

Preliminary *Ex-vivo* and an Animal Model Evaluation of *Ocimum sanctum*'s Essential Oil Extract for its Antibacterial and Anti- Inflammatory Properties

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Abstract

Aim: To evaluate the essential oil extract of the au *Ocimum sanctum* for its antibacterial and anti inflammatory efficacy.

Methods: The essential oil extract was prepared in the Clevenger's apparatus. Antibacterial efficacy was tested against *Enterococcus faecalis* at two concentrations i.e. 100 & 50%. Bacterial growth was measured spectrophotometrically and percentage inhibition was calculated. Anti- inflammatory efficacy was tested on an established adult albino rat model. Autoclaved cotton pellets were used for inducing the chronic inflammatory conditions for 7 days. On day 8, the cotton pellets induced granuloma were excised, dried and weighed. The percentage inhibition of the granuloma was calculated.

Results: The essential oil extract of *Ocimum sanctum* showed antibacterial efficacy, which improved with an increase in concentration and contact period. It also had a significant ($p=0.034$) anti- inflammatory action.

Conclusion: This *ex-vivo* and an animal model study documented the antibacterial and anti- inflammatory properties of the essential oil extract of *Ocimum sanctum*, for its proposed use as an intracanal medicament.

Key Words: *E. faecalis*, Herbal Medicine, Endodontics, *Ocimum sanctum*

Introduction

Role of microorganisms in the development and maintenance of pulpal and periapical inflammation have been well documented. Primary root canal infections are polymicrobial, typically dominated by obligate anaerobic bacteria [1]. The success of endodontic therapy largely depends on the elimination of these microbial contaminations from the root canal system. The objectives of root canal instrumentation are thorough debridement, creation of an optimum space for delivery of antimicrobial substances and to facilitate 3D obturation of the root canal system to prevent recolonization by oral microbiota [2]. Mechanical instrumentation in combination with a chemically inert irrigating solution cannot adequately reduce viable microorganisms in the infected root canal system [3]. With the current nickel titanium and traditional stainless-steel instruments, almost half of the root canal walls were shown to be left unprepared [4]. Hence the use of intracanal medicaments has been widely advocated to help eliminate these remaining bacteria. 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 [5]. 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 [6].

Intracanal medicaments that exert their antibacterial action in a vapour form are formocresol, camphorated monochlorophenol, merthiolate, metacresylacetate, beechwood creosote, and glutaraldehyde [6,7]. Although, they are effective against certain microorganism implicated in periradicular

disease, alpha hemolytic streptococci and enterococci have been found to be resistant to the vapors of these medicaments. Moreover they have a limited role because they are extremely toxic, antigenic and its effect is lost after a few days.

The medicaments reduce periapical inflammation, pain and induce healing [7]. It also aids in controlling inflammatory root resorption and prevent contamination between appointments by acting as a physicochemical barrier, precluding the proliferation of residual intracanal microorganisms and preventing reinfection of the root canal by bacteria from the oral cavity [8].

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

Ocimum sanctum (Holy basil, Tulsi) is a plant native to India with known medicinal properties since the Vedic period. It is classified as a "rasayana", - a herb that nourishes a person's growth to perfect health and promotes long life. It has known antibacterial [9], antifungal [10] and antiviral properties [11]. Extracts of *Ocimum sanctum* inhibits acute as well as chronic inflammation and is used in the treatment of arthritis [12]. In addition, this oil has a strong analgesic effect [13]. The dried leaves of the plant can be powdered, mixed with mustard oil to make a dentrifice. It can prevent dental caries and aphthous ulcers in the mouth [14]. There are few studies documenting the use of herbal extracts as intracanal medicaments [15,16], however no study has been reported on essential oil extract of *Ocimum sanctum* as an intracanal medicament The aim of the present preliminary study was to evaluate the essential oil extract of *Ocimum sanctum* for its antibacterial, anti inflammatory property for its proposed use as an intracanal medicament.

Subjects and Methods

Aim

To evaluate the essential oil extract of *Ocimum sanctum* for its antibacterial and anti granulomatous efficacy.

Methods

Preparation of essential oil extract of *Ocimum sanctum*: The oil was prepared in Clevenger's apparatus (Clevenger, 1928) by steam distillation. Fresh leaves of *Ocimum sanctum* were collected, cleaned, sun dried and powdered. 100 grams of the prepared powder was mixed with 75 ml of glycerine and 175 ml of triple distilled water. The mix was then transferred into a distilling flask of the Clevenger apparatus. 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 1).

Antibacterial efficacy of *Ocimum sanctum*'s oil extract against *Enterococcus faecalis*: The oil extract was tested against *E. faecalis* (ATCC 29212) at two concentrations, i.e., 100% and 50%.

The bacterium was cultured on nutrient agar plates and incubated at 37°C for 24 hours. Two to three colonies were picked and suspended in 5 ml of Muller Hinton broth (Cheongmeak, Korea). These were incubated for 3-4 hours till a growth equivalent to 0.5 Mc Farland (1.5×10⁸ CFU/ml) was obtained. The experiment was divided into two sets:

Set 1: 2 ml of 100% essential oil extract of *Ocimum sanctum* was taken in a test tube. To this, 500 µl of 0.5 Mc Farland bacterial suspension was added.

Set 2: 50% essential oil extract of *Ocimum sanctum* was made by mixing 1 ml of pure extract with 1 ml of Muller Hinton broth. To this, 500 µl of 0.5 Mc Farland bacterial suspension was added.

Bacterial growth was measured spectrophotometrically



Figure 1. 100% Essential oil extract of *Ocimum sanctum*.

(Thermo scientific UV-1, Helios Omega Single-Beam UV-Visible Spectrophotometer, United states). The readings (520 nm) were noted immediately and subsequent to incubation after specific contact periods of 48, 72 and 96 hours for both the experimental groups. The mean values were taken as final Optical Density (OD). 100 and 50% essential oil extract of *Ocimum sanctum* served as a negative control for set 1 and set 2 respectively. 0.5 Mc Farland bacterial suspension was positive control for both the sets. The entire experiment was repeated in triplicate.

For each set, the immediate mean OD value was subtracted from the mean OD value at 48, 72, and 96 hours (Table 1). This growth was taken as 100% and percentage inhibition was calculated as follows

$$\% \text{ Inhibition} = \frac{1 - \text{OD test well}}{\text{OD of corresponding control well}} \times 10$$

Anti granulomatous efficacy: Prior approval was obtained from the Institutional Animal Ethics Committee. Two well trained experts in the field of pharmacology and zoology took part in the study. Six adult albino rats (avg. wt. 180-200 grams) were anaesthetized using diethyl ether (Figure 2). Preweighed (30±0.1 mg) autoclaved cotton pellets were used for inducing the chronic inflammation. Bilateral linear axillary incisions were made under aseptic conditions (Figure 3). Right and left axillary regions served as group 1 (Test - cotton pellets dipped in 1 ml of essential oil extract of *Ocimum sanctum*) and group 2 (control- cotton pellets dipped in 1 ml of normal saline) respectively (Figure 4).

The incisions were sutured using 3-0 silk and the rats were maintained under aseptic conditions for 7 days and fed well. On day 8, they were sacrificed by an overdose of diethyl

Table 1: Spectrophotometric optical density readings (520 nm) for 100 and 50% essential oil extract of *Ocimum sanctum* at 0, 48, 72 and 96 hours.

Time Period	Test groups	OD Set 1 ⁺	OD Set 2 ⁺⁺
Immediate	<i>O. sanctum</i> extract + bacterial growth	2.586	0.395
	Positive control ^{**}	0.265	0.307
	Negative control ^{***}	0.001	0.001
48 Hrs	<i>O. sanctum</i> extract + bacterial growth	2.616	0.510
	Positive control	0.511	0.511
	Negative control	0.001	0.002
72 Hrs	<i>O. sanctum</i> extract + bacterial growth	2.626	0.522
	Positive control	0.623	0.623
	Negative control	0.001	0.002
96 Hrs	<i>O. sanctum</i> extract + bacterial growth	2.642 #	0.565###
	Positive control	0.865	0.865
	Negative control	0.002	0.002

⁺Optical density for Pure extract, ⁺⁺Optical density for 50% extract
^{###}Increase in Optical Density (OD) signifies increased lysis of bacteria (bactericidal potency)

*Positive control - 0.5 Mc Farland bacterial suspension

**Negative control - Muller Hinton Broth

Blank: Solution used for calibration

For test solution with bacterial suspension – 100 and 50% essential oil extract of *O. sanctum* for set1 and set 2 respectively

For positive control – Muller Hilton Broth

For negative control - Muller Hilton Broth

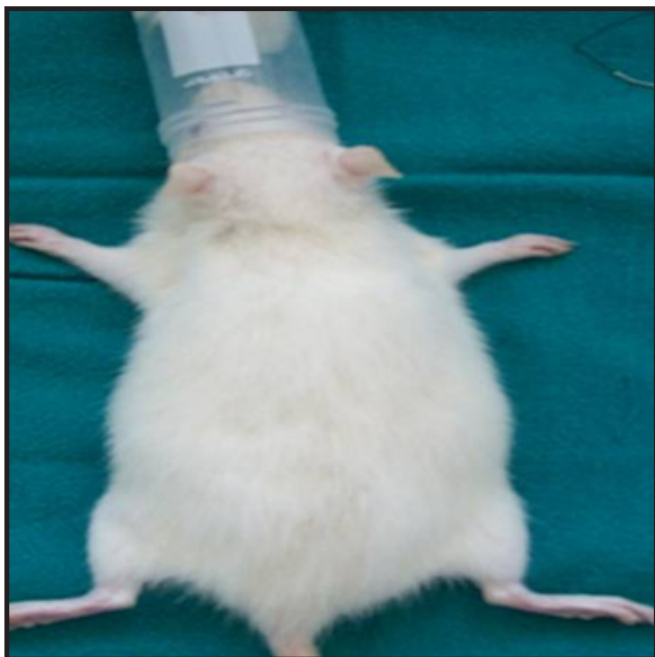


Figure 2. Albino rats anaesthetized using diethyl ether.



Figure 3. Bilateral axillary incisions.

ether. The cotton pellets along with the induced granuloma was dissected from the adjacent healthy tissue (Figures 5 and 6). These were subsequently dried in a hot air oven (Thermo Scientific Lindberg/Blue M Performance ovens, Gravity convection, USA) at 60°C for 8 hours and weighed. The increase in weight was considered as the dry granuloma weight (Winter et al., 1958). Both groups were subjected to statistical comparison using unpaired student's 't' test with significance level set at $p < 0.05$. This test is used to compare two independent population means inferred from two samples (independent indicates that the values from both samples are numerically independent of each - there is no correlation between corresponding values) i.e. same subject is exposed to 2 different experimental treatments.

Results

Increase in Optical Density (OD) was directly proportional to

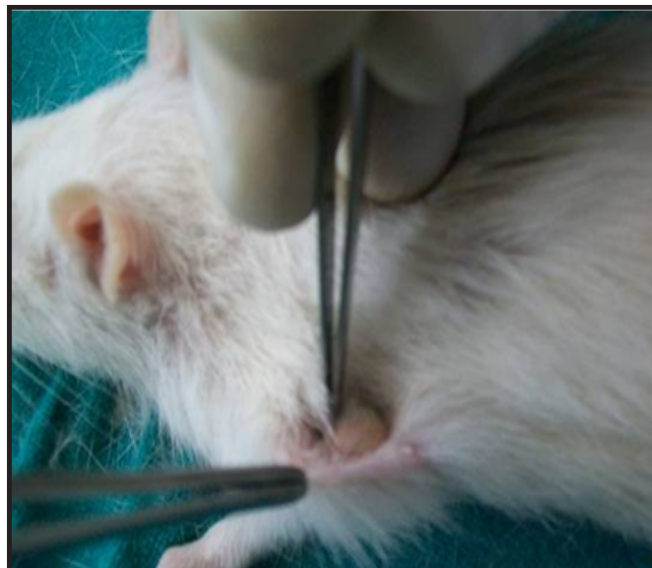


Figure 4. Pre-weighed autoclaved cotton pellets implanted.



Figure 5. Excised cotton pellets with attached granulomatous tissue (Control).



Figure 6. Excised cotton pellets with attached granulomatous tissue (Test).

the antibacterial efficacy of the essential oil extract of *Ocimum sanctum*. The antibacterial efficacy of essential oil extract of

Ocimum sanctum improved with an increase in concentration and contact period (Table 2).

Increase or decrease in pre and post implantation cotton pellets weight (mgs) implied a larger or a smaller granuloma formation (Table 3). Both groups were subjected to statistical comparison using unpaired student's 't' test with significance level set at $p < 0.05$. There was a statistically significant decrease in dry granuloma weight in group 1 (Test), $p = 0.034$, as compared to group 2 (control). Percentage inhibition of granuloma formation was calculated as follows

$$1 - \frac{\text{Average weight of implanted cotton pellet with Test solution}}{\text{Average Weight of implanted cotton pellet with saline}} \times 100$$

The percentage inhibition of granuloma was 35.8%.

Discussion

The use of an intracanal medicament after canal preparation is generally recommended in multiple sitting endodontics [17]. Calcium hydroxide is the gold standard with a high degree of success [18]. However, specific microorganisms such as *Enterococcus faecalis* have shown resistance against this [19] and other commonly used intracanal medicaments [18].

Increase in antibiotic resistant strains and side effects caused by various synthetic drugs have prompted the researchers to look for herbal/botanical therapy. One such product with wide usage in Indian context is *Ocimum sanctum* (*Tulsi*). It belongs to the Labiateae family and has three varieties: Sri, Krishna and Vana *Tulsi*. It has the following common names: *Tulsi* (Hindi), Manjari (Sanskrit) and Holy Basil (English). It is a branched, erect, stout and aromatic herb. It grows up to the height of about 75 to 90 cm and is hairy all over. The chief source of the essential oil is leaves, followed by inflorescence and stem. The oil extracted from seeds is called 'fixed oil' and mainly contains fatty acids and sitosterol [20].

The oil can be obtained by steam, petroleum ether or benzene distillation [21,22]. In the present study steam

Table 2: Comparative percentage inhibition of *E. faecalis*.

Contact Period	Set 1*	Set 2**
48 hrs	87.8%	43.7%
72 hrs	88.83%	59.9%
96 hrs	90.67%	69.54%

* Pure essential oil extract of *Ocimum sanctum*

** 50% essential oil extract of *Ocimum sanctum*

Table 3: Change in cotton pellets weight (in mgs)* before and after implantation.

Group 1 **	Group 2***
31.2	32.5
21.0	42.7
18.1	51.4
32.0	32.1
26.5	34.5
25.0	41.5

* Increase in weight denotes larger granuloma formation and vice-versa

**Test- preweighed cotton pellet (30 ± 0.1) mg dipped in 1 ml of essential oil extract of *Ocimum sanctum*

***Control- preweighed cotton pellet (30 ± 0.1) mg dipped in 1 ml of saline

distilled essential oil extract of *O. sanctum* was prepared. This is a volatile oil.

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 [18]. It has been suggested that use of the medicament's vapor might be the best method to regulate the dose and still get the drug into all the irregularities within the root canal system for successful disinfection [3].

Intracanal medicaments that exert their antibacterial action in a vapour form are formocresol, camphorated monochlorophenol, merthiolate, metacresylacetate, beechwood creosote, and glutaraldehyde [23-25]. Although, they are effective against certain microorganism implicated in periradicular disease, alpha hemolytic streptococci and enterococci have been found to be resistant to the vapors of these medicaments. Moreover they have a limited role because they are extremely toxic, antigenic and its effect is lost after a few days [26].

Enterococcus faecalis is the most commonly isolated or detected species from failed root canal therapy [17]. Its high resistance to antibacterial substances is widely documented [27] and is attributed to its ability to enter into a viable but nonculturable state during environmental stresses [28,29]. Furthermore, it can penetrate deeply into the dentinal tubules and resist bactericidal substances commonly used in endodontic procedures [30]. Recognizing the potential role of *E. faecalis* in the failure of root canal therapy and its resistance for various drugs makes it a good test model for evaluating the antibacterial efficacy of essential oil extract of *Ocimum sanctum*.

The present study utilized Spectrophotometric system for percentage inhibition assay because it is less subjective, cheaper and has a greater sensitivity than the standard well and disc diffusion methods. The antibacterial effect of *Ocimum sanctum* is due to the presence of linoleic acid, linolenic acid, eugenol (1-hydroxy-2-methoxy-4-allylbenzene), and carvacrol [31]. 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 bond formation occurs in the bacterial DNA and RNA [32] resulting in inhibition of replication of DNA and subsequent bacterial death. Another hypothesized 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 and culminating in their death [33].

A herb *Aloe vera*, in a gel form was used as an intracanal medicament against *E. faecalis*. It was found that only (22.5%) of the teeth medicated with this herbal gel yielded negative cultures. It was concluded that the gel is ineffective against the organism and hence cannot be used as intracanal medicament [34]. However, the result of the present study showed that the antibacterial efficacy of essential oil extract of *Ocimum sanctum* was directly proportional to an increase in concentration and contact period. 100% essential oil had greater antibacterial efficacy tested at all the contact periods (Table 2).

Periapical dental granuloma is a growing mass of granulation tissue surrounding the apex of a nonvital tooth and is a sequelae to pulp necrosis. It is a result of a successful attempt of periapical tissues to neutralize and confine the irritating toxic products escaping from the root canal. Normally, thorough chemomechanical preparation reduces the bacterial load of the canal allowing healing to commence. However this can take weeks or months [35].

In the present study an adult albino rat model was used to evaluate the anti-granulomatous effect. Cotton pellet-induced granuloma in rats [36] is a widely used model to evaluate components of chronic inflammation. The moist weight of the cotton pellet correlates with the transudate and the dry weight of the pellet correlates with the amount of granulomatous tissue.

The present study documented a potent anti-inflammatory action against chronic inflammation induced granuloma. There was a statistically significant decrease in dry granuloma weight in group 1 (Test), $p=0.034$ (Table 3). The percentage inhibition of granuloma was 35.8%.

The anti inflammatory action of essential oil extract of *Ocimum sanctum* is attributed 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 a dual inhibitory action. 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 and leukotrienes. It also acts on histamine, serotonin and kinins.

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Conclusion

Based on the encouraging results of this *ex-vivo* and animal model study, which documented the beneficial properties of the essential oil extract of *Ocimum sanctum*, further research in humans is needed to extrapolate these and the other potential beneficial properties for its potential use in endodontics as an intracanal medicament.

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