conditions and a low-oxygen environment colonize the root canal system.^[1] Primary root canal infections are typically polymicrobial, dominated by obligate anaerobic bacteria,^[2] which can usually be eliminated during routine root canal treatment. However, facultative bacteria such as Streptococci, Enterococci, and Lactobacilli are more resilient and can often withstand chemomechanical instrumentation and intracanal medication.^[3] Among these, Enterococcus faecalis has drawn considerable attention in endodontic literature due to its frequent isolation from persistent infections and nonsurgical retreatment cases.^[4,5] The success of endodontic therapy fundamentally depends on eradicating these microbial contaminants from the root canal system. The objectives of root canal instrumentation include thorough debridement, creation of an optimal space for antimicrobial delivery, and

enabling three-dimensional obturation to prevent recolonization by oral microbiota.^[6] Nevertheless, mechanical instrumentation alone, even when combined with irrigation, fails to completely eliminate viable microorganisms,^[7,8] and cannot fully prevent the formation of a smear layer.^[9] Studies have shown that nearly half of the root canal walls remain untouched regardless of whether nickel–titanium rotary systems or traditional stainless-steel hand files are used.^[10] To overcome these limitations, the use of interappointment medicaments has been advocated. These agents assist in eliminating residual bacteria after chemomechanical preparation, reducing periapical inflammation and pain, and

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promoting healing.^[11] They also help reduce apical exudation, control inflammatory root resorption, and prevent interappointment contamination by functioning as a physicochemical barrier against residual and invading microorganisms.^[12] An ideal intracanal medicament should be biocompatible, non-staining, easily retrievable, compatible with obturating materials, and possess pulp tissue dissolving ability. Based on chemical composition, intracanal medicaments have traditionally been classified into phenolic compounds (e.g., eugenol, camphorated monochlorophenol), aldehydes (formocresol), halides (iodine, potassium iodide), calcium hydroxide, antibiotics, and various combinations.^[13] However, most of these agents are now rarely used due to concerns regarding toxicity, immune suppression, and the emergence of resistant microbial strains.^[14] A crucial property of irrigants and medicaments is their pulp tissue-dissolving efficacy. Calcium hydroxide, a commonly used medicament, demonstrates only limited pulp tissue dissolution, whereas sodium hypochlorite (NaOCl), the gold standard irrigant, exhibits strong tissue-dissolving capacity. However, NaOCl is known for its cytotoxicity and caustic effects on periapical and oral tissues. This necessitates the exploration of safer alternatives with comparable efficacy. Herbal medicine, once considered outside mainstream medicine, has gained renewed attention as scientific evidence increasingly validates its role in disease treatment and prevention.^[15] Herbal medicine, also referred to as botanical medicine or phytomedicine, employs plant parts such as leaves, roots, seeds, flowers, and bark for therapeutic purposes. The World Health Organization estimates that nearly 80% of the global population relies on herbal medicines for some aspect of primary healthcare.^[16] Their advantages include abundance, biocompatibility, minimal side effects, and the absence of resistance even after prolonged use.^[17] *Ocimum sanctum* (Holy Basil, Tulsi), a plant native to India and revered since the Vedic period, is classified as a “*rasayana*”, a rejuvenating herb believed to promote longevity and holistic health. It exhibits diverse pharmacological properties, including antibacterial, antifungal, antiviral, and anti-inflammatory effects.^[18,19] It has shown inhibitory activity against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus anthracis*, and *Mycobacterium tuberculosis*, as well as therapeutic benefits in conditions such as viral hepatitis, viral encephalitis, and arthritis.^[18,19] Additionally, *Ocimum sanctum* demonstrates strong analgesic properties, providing relief from headaches and body aches.^[20] Traditionally, powdered Tulsi leaves have been used as a dentifrice, often mixed with mustard oil for gum massage and tooth cleansing. These applications are believed to reduce bad breath, prevent dental caries, and promote oral health by alleviating infections and ulcers.

Although a few studies have evaluated herbal extracts as intracanal medicaments,^[21-23] no research has yet investigated the pulp tissue-dissolving efficacy of essential oil extracted from *Ocimum sanctum*. Hence, the present pilot ex vivo study was undertaken to evaluate the tissue-dissolving potential of *Ocimum sanctum* essential oil and compare its efficacy with sodium hypochlorite, with the aim

of proposing it as a safer alternative for use as an intracanal irrigant or medicament.

The present study aimed to evaluate the pulp tissue dissolving efficacy of *Ocimum sanctum* (Tulsi) essential oil extract. Specifically, it sought to assess, under ex vivo conditions, its tissue-dissolving ability at two different concentrations, 50% and 100%.

MATERIALS AND METHODS

The present study was a pilot ex vivo experimental study that was conducted in the Department of Conservative Dentistry and Endodontics, Post Graduate Institute of Dental Education and Research (PGIDER), and Department of Pharmacology, Indira Gandhi Institute of Medical Sciences (IGIMS), Patna, Bihar, India.

Preparation of *Ocimum sanctum* Essential Oil Extract: The essential oil of *Ocimum sanctum* was prepared in the Department of Pharmacology using a Clevenger apparatus [Figure 1]. Fresh leaves were collected, thoroughly cleaned, shade-dried, and finely powdered. A total of 100 g of the powdered leaves was mixed with 75 mL of glycerin and 175 mL of distilled water in a 1 L distilling flask. A porous earthenware piece and strips of filter paper (15 cm in diameter, cut into 7–12 mm width) were added to aid in distillation. The flask was then connected to the still head of the Clevenger apparatus.^[24]

Before attaching the condenser, water was introduced into the graduated receiver with tap T kept open until overflow occurred at point P. Air bubbles in the India rubber tubing (a–b) were carefully removed, following which the condenser was attached. The contents were heated with intermittent agitation until boiling commenced. Distillation was carried out at a rate sufficient to keep the condenser cool, and the flask was rotated occasionally to prevent residue adherence to the sides.

At the end of each cycle, heating was discontinued and the apparatus was cooled for 10 minutes. Tap T was opened, and tube L1 was lowered to allow the oil layer to enter the graduated receiver, where its volume was recorded. The oil was then returned to the bulb, and distillation was continued for another hour. This process was repeated until successive readings showed no variation, at which point the yield was recorded as the volatile oil content of *Ocimum sanctum* [Figure 2]. The extracted oil was stored in amber-colored bottles at 4°C until further use.

Collection and Preparation of Pulp Tissue: Six freshly extracted impacted human third molars, indicated for removal on therapeutic grounds, were collected after obtaining informed consent. Immediately after extraction, the teeth were grooved longitudinally with a fissure bur and split into halves. Pulp tissue was carefully extirpated using a sterile spoon excavator, rinsed in distilled water to remove blood and debris, and sectioned into 12 uniform fragments. Each fragment was weighed on an analytical balance (Denver SI-S234, Germany) with an accuracy of 0.001 g.

Experimental Groups:

The tissue samples were randomly allocated into four experimental groups:

- **Group I:** 100% *Ocimum sanctum* essential oil extract.
- **Group II:** 50% *Ocimum sanctum* essential oil extract (prepared by mixing 2 mL of 100% extract with 2 mL of

- dimethyl formamide, an inert solvent).
- Group III (Positive Control):** 5.25% sodium hypochlorite (NaOCl) solution.
- Group IV (Negative Control):** Distilled water.

Each pulp fragment was immersed in 4 mL of the respective solution in sterile test tubes, which were sealed and incubated at 37°C to simulate physiological conditions.

Evaluation of Pulp Dissolution: The pulp fragments were observed at intervals of 24, 48, and 96 hours by an independent evaluator blinded to the test groups. The dissolution time was recorded as the period between immersion of the pulp fragment in the test solution and its complete disappearance. The dissolution speed was calculated by dividing the initial weight of the pulp fragment by the time taken for complete dissolution.

RESULTS

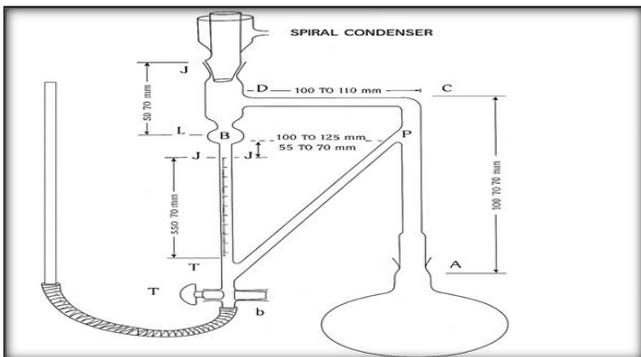


Figure 1: Clevenger apparatus: Steam distillation for Essential oil extract of Ocimum Sanctum (Tulsi).

Pulp Tissue Dissolving Efficacy of Ocimum sanctum Extract: Neither the 50% nor the 100% concentrations of Ocimum sanctum essential oil extract demonstrated any pulp tissue dissolving activity at any of the evaluated time intervals (24, 48, and 96 hours). Complete absence of dissolution was consistently observed in both groups. In contrast, the positive control group (5.25% sodium hypochlorite) showed complete dissolution of pulp tissue within a comparatively short period, confirming its well-documented tissue-dissolving capacity. The negative control group (distilled water) did not exhibit any dissolution, as

expected.

Taken together, these findings indicate that Ocimum sanctum essential oil extract lacks pulp tissue-dissolving efficacy. While it cannot serve as an alternative to sodium hypochlorite for tissue dissolution, its antimicrobial and anti-inflammatory properties may still justify its role as an intracanal medicament rather than as a primary irrigant [Table 1 and Figure 3].



Figure 2: 100% Essential Oil Extract of Ocimum sanctum (Tulsi).

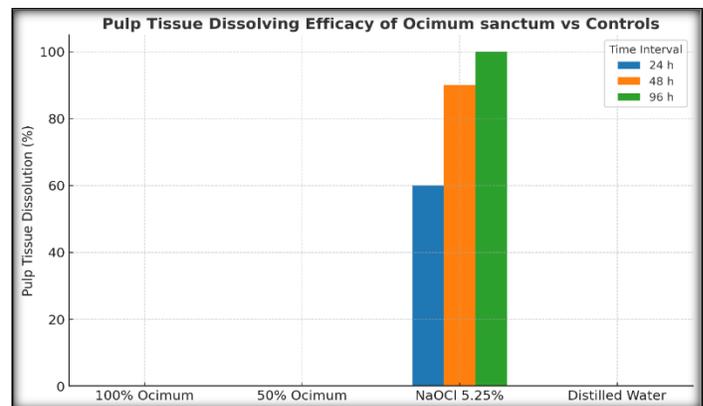


Figure 3: Pulp tissue dissolution (%) of different test solutions at 24, 48, and 96 hours.

Table 1: Pulp tissue dissolution outcomes in different experimental groups

Group	Test Solution	Dissolution at 24 h	Dissolution at 48 h	Dissolution at 96 h	Final Outcome
I	100% Ocimum sanctum essential oil	No dissolution	No dissolution	No dissolution	No pulp tissue dissolution
II	50% Ocimum sanctum essential oil	No dissolution	No dissolution	No dissolution	No pulp tissue dissolution
III	5.25% Sodium hypochlorite (Positive control)	Partial dissolution	Almost complete dissolution	Complete dissolution	Complete pulp tissue dissolution
IV	Distilled water (Negative control)	No dissolution	No dissolution	No dissolution	No pulp tissue dissolution

DISCUSSION

The antimicrobial and anti-inflammatory properties of Ocimum sanctum have already been demonstrated in our

previous work, and therefore, in this study, the focus was limited to evaluating its pulp tissue dissolving potential. Ocimum sanctum (Tulsi), native to India and known since the Vedic

period, belongs to the family Labiatae and exists in three varieties: Sri Tulsi, Krishna Tulsi, and Vana Tulsi. It is commonly referred to as Tulsi (Hindi), Manjari (Sanskrit), and Holy Basil (English).^[25,26] The plant is a stout, erect, aromatic herb growing up to 75–90 cm in height, covered with fine hairs. Its tender leaves, usually smooth, measure up to 2.5 cm, and it bears inflorescences in the form of long spikes with small purple flowers. The plant is characterized by its bitter and pungent taste. Chemically, it contains essential oils such as eugenol, eugenal, carvacrol, methyl chavicol, limatrol, and caryophylline, along with several biologically active compounds including ursolic acid, apigenin, and luteolin.^[27,28]

Bystrom et al. emphasized that the use of biocompatible intracanal medicaments with both antimicrobial and tissue-dissolving properties can help eliminate or reduce bacteria within the root canal system between appointments, thereby improving treatment outcomes.^[29] For an intracanal medicament to be effective, it must diffuse into dentinal tubules at concentrations sufficient to overcome the dentin's buffering capacity.^[29] It has been suggested that vapor-based delivery of medicaments may be advantageous in achieving adequate distribution into canal irregularities, thus enhancing the effectiveness of root canal therapy.^[30] Examples of vaporizing intracanal medicaments include formocresol, camphorated monochlorophenol, merthiolate, metacresyl-acetate, beechwood creosote, and glutaraldehyde.^[31–33] Although these are effective against some microorganisms associated with periradicular disease, such as *Streptococcus* species, they have shown limited efficacy against *Enterococcus faecalis*.^[34] Furthermore, their clinical use is restricted due to high toxicity, strong antigenicity, and loss of effect after only a few days.^[35,36]

Other conventional agents like calcium hydroxide and antibiotic-containing pastes, though widely used, lack vaporizing action and therefore cannot effectively penetrate the complexities of the root canal system.^[22,23] This limitation has driven research towards safer, biocompatible alternatives with stronger antimicrobial activity and potential tissue-dissolving effects. One such herbal option is *Ocimum sanctum* (Tulsi), which has been extensively used in traditional Indian medicine. The bioactive ingredients responsible for its antimicrobial properties include linoleic acid, linolenic acid, eugenol (1-hydroxy-2-methoxy-4-allylbenzene), and carvacrol.^[37]

The antibacterial mechanism of Tulsi oil has been linked to malondialdehyde, a breakdown product of peroxidized polyunsaturated lipids such as linoleic and linolenic acids. Malondialdehyde acts as a cross-linker that induces oxidative damage in bacterial DNA and RNA, ultimately preventing replication and causing bacterial death.^[38] Another mechanism involves malondialdehyde cross-linking with amino groups of bacterial enzymes, disrupting metabolic processes and leading to cell death.^[39]

The antimicrobial efficacy of Tulsi's volatile essential oil extract has been studied against *Enterococcus faecalis*, a Gram-positive, facultative anaerobe frequently implicated in failed root canal treatments. This bacterium is known for its resilience to antimicrobial agents and ability to survive in a

viable but nonculturable state under stress.^[40] Multiple studies have reported the limited effectiveness of calcium hydroxide and other intracanal medicaments against *E. faecalis*. Pooja et al. demonstrated that an ethanolic extract of *O. sanctum* at 4% concentration effectively inhibited the growth of *Streptococcus mutans*.^[41] Despite such promising results, many conventional medicaments and herbal extracts have shown shortcomings in terms of antibacterial activity, penetration, biocompatibility, or long-term substantivity.^[22,23] The anti-inflammatory properties of *O. sanctum* are attributed to alpha-linolenic acid, an omega-3 fatty acid that inhibits both cyclooxygenase and lipoxygenase pathways, thus demonstrating dual action.^[42] Alpha-linolenic acid undergoes metabolic conversion to compounds such as stearidonic acid and eicosapentaenoic acid (EPA). EPA, in particular, has been shown to competitively inhibit the synthesis of prostaglandins and leukotrienes while modulating mediators like histamine, serotonin, and kinins. Additionally, Tulsi oil may exert anti-granulomatous effects by regulating AP-1 transactivation, thereby inhibiting epidermal and fibroblast growth factors, which contribute to its antiproliferative properties.^[43]

In the present study, the pulp tissue dissolution potential of 50% and 100% essential oil extracts of *Ocimum sanctum* was evaluated. However, no tissue dissolution was observed at 24, 48, or 96 hours. These findings suggest that although *O. sanctum* oil possesses significant antimicrobial and anti-inflammatory properties, it lacks pulp tissue dissolving capability. Hence, while it may serve as a useful intracanal medicament, it cannot replace agents with proven tissue-dissolving efficacy.^[44]

Limitations of the study: This study was conducted on a limited sample size under ex vivo conditions, which, while providing controlled observations, may not fully replicate the complexities of in vivo scenarios. Further large-scale clinical studies are encouraged to validate and expand these findings.

CONCLUSION

The essential oil extract of *Ocimum sanctum* (Tulsi), at both 50% and 100% concentrations, does not possess pulp tissue dissolving efficacy at any of the tested contact periods (24, 48, and 96 hours). While *Ocimum sanctum* demonstrates proven antimicrobial and anti-inflammatory properties, its inability to dissolve pulp tissue restricts its role as a primary irrigant or tissue-dissolving agent in root canal therapy. However, its biocompatibility, antimicrobial activity, and anti-inflammatory potential support its use as an intracanal medicament, where it may serve as an adjunct in root canal disinfection and periapical healing.

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Conflicts of interest

There are no conflicts of interest.

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