

Evaluation of Pulp tissue dissolving ability of Ocimum Sanctum's (Tulsi) Essential Oil Extract for Its Proposed Use as an Intracanal Irrigant/Medicament. A pilot ex–vivo study

¹Dr Navin Mishra, Asst Professor Conservative Dentistry and Endodontics, Dept of Dentistry, Sub Dean Research , IGIMS, Patna

²Dr Krishna Biswas ,Senior Resident, Lady Harding Medical College, New Delhi

³Dr Isha Narang Consultant, Endodontist HCMS, Haryana, Civil Hospital, Gurugram

⁴Dr Jawed Iqbal, Maxillofacial Surgeon Dept of Dentistry, IGIMS, Patna

Corresponding Author: Dr Krishna Biswas, Senior Resident, Lady Harding Medical College, New Delhi

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Abstract

Ocimum sanctum (O.sanctum) is a plant, native to India and has been known from as early as Vedic period. It belongs to Labiateae family having three varieties: Sri Tulsi, Krishna Tulsi and Vana Tulsi and is also known by the following common names Tulsi (Hindi), Manjari (Sanskrit), Holy Basil (English).

In this present study pulp tissue dissolving ability Ocimum Sanctum's (Tulsi) essential oil extract in concentrations of 100 % and 50 % was done after steam distillation through cleverger apparatus and was tested for contact period of 24, 48 and 96 hours. This study showed that there was no pulp tissue dissolution at all contact periods but still it can be used as intra canal medicament for its anti-inflammatory and antimicrobial properties.

Keywords: Ocimum Sanctum, Essential Oil Extract, Tulsi, Pulp Tissue

Introduction

Role of microorganisms in the development and maintenance of pulpal and periapical inflammation have been well documented. As the host defense loses its access to the necrotic pulp space, opportunistic microorganisms selected by harsh ecological conditions and the low-oxygen environment aggregate in the root canal system.¹ Primary root canal infections are polymicrobial, typically dominated by obligate anaerobic bacteria.² These are rather easily eradicated during root canal treatment. On the other hand, facultative bacteria such as Streptococci, Enterococci, and Lactobacilli, once established, are more likely to survive chemomechanical instrumentation and root canal medication.³ In particular Enterococcus faecalis, has gained attention in the endodontic literature, as it can frequently be isolated from root canals of non surgical retreatment cases.^{4,5} The success of endodontic therapy

largely depends on the elimination of these microbial contamination from the root canal system.

The objectives of root canal instrumentation are thorough debridement, creation of optimum space for delivery of antimicrobial substances and to facilitate 3D obturation of the root canal system to prevent recolonization by oral microbiota.⁶

Mechanical instrumentation alone or in combination with an irrigating solution neither adequately reduce viable microorganisms in the infected root canal system^{7, 8} nor the formation of a smear layer can be prevented.⁹ With both current nickel titanium instrumentation systems and traditional stainless-steel hand instruments, almost half of the root canal walls are left unprepared.¹⁰

Hence use of inter appointment medicament has been widely advocated to help eliminate remaining bacteria within the root canal system left after chemomechanical preparation, reduce periapical inflammation, pain and induce healing.¹¹ They also help to reduce apical exudates, control inflammatory root resorption, and prevent contamination between appointments¹² by acting as a physico chemical barrier, precluding the proliferation of residual microorganisms and preventing reinfection of the root canal by bacteria from the oral cavity.

Ideal requirements for an intra canal medicament are that they should be biocompatible, easily retrievable, non staining and have no effect on obturating materials and have pulp dissolving abilities.

Intra canal medicaments can be classified on the basis of their chemical composition into phenolic compounds (e.g. eugenol and camphorated monochlorophenol), aldehydes (Formocresol), halides (e.g. iodine potassium iodide), calcium hydroxide, antibiotics, and various combinations.¹³ The majority of these preparations are not used in contemporary endodontic practice due to reported

toxicity, development of resistant strains, and suppression of the immune system.¹⁴

A very important characteristic of Irrigant/ Medicament is to have pulp tissue dissolving efficacy. Calcium Hydroxide is an intracanal medicament which has slight pulp tissue dissolving efficacy while Irrigant such as sodium hypochlorite is most efficacious agent having greatest dissolving efficacy but it is caustic and harsh to oral tissues.

Change is the law of nature. Long practiced outside of conventional medicine, herbal therapy is getting importance as research shows their value in the treatment and prevention of diseases.¹⁵ Herbal medicine, also called “botanical medicine” or “phytomedicine”, refers to the use of plant seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. Recently, the World Health Organization estimated that 80% of people worldwide relied on herbal medicines for some aspect of their primary healthcare.¹⁶

The benefits of herbal medicine are its abundance, biocompatibility with minimum side effects and no resistance development on its prolonged and repeated use.¹⁷

Ocimum sanctum (Holy basil, Tulsi) is a plant native to India and has been known to have medicinal properties from as early as Vedic period. It is classified as a “**rasayana**”, - a herb that nourishes a person’s growth to perfect health and promotes long life.

Several medicinal properties have been attributed to Ocimum sanctum. It has antibacterial, antifungal and antiviral properties. It inhibits the growth of Staphylococcus aureus, Escherichia coli, Bacillus anthracis, Mycobacterium tuberculosis etc. It significantly shortens the course of illness, clinical symptoms and the biochemical parameters in patients with viral hepatitis and viral encephalitis.¹⁸ Extract of Ocimum sanctum has been

found to inhibit acute as well as chronic inflammation and is found useful in the treatment of arthritis.¹⁹ It has a strong analgesic effect, reduces headache and body ache.²⁰ The leaf, dried in the sun and powdered, has been used as a dentrifice. It can be mixed with mustard oil to make a paste and used as toothpaste. The herb is useful in counter acting bad breath and for massaging the gums. It prevents dental caries and has a therapeutic effect in oral infections and ulcers in the mouth.²¹

There are few studies on the use of herbal extract as intracanal medicament.^{22, 23} however no study has yet been reported on essential oil extract of *Ocimum sanctum*.

The aim of the present study was to evaluate the essential oil extract of *Ocimum sanctum* for its tissue dissolving property for its proposed use as an intracanal Irrigant/medicament as is non toxic to oral tissues in comparison to sodium hypochlorite.

Aim:

This study was undertaken to evaluate the essential oil extract of *Ocimum sanctum* for its pulp tissue dissolving property.

Objectives

1. To evaluate ex-vivo its pulp tissue dissolving efficacy at 50% and 100 % concentration.

This study was undertaken in the Department of Conservative Dentistry and Endodontics, Center for Dental Education and Research in collaboration with the Department of Pharmacology, All India Institute of Medical Sciences, New Delhi, India.

Materials and Methodology

Preparation of essential oil extract of *Ocimum sanctum*:

The oil was prepared in the Department of Pharmacology using Clavinger apparatus (line drawing of the apparatus in figure 1)

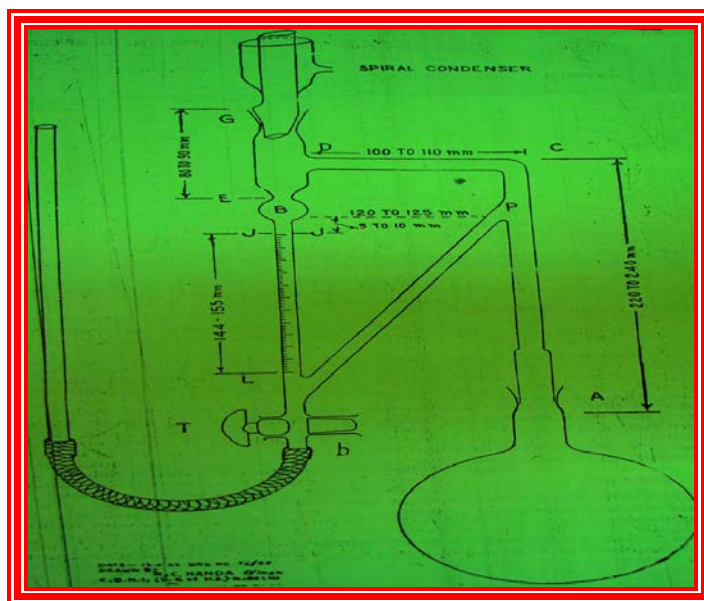


Figure 1. Clavenger apparatus: Steam distillation for Essential oil extract of *Ocimum Sanctum* (Tulsi).

Fresh leaves of *Ocimum sanctum* were collected, cleaned, dried and powdered. 100 grams of the prepared powder was mixed with 75 ml of glycerin and 175 ml of water. The mix was then taken in a 1litre capacity distilling flask. In this, a porous earthen ware and a filter paper measuring 15 cm in diameter which was cut into small strips, of 7 to 12 mm wide were put. It was then connected to the still head of the Clavenger apparatus.²⁴ Before attaching the condenser, water was run into a graduated receiver, keeping the tap T open until the water overflowed at P. Any air bubbles in the India rubber tubing (a-b) were carefully removed by pressing the tube. The tap was then closed and the condenser attached. The contents of the flask were heated and stirred by frequent agitation until ebullition commenced. The distillation was continued at a rate to keep the lower end of the condenser cool. The flask was rotated occasionally to wash down any material that adhered to its sides.

At the end of specified time heating was discontinued. The apparatus was allowed to cool for 10 minutes and the tap T was opened and the tube L1 lowered slowly. As the layer of the oil completely entered into the graduated part

of the receiver the tap was closed and the volume was read.

The tube L1 was raised till the level of water in it was above the level of B, then the tap T was slowly opened to return the oil to the bulb. The distillation was continued for another hour and the volume of oil was again read, after cooling the apparatus as before. The distillation was continued until successive readings of the oil did not differ. The measured yield was taken to be the content of volatile oil in the drug. Figure 2.



Figure 2. Essential oil extract of Tulsi 100%

Pulp tissue dissolving efficacy of 50% and 100% extract of Ocimum sanctum

The pulp tissues were obtained from six impacted human third molars slated for extraction. Immediately after extraction, six teeth were longitudinally grooved with a fissure bur and then split in half. The dental pulps were extracted carefully using a spoon excavator. All the pulp tissue samples were removed, washed with distilled water and divided into 12 fragments. All the fragments were weighed in an analytical balance (Denver SI-S234, Germany) and placed in contact with 4 ml of 50% and 100% essential oil extract of Ocimum sanctum in all six

test tubes. 50% extract was prepared by adding 2ml of inert solvent dimethyl formamide to 2ml of 100% extract. The readings of the samples were taken at 24, 48 and 96 hours by an impartial evaluator.

Time of the dissolution was assessed from the time when the pulp tissue fragments were placed in the test tubes in contact with 100% extract of essential oil of *O. sanctum* till total dissolution of the specimens was observed. Dissolution speed was calculated as weight of fragment divided by dissolution time.

Results

Pulp tissue dissolving efficacy of 50% and 100% extract of Ocimum sanctum

There was no pulp tissue dissolving efficacy exhibited either by 50% or 100% essential oil extract of *Ocimum sanctum* at all contact periods. Hence our current research to test the pulp dissolving efficacy of essential oil extract of *Ocimum Sanctum* showed it has no pulp dissolving action and hence can only be used as an antimicrobial agent or as an anti-inflammatory agent in root canal treatment as an intracanal medicament.

Discussion

The antimicrobial and anti inflammatory properties have been tested and proved in our previous study and hence in this study only pulp tissue dissolving efficacy was tested.⁴⁴ *Ocimum sanctum* (*O. sanctum*) is a plant, native to India and has been known from as early as Vedic period. It belongs to Labiateae family having three varieties: **Sri Tulsi, Krishna Tulsi and Vana Tulsi** and is also known by the following common names Tulsi (Hindi), Manjari (Sanskrit), Holy Basil (English).^{25,26} It is a branched, erect stout and aromatic herb. It grows up to the height of about 75 cm to 90cm and is hairy all over. The plant has tender leaves, usually smooth, and measures up to 2.5cm in length. This herb is bitter and pungent. The inflorescence is a long spike with tiny purple flowers. The essential oils

are eugenol, eugenal, carvacrol, methyl-chavicol, limatrol and caryophylline and many more biologically active compounds like ursolic acid, apigenin and luteolin.^{27,28}

Bystrom et al. emphasized the fact that the use of a **biocompatible** intracanal medicament possessing antimicrobial properties and tissue dissolving properties between appointments may reduce or eliminate bacteria in the root canal system and increase the success of root canal treatment.

For intracanal medicaments to be effective against bacteria they should be able to diffuse into the dentinal tubules at sufficient concentrations and exceed the dentin buffering ability.²⁹ It has been suggested that use of the medicament's vapors might be the best method to regulate the dose and still get the medicament into all the irregularities within the root canal system for successful outcome of root canal therapy.³⁰

Intracanal medicament having vapourizing action are formocresol, Camphorated mono chloro phenol, Merthiolate, metacresylacetate, beechwood creosote, and glutaraldehyde.^{31,32,33} Although they are effective against certain microorganism implicated in peri radicular disease, alpha hemolytic streptococci and enterococci have been found to be resistant to the vapors of these medicaments.³⁴ Moreover they have a limited role because they are extremely toxic and antigenic and its effect is lost after a few days.^{35, 36} The other commonly used intracanal medicaments like calcium hydroxide and antibiotic containing paste have no vapourizing action and hence limited access to the complexities of the root canal system to exert their antibacterial action.

Search for biocompatible or least toxic medicament with high antimicrobial potency and pulp tissue dissolving efficacy has led the researchers towards herbal/botanical therapy. One such product with wide usage in Indian context is *Ocimum sanctum* (Tulsi).

Ingredients of Tulsi having Anti-microbial activities are linoleic acid, linolenic acid, eugenol (1-hydroxy-2-methoxy-4-allylbenzene), and carvacrol.³⁷ The mechanism of antibacterial action of this oil is due to the formation of **Malondialdehyde**, an aldehyde formed as a breakdown product of, Linoleic acid and linolenic acid which are peroxidized polyunsaturated lipids. **Malondialdehyde** is a cross linker and initiates oxidation reactions in which undesirable bonds formation occurs in bacterial DNA and RNA³⁸ resulting in inhibition of replication of DNA and bacterial death. The other proposed mechanism of action of the extract is that **Malondialdehyde** crosslink with different amino groups of various bacterial enzymes jeopardizing the metabolic processes in the bacteria culminating in their death.³⁹

Antimicrobial efficacy of volatile essential oil extract of *Ocimum sanctum* was tested against *Enterococcus faecalis*, which are Gram-positive, nonspore-forming, non motile, fermentative, facultative anaerobic cocci. It is the most commonly isolated or detected species from failed root canal therapy. Its high resistance to antibacterial substances is widely documented, and this bacterium can enter in a viable but nonculturable state during environmental stress.⁴⁰ There are several studies on the ineffectiveness of calcium hydroxide and other commonly used intracanal medicaments against it.

Pooja et al studied the antimicrobial action of different concentrations *O. sanctum*'s ethanolic extract on *Streptococcus mutans*. They found that 4% of the extract was most effective in curtailing their growth.⁴¹

Various medicaments and few herbal extract are tried as root canal medications or irrigants, but they showed ineffectiveness either as an antibacterial agent or in terms of penetrability, biocompatibility and substantivity.^{22, 23}

The anti inflammatory action of essential oil extract of *Ocimum sanctum* is due to the presence of alpha linolenic

acid. It is an omega-3 fatty acid which inhibits both lipoxygenase and cyclooxygenase pathways i.e. it has dual inhibitory actions.⁴² The alpha linolenic acid is progressively metabolized to 6, 9, 12, 15 octadecatetraenoic acids, stearadonic acid and eicosapentaenoic acid, which are the end products. Eicosapentaenoic has the capacity to competitively inhibit the formation of prostaglandins, leukotrienes and also acts on histamine, serotonin and kinins. Another proposed mechanism for its antigranulomatous efficacy is its ability to regulate the AP-1 transactivation.⁴³ This results of inhibition of epidermal and fibroblast growth factors, gives the oil its anti proliferative property.

In the present study, pulp tissue dissolving efficacy of 50% and 100% essential oil extract of *Ocimum sanctum* was evaluated. However, it showed no dissolution of tissues at 24, 48 and 96 hrs contact period. However *Ocimum sanctum* oil has both antimicrobial properties and anti inflammatory properties but no pulp tissue dissolving capabilities and can be used as an Intracanal medicament.⁴⁴

Conclusion

This study was undertaken to evaluate the pulp tissue dissolving efficacy of essential oil extract of *Ocimum sanctum*. The oil extract was prepared in the Clavingers apparatus and its pulp tissue dissolving efficacy was tested ex-vivo for two concentrations 50% and 100% at 24, 48 and 96 hours contact period.

There was no pulp tissue dissolving efficacy exhibited either by 50% or 100% essential oil extract of *Ocimum sanctum* at all contact periods.

References

1. Nair PN. Pathogenesis of apical periodontitis and the causes of endodontic failures. Crit Rev Oral Biol Med 2004; 15:348–81

2. Sundqvist G. Taxonomy, ecology, and pathogenicity of the root canal flora. Oral Surg Oral Med Oral Pathol 1994; 78:522–30
3. Chavez LE, Dahlén G, Molander A, Moller A, Bergenholtz G. Bacteria recovered from teeth with apical periodontitis after antimicrobial endodontic treatment. Int Endod J 2003; 36:500–8
4. Engstrom B. The significance of Enterococci in root canal treatment. Odontol Revy 1964; 15:87–106
5. Haapasalo M, Ranta K, Ranta H. Facultative Gram-negative enteric rods in persistent periapical infections. Acta Odontol Scand 1983; 91:458–63
6. Waltimo TM, Sirén EK, Torkko HL, Olsen I, Haapasalo MP. Fungi in therapy-resistant apical periodontitis. Int Endod J 1997; 30:96–101
7. Schilder H. Cleaning and shaping the root canal. Dent Clin North Am 1974; 18:269-96
8. Grahén H, Krasse B. The effect of instrumentation and flushing of non-vital teeth in endodontic therapy. Odontol Revy 1962; 13:167–77
9. Bystrom A, Sundqvist G. Bacteriologic evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy. Scand J Dent Res 1981; 89:321–8
10. Mayer BE, Peters OA, Barbakow F. Effects of rotary instruments and ultrasonic irrigation on debris and smear layer scores: a scanning electron microscopic study. Int Endod J 2002; 35:582–9
11. Peters OA. Current challenges and concepts in the preparation of root canal systems: a review. J Endod 2004; 30:559–67
12. Bystrom A, Sundqvist G. Bacteriologic evaluation of the effect of 0.5 percent sodium hypochlorite in endodontic therapy. Oral Surg Oral med Oral Pathol 1983; 55:307-1

13. Ikhlas EL, John K. The antimicrobial effect of root canal irrigation and medication. *Oral Surg Oral Med Oral Pathol* 2007;103:560-69
14. Harrison W. The clinical toxicity of endodontic medicaments. *J Endod* 1979; 5:2-6
15. Santos LL, Vilhena D E, Toledo, Medeiros S, P Souza. The use of herbal medicine in Alzheimer's disease-a systematic review. *Evid Based Complement Alternat Med* 2006; 3(4):441-5
16. Pulok K. Mukherjee A, Wahile L. Integrated approaches towards drug development from Ayurveda and other Indian system of medicines. *Jour Ethnopharmacol* 2006; 103: 25-3
17. Chomna, Mullika, Gritsanapan W. Antimicrobial effects of Thai medicinal plants against acne inducing bacteria. *Journ Ethno Pharmacol* 2005; 101:330-333
18. Rajeshwari S. *Ocimum sanctum*- The Indian home remedy. *Current Medical Scene* 1992;12: 21-23
19. Singh and Majumdar D K. Effects of fixed oil Of *Ocimum sanctum* against experimentally induced arthritis and joint edema in laboratory animals. *Int J Pharmacog* 1996; 34: 218
20. Singh and Majumdar D K, Analgesic activity of *Ocimum sanctum* and its possible mechanism of action. *Int J Pharmacog* 1995; 33:188-91
21. Prabhakar S. Herbal remedies. 2008; 43: 12-13
22. Badr AE, Omar F A. A laboratory evaluation of the antibacterial and cytotoxic effect of Liquorice when used as root canal medicament. *Int Endod J* 2010; 43:1176-79
23. Prabhakar J, Senthilkumar M, Priya S, Mahalakshmi K, Sehgal P.K, Sukumaran G. Evaluation of Antimicrobial Efficacy of Herbal Alternatives (Triphala and Green Tea Polyphenols), MTAD, and 5% Sodium Hypochlorite against *Enterococcus faecalis* Biofilm Formed on Tooth Substrate: An In Vitro Study. *J Endod* 2010; 36:83-86
24. Mukherjee B. *Indian Pharmacopeia*. 2nd edition 1966: 944-47
25. Ghosh G. *New Approaches to Medicine and Health*. 1995; 3: 23-29.
26. Phillip MP, Damodaran NP. Chemo-types of *Ocimum sanctum*. *Indian Perfumer* 1985; 29: 49-56
27. Skaltsa H, Philianos S, Singh M. Phytochemical study of the leaves of *Ocimum sanctum*. *Fitoterapia* 1987; 8: 286
28. Norr H, Wanger H. New constituents from *Ocimum sanctum*. *Planta Med* 1992; 58: 574
29. Siquerira JF, Lopes H. Mechanisms of antimicrobial activity of calcium hydroxide: a critical review. *Int Endod J* 1999; 32:361-9
30. Schilder H. Cleaning and shaping the root canal. *Dent Clin North Am* 1974; 18:269-96
31. Wesley, Marshall D.J, Rosen F.J. The quantization of formocresol as a root canal medicament. *Oral Surg* 1965. 29:603-6
32. Bender I.B, Seltzer S, Turkenk S. To culture or not to culture. *Oral Sur* 1964.18:527
33. Pear J.R. Bactericidal effects of some drugs used in pulp canal therapy. 1970. *J Am Dent Assoc* 29:244-47
34. Cwikla J.R. The vaporization and capillarity effect of endodontic medicaments. *Oral Surg* 1972; 34:11
35. Vander W, Dowson G.L, Shipman C. Antibacterial efficacy and cytotoxicity of three drugs. *Oral Surg* 1971; 33:230
36. Harrison, J.W, Madonia J.V. The toxicity of parachlorophenol. *Oral Surg* 1971; 32:9
37. Singh S, Malhotra M, Majumdar D K, Antibacterial activity of *Ocimum sanctus* L. fixed oil, *Indian J Exp Biol* 43 (2005) 83

38. Hall E D, Oostveen J A, Andrus P K, Anderson D K and Thomas C E, Immunocytochemical method for investigating in vivo neuronal oxygen radical induced lipid peroxidation. *J Neurosci Methods* 1997; 76 :115-17
39. Luczaj W and Skrzydlewska E, A damage caused by lipid peroxidation products, *Cel Mol Biol Let* 2003; (8): 391-93
40. Spangberg L, Engstrom B, Langeland K. Biologic effects of dental materials and toxicity and antimicrobial effect of endodontic antiseptics in vitro. *Oral Surg* 1973; 36:856
41. Goldstein SA, Shemano L, Daweo R, Betler J. Cotton pellet ganuloma pouch method for evaluation of anti-inflammatory activity. *Arch Pharmacodyamic Ther* 1976; 294-301
42. Di Martino M J, Campbell G, Wolff C, Hanna N, The pharmacology of arachidonic acid-induced rat paw edema, *Agents Actions* 1987; 21: 303-4
43. Liu G, Bibus D, Bode A, Ma W, Holman R, Dong Z, Omega -3 but not omega-6 fatty acids inhibits AP-1 activity and cell transformation in JB6 cells. *Med Sci* 2001;98: 75-80
44. Mishra N et al, Preliminary Ex-vivo and an Animal Model Evaluation of Ocimum sanctum's Essential Oil Extract for its Antibacterial and Anti- Inflammatory Properties. *Or He Dent Mang* 2013; 12(3):174-9