

# Assessment of Antimicrobial Activity of Ocimum Sanctum Against Oral Microbes- A Systematic Review

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## Abstract

Aim of this systematic review: To systematically evaluate the antimicrobial effect of Ocimum sanctum against oral and systemic pathogens. Methodology: Structured questions were framed; inclusion and exclusion criteria were used to filter the studies. Data bases like Pubmed, Scopus, Web of science, Science direct, Cochrane were used to search and limit the studies with respect to aim of the study. After search and removing the duplicates 220 articles were obtained and after reading the abstract 205 articles were considered as irrelevant and excluded. Full text was searched for the 15 articles and after search, for five articles full text was not available and were excluded. Ten articles considered for systematic review. Conclusion: After analysing the articles for the type of bacteria evaluated and concentrations of Ocimum sanctum used its concluded that lots of heterogeneity exists in the studies and following observations were made: Ocimum sanctum extract is effective against Gram positive organisms like *S.sanguis*, *S.aureus* and gram negative organisms like *A.actinomycetem comitans*, *P.gingivalis*. Concentrations 2%,4%,6% are effective against microorganisms. Based on toxicity levels of this concentration best suitable concentration can be selected in future clinical studies. Further studies are required to evaluate the effect of Ocimum extracts against candida, virus and other organisms like Salmonella, Klebsiella, Pseudomonas.

**Key words:** Ocimum sanctum, Tulsi extract, Periodontal disease, Anti-Microbial Effect, Periodontiti

## 1. Introduction

Periodontitis is an infectious disease resulting in inflammation within the supporting tissues of the teeth, progressive attachment and bone loss and is characterized by pocket formation and/or recession of the gingiva. [1] Non-surgical periodontal therapy generally aims to eliminate subgingival microbes and to remove their deposits from the root surfaces, thereby controlling the progression of periodontal destruction, reducing aetiologic agents and creating a healthy subgingival environment.[2] Scaling and root planing (SRP) is the most common periodontal treatment which has proven clinical effectiveness in terms of reducing inflammation, decreasing the probing pocket depth and improving the clinical attachment level (CAL).[3,4] But, SRP has some limitations, such as difficulties in accessing deeper pockets, furcation areas and root concavities[5,6] and difficulty to remove microbial pathogens that are penetrated into dentin tubules and which are residing in lacunae and concavities.[7]

Antiseptics and antibiotics delivered locally had been used as adjunct to SRP procedures in order to control the subgingival microbes and thereby improve the treatment outcome because mechanical

root debridement alone is a technically demanding procedure. The results presented in the literature, however, are inconclusive. [8]

Various anti-bacterial agents such as chlorhexidine (CHX), metronidazole, tetracyclines and povidone iodine have been tried in the recent past and were proven to be effective in improving the clinical parameters when used site specifically. [9]

In recent times, there has been renewed interest in naturally occurring products. There are several natural products and herbs that claim to have better properties and less side effects than the chemical commercially available agents/drugs.

### Aim of the review

To systematically evaluate the antimicrobial effect of Ocimum sanctum against oral and systemic pathogens.

### Structured question

What is the spectrum of activity of Ocimum sanctum and which pathogens are highly susceptible to Ocimum sanctum ?

## 2. Materials and methods

### Selection criteria

All Invitro studies pertaining to evaluation of

antimicrobial activity of Ocimum sanctum against oral and systemic microbes.

**Inclusion criteria:**

- Articles demonstrating antimicrobial activity of Ocimum sanctum against oral diseases like dental caries, periodontitis and systemic diseases
- Articles using Ocimum sanctum extract in culture technique to demonstrate antimicrobial activity

**Exclusion criteria**

- Articles that have used combination of natural products
- Articles which haven't mentioned antimicrobial effect in the manuscript
- Articles which demonstrate antimicrobial effect against other species of Ocimum

Variables of interest:	
SL:NO	VARIABLES OF INTEREST
1.	Anti-Bacterial Effect
2.	Anti-Viral Effect
3.	Anti-Fungal Effect

Sources of electronic search:

- Pubmed
- Pubmed central

- Cochrane library
- Google scholar
- Medline
- Science direct

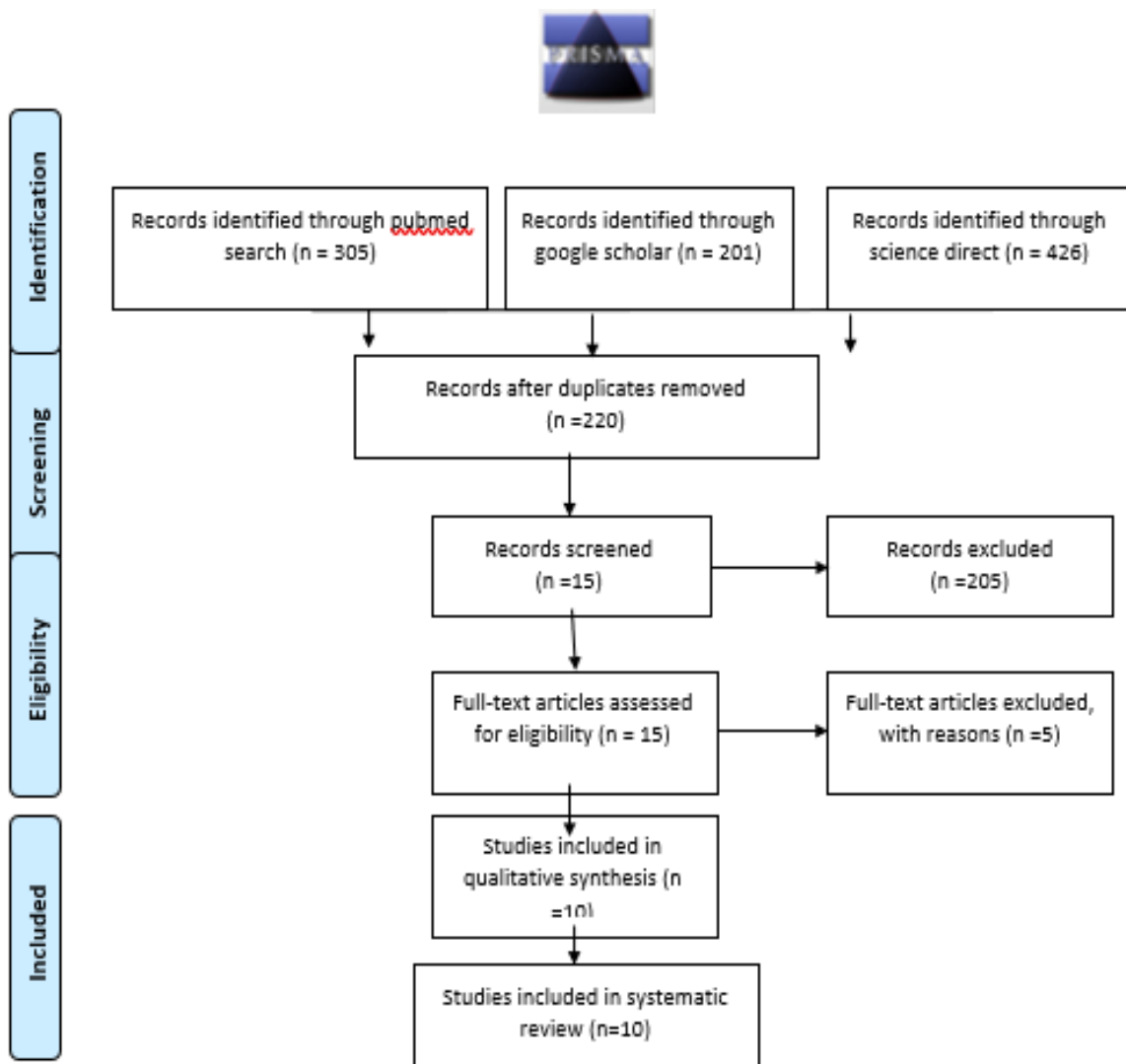
**Sources of hand search**

- Contemporary clinical dentistry
- Journal of periodontology
- Journal of periodontal research
- Search methodology(pubmed)

**Search strategy**

((("Plants, Medicinal"[Mesh]) OR "herbal preparation PC-CARE" [Supplementary Concept]) OR ("Drug Delivery Systems"[Mesh] OR "Nanoparticle Drug Delivery System"[Mesh] OR ("Ocimum basilicum"[MeSH Terms] OR "eugenol synthase ocimum basilium"[Supplementary Concept] OR "Ocimum"[MeSH Terms]) AND "Anti-Infective Agents"[MeSH Terms]) OR ("Anti-Infective Agents"[Pharmacological Action] OR "anti-infective agents, local"[MeSH Terms]) OR ("Ocimum"[MeSH Terms] AND ("Anti-Infective Agents"[MeSH Terms] OR "Anti-Infective Agents"[Pharmacological Action] OR "anti-infective agents, local"[MeSH Terms]) OR ("anti-infective agents local"[Pharmacological Action] OR "Biofilms"[MeSH Terms]))

**Prisma flow chart**



Evaluation of articles				
Sl.No	Title of the study	Journal	Methodology	Outcome
1	Evaluation of the antimicrobial activity of various concentrations of Tulsi ( <i>Ocimum sanctum</i> ) extract against <i>Streptococcus mutans</i> : an in vitro study.	Indian Journal of Dental Research 2010	Culture: <i>Ocimum sanctum</i> with concentrations 0.5%, 1%, 1.5%, 2%, 2.5%, 3%, 3.5%, 4%, 4.5%, 5%, 6%, 7% 8%, 9%, 10% of the extract were used. Microbe analysed was <i>Streptococcus mutans</i>	At the 4% concentration of Tulsi extract, a zone of inhibition of 22 mm was obtained. This was the widest zone of inhibition observed among the 15 different concentrations used.
2	The antimicrobial activity of <i>Azadirachta indica</i> , <i>Mimusops elengi</i> , <i>Tinospora cardifolia</i> , <i>Ocimum sanctum</i> and 2% chlorhexidine gluconate on common endodontic pathogens: An in vitro study	European Journal of Dentistry 2014	The agar diffusion test was used to check the antimicrobial activity of the Methanolic extracts of the medicinal plants along with CHX. Six different concentrations of the tested agents were used for the study	All the plants extracts showed considerable antimicrobial activity against selected endodontic pathogens. At 3mg. concentration, <i>O. sanctum</i> was the most effective against <i>S. mutans</i>
3	Sunlight-induced rapid and efficient biogenic synthesis of silver nanoparticles using aqueous leaf extract of <i>Ocimum sanctum</i> Linn. with enhanced antibacterial activity	Organic and Medicinal Chemistry Letters 2014	Antimicrobial spectra for each concentration (5%, 7%, and 10%) of aqueous leaf extracts of <i>O. sanctum</i> as well as silver nano formed by these extracts upon sunlight induction were studied against four Gram-positive and four Gram-negative bacteria	<i>O. sanctum</i> nano particles showed prominent activity on Gram-negative human pathogenic <i>Salmonella typhimurium</i> , <i>Escherichia coli</i> , <i>Pseudomonas aeruginosa</i> .
4	Use of an Extract of Indian Sacred Plant <i>Ocimum Sanctum</i> as an Anticariogenic Agent: An in vitro Study	International Journal of Clinical Pediatric Dentistry 2015	Culture Ethanol extract of <i>Ocimum sanctum</i> was prepared by the hot extraction method. 3 different concentrations of <i>O. sanctum</i> (2.5, 5 and 10%) of the extract were used. Tested pathogens were <i>S. mutans</i> , <i>S. mitis</i> , <i>S. sanguis</i> , <i>L. acidophilus</i>	<i>Ocimum sanctum</i> leaf extract demonstrated maximum antimicrobial activity against microorganisms responsible for dental caries at the 10% concentration level. Maximum activity was seen against <i>S. mutans</i> and <i>S. sanguis</i> with 10% extract.
5	Anti-microbial Activity of Tulsi [ <i>Ocimum Sanctum</i> (Linn.)] Extract on a Periodontal Pathogen in Human Dental Plaque: An Invitro Study.	Journal of Clinical Diagnostic Research 2016	Culture Anti-microbial activity of <i>Ocimum sanctum</i> (Linn.) extract was tested by agar well-diffusion method against 0.2% chlorhexidine as a positive control and dimethyl sulfoxide as a negative control. The zone of inhibition was measured in millimeters using Vernier callipers.	At the 6% w/v concentration of <i>Ocimum sanctum</i> (Linn.) extract, a zone of inhibition of 22 mm was obtained.
6	Antimicrobial efficacy of Tulsi leaf ( <i>Ocimum sanctum</i> ) extract on periodontal pathogens: An in vitro study.	Journal of Indian Society of Periodontology. 2016	Culture: Extract was diluted with an inert solvent, dimethyl formamide, to obtain five different concentrations (0.5%, 1%, 2%, 5%, and 10%). Agar well diffusion method was employed to determine the concentration at which Tulsi gave an inhibition zone, similar to doxycycline	At 5% and 10% concentrations, Tulsi extracts demonstrated antimicrobial activity against <i>A.a</i> similar to doxycycline with similar inhibition zones ( $P > 0.05$ ). <i>P. gingivalis</i> and <i>P. intermedia</i> , however, exhibited resistance to Tulsi extract that showed significantly smaller inhibition zones ( $P < 0.05$ ).
7	Evaluation of antiviral activity of <i>Ocimum sanctum</i>	BMC Complement	The antiviral efficacy of different leaves extracts	All the extracts of <i>O. sanctum</i> (crude extract, terpenoid and

	and Acacia arabica leaves extracts against H9N2 virus using embryonated chicken egg model.	Altern Med. 2018	was systematically studied in three experimental protocols viz. virucidal (dose-dependent), therapeutic (time-dependent) and prophylactic (dose-dependent) activity employing in ovo model. antiviral efficacy was determined in terms of reduction in viral titres, measured by Haemagglutination (HA) and RT-PCR	polyphenol) showed significant virucidal activity against H9N2 virus
8	In vitro Antimicrobial Activity of Ocimum sanctum (Tulsi) Extract on Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis.	Journal of Contemporary Dental Practice 2018	Various concentrations (2, 4, 6, and 8%) A 0.2% chlorhexidine served as the positive control, Zones of inhibition were measured, each for A. and P. gingivalis	The 8% concentration of the tulsi extract showed maximum zone of inhibition against A. actinomycetemcomitans and P. gingivalis ( $40.10 \pm 0.90$ , $33.79 \pm 1.82$ mm), followed by the 6, 4, and 2% concentrations
9	Antibacterial property of Azadirachta indica, Ocimum sanctum, and Vitex negundo against oral microbes.	Journal of Conservative Dentistry 2019	Plant extract was prepared with hot continuous extraction method by the Soxhlet Apparatus. Microorganisms isolated from the oral cavity and identified by Vitek-2. Bacterial inoculums poured and spread into Mueller Hinton plates. Plant extract was poured into prepared wells taking ciprofloxacin as the positive control and dimethyl sulfoxide as the negative control.	All the three plants showed antibacterial potency against the isolated organisms. Acetone group showed better efficacy than the aqueous extract group.
10	Antifungal activity of solvent extracts of Piper betle and Ocimum sanctum Linn on Candida albicans: An in vitro comparative study.	Journal of Oral Maxillofacial Pathology 2019	Subcultures of C. albicans obtained from oral thrush patients were used in the present study. Ethanolic and ethyl acetate extracts of P. betle (betel) and O. sanctum L. (tulsi) leaves were prepared by cold extraction method	Both the extracts exhibited anticandidal activity on the subcultures of C. albicans. The ethyl acetate extract of mature betel leaf showed a maximum zone of inhibition (26 mm) when compared with Ocimum and fluconazole (13 mm).

Evaluation of Anti-microbial effect of Ocimum sanctum:				
Sl no	Authors	Bacteria	Fungi	Virus
1	Eshwar Pranati et al	Effective against Actinobacillus actinomycetem comitans	-	-
2	Ghoke et al	-	-	Effective against H9N2 virus
3	Mallikarjun et al	Effective against Actinobacillus actinomycetem comitans	-	-
4	Gautam bramachari et al	Effective against Salmonella typhimurium, Escherichia coli, Pseudomonas aeruginosa	-	-
5	Pai et al	Effective against S.mutans and S.sanguis	-	-
6	Aqarwal et al	Effective against S.mutans	-	-
7	Jayanthi et al	Effective against A.a and P.gingivalis	-	-
8	Mistry ks et al	Effective against S.mutans, S.aureus, E.faecalis	-	-
9	Sivareddy et al	-	Effective against Candida albicans	-
10	Kalita et al	Klebsiella oxytoca, Kochuria kristinae, Acinetobacter boumani, Sphingomonas paucimobilis, Pseudomonas fluorescens, Streptococcus gordonii, Enterococcus faecalis, and Bacillus subtilis	-	-

## Figures:

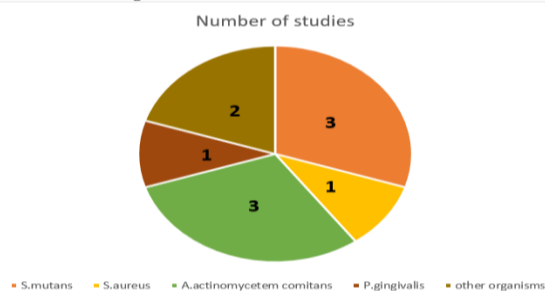


Figure 1 shows the pie chart of number of studies done to evaluate anti-microbial effect of *Ocimum sanctum* against various microorganisms

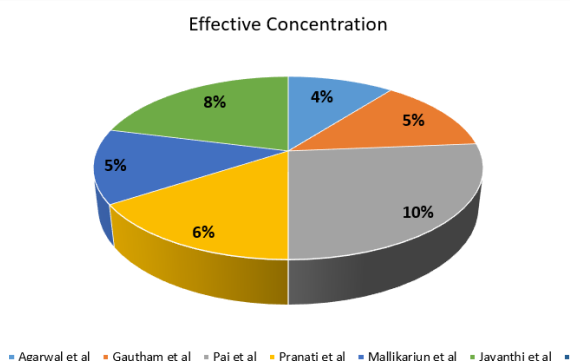


Figure 2 shows Evaluation of various *Ocimum sanctum* concentrations used in studies:

### 3. Discussion

In this systematic review, we have selected 10 articles based on our inclusion criteria, relevance of the subject, variables of interest. Out of this, three of the articles evaluated antimicrobial activity against *Actinobacillus actinomycetem comitans* (Currently named as *Aggregatibacter actinomycetem comitans*), three articles against *Streptococcus mutans*, one against *Porphyromonas gingivalis*, *Staphylococcus aureus* and two articles against *Salmonella*, *Klebsiella*, *Virus*, *Candida* species (Refer Figure 1).

Pranati et al [10] evaluated the antimicrobial activity of *Ocimum sanctum* against A.a. A lab based invitro experimental study design was adopted. Ethanolic extract of *Ocimum sanctum* (Linn.) was prepared by the cold extraction method. The extract was diluted with an inert solvent, dimethyl sulfoxide, to obtain ten different concentrations (1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%) of extract. Plaque sample was collected from 05 subjects diagnosed with periodontal disease. Isolation of *Actinobacillus actinomycetemcomitans* from plaque samples was done using Tryptic Soy Serum Bacitracin Vancomycin agar (TSBV) medium. Identification of *Actinobacillus actinomycetemcomitans* was done based on cultural, microscopic, biochemical characterization and multiple drug resistance patterns. Anti-microbial activity of *Ocimum sanctum* (Linn.) extract was tested by agar well-diffusion method against 0.2% chlorhexidine as a positive control and dimethyl sulfoxide as a negative control. The zone of inhibition

was measured in millimeters using Vernier callipers. At the 6% w/v concentration of *Ocimum sanctum* (Linn.) extract, a zone of inhibition of 22 mm was obtained. This was the widest zone of inhibition observed among all the 10 different concentrations tested.

Ghoke et al [11] evaluated the antiviral effect of *Ocimum sanctum* against H9N2 virus. He didn't check for anti-bacterial effect. He studied the effects with parameters like dose dependent and time dependent variations. Also, he made difference in preparation methods also, he used crude extract, terpenoid and polyphenol and checked any variation among these. Results showed all the three preparations showed anti-viral effect and hence preparation protocol doesn't have an impact on the results.

Mallikarjun et al [12] evaluated antimicrobial effect against A.and P.g. He showed that *Ocimum sanctum* is very effective against A.a. P.g showed minimal inhibition zone to *Ocimum* extract. Eventhough they got minimal inhibition zone to P.g it can be correlated clinically as *Ocimum* extracts can reduce the growth of these organisms and load will be reduced and therapeutic benefit will be there.

Gautham et al [13] did a study on antimicrobial effect of *Ocimum* extracts on pathogenic bacteria like *Salmonella*, *E. coli*, *Pseudomonas*. He used nano particles mixed with *Ocimum* extracts to enhance its activity. *O.sanctum* nano particles showed prominent activity on Gram-negative human pathogenic *Salmonella typhimurium*, *Escherichia coli*, *Pseudomonas aeruginosa*. This study didn't evaluate against periodontal pathogens like A.a, P.g. Pai et al [14] and Agarwal et al [15] did study on antimicrobial effect of *Ocimum sanctum* extracts on gram positive bacteria like *S.mutans*, *S.sanguis*. They tried different concentrations from 1% to 9%. 2%, 4% are effective against these bacteria, higher concentrations are also effective but optimum activity is achieved in those two concentrations. Mistry et al [17] also demonstrated antimicrobial activity against *S.mutans*.

Jayanthi et al [16] Tulsi extract with ethanol was prepared using cold extraction method in the present in vitro study. Various concentrations (2, 4, 6, and 8%) were then obtained by dilution with dimethylformamide. A 0.2% chlorhexidine served as the positive control, whereas the negative control was dimethylformamide. Zones of inhibition were measured, each for *A.actinomycetemcomitans* and *P. gingivalis*. 2% concentration showed inhibition zone of 16mm against A.and 14mm against P.g, whereas 40mm inhibition zone was achieved at 8% concentration. Hence tulsi can be used effectively in 2% concentration itself.

Sivareddy et al [18] did study on antimicrobial effect of *Ocimum* extract on *Candida* subcultures of *C. albicans* obtained from oral thrush patients were used. *Ocimum* extracts exhibited anticandidal activity on the subcultures of *C. albicans*. Not many studies done on candida species to demonstrate it

effect. Kalita et al [19] demonstrated antimicrobial activity of *Ocimum sanctum* against organisms like *Klebsiella*, *Pseudomonas*, *Sphingomonas*, *E. faecalis* species.

In this systematic review, there are heterogeneity in studies in relation to type of bacteria analysed, concentration of *Ocimum* extract used. Also limited number of studies available in demonstrating anti-fungal and anti-viral effect of *Ocimum sanctum*. Hence cannot come to a conclusion on the effectiveness of *Ocimum sanctum* on all the microorganisms. Some recent research have highlighted the anti-oxidant effect, anti-inflammatory effect of *Ocimum sanctum* and mentioned along with the anti-microbial effect these effects provides added benefit in the treatment of periodontal disease. [20,21] *Ocimum sanctum* extract mixed with copper nano particles also used in a study and it demonstrated enhanced antioxidant and anti-inflammatory effect of *Ocimum sanctum* alone.[22]. Clinical study done on *Ocimum sanctum* demonstrated superior clinical benefits in the treatment of gingivitis compared to Chlorhexidine and since it has very less side effects it could be an alternative for the management of periodontal disease. [23]

Following conclusions can be arrived from this systematic review, *Ocimum sanctum* extract is effective against Gram positive organisms like *S. sanguis*, *S. aureus* and gram negative organisms like *A. actinomycetem comitans*, *P. gingivalis*. Concentrations 2%,4%,6% are effective against microorganisms. Based on toxicity levels of this concentration best suitable concentration can be selected in future clinical studies. Further studies are required to evaluate the effect of *Ocimum* extracts against candida, virus and other organisms like *Salmonella*, *Klebsiella*, *Pseudomonas*.

#### 4. Limitation

This systematic review has more studies against bacterial species but limited studies against virus and fungi, hence cannot conclude the effect against those pathogens with the limited number of available studies. Also this systematic review didn't have articles against other periodontal pathogens like *T. forsythia* and *T. denticola*.

#### Future Scope

*Ocimum sanctum* can be used as an alternative for the standard drug chlorhexidine to treat oral infections and with lesser side effects it could be a viable alternative too.

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