ORIGINAL RESEARCH

Evaluation of the Antimicrobial Effect of *Ocimum sanctum* L. Oral Gel against Anaerobic Oral Microbes: An *In Vitro* Study

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Abstract

Aim: The aim of the present study was to evaluate the antimicrobial activity of *Ocimum sanctum* (*Tulsi*) gel against anaerobic oral microbes.

Materials and methods: Subgingival plaque samples were collected from a patient diagnosed with periodontitis. The plaque sample was immediately transferred to an Eppendorf tube containing thioglycollate broth. The sample was incubated in anaerobic conditions at 37° for 24 hours. A total of 20 µL of the cultured broth was subcultured into the test *O. sanctum* L. (*Tulsi* gel) and control group gels [chlorhexidine (CHX) gel]. The subcultured gels were of five different concentrations (50, 100, 150, 200, and 250 µL). The subcultured plaque samples were plated onto a Petri dish containing brain heart infusion (BHI) agar. The Petri dishes were incubated at 37° for 24 hours under anaerobic conditions. An anaerobic gas pack was placed in an anaerobic jar to create an anaerobic condition. After 24 hours, the number of colonies formed was observed and noted.

Results: *O. sanctum* L. (*Tulsi*) gel demonstrated effective antimicrobial activity-against anaerobic oral microbes at 20 and 25%. A higher concentration of *O. sanctum* L. (*Tulsi*) gel is effective in demonstrating its potential use as an efficient and supplement for the quality level of treatment in periodontitis (periodontal condition). CHX gel showed no activity in comparison with the *O. sanctum* L. (*Tulsi*) gel.

Conclusion: *O. sanctum* L. (*Tulsi*) plant extract illustrates antimicrobial efficacy against anaerobic oral microbes. *O. sanctum* L. (*Tulsi*) exhibited efficient antimicrobial activity against anaerobic oral microbes proving its potential use as an efficient and standard supplement in the treatment of periodontal conditions.

Clinical significance: Periodontitis is an inflammation of a periodontal organ complex with various types of diseases which are linked with particular bacteria that occupy the subgingival portion. Extensive utilization of drugs developed side effects. It resulted in uncommon infections and also resulted in resistance. In various clinical situations, *O. sanctum* L. (*Tulsi*) is used as herbal medicine. Hence it was found to be an appropriate substitute to control the situations which are altering the oral cavity.

Keywords: Anaerobic microorganisms, Antimicrobial, Chlorhexidine, Dental plaque, *Tulsi* extract.

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Introduction

Oral infections, mainly periodontitis and periodontal conditions, are correlated with aerobic and anaerobic microorganisms. The oral cavity (mouth) accommodates more than 500 various forms of microorganisms that are frequently correlated with various oral infections.[1] The main etiological factor for periodontitis is dental plaque and biofilm that is present on the tooth surfaces.[2] Bacteria in dental plaque produce noxious products, which in turn activate the inflammatory process in the tissues of the periodontium.

Dental plaque is defined as various communities of microorganisms present in the form of biofilm on the tooth surface. They are present in the extracellular matrix of polymers of the host and are pure of microbial origin. "Dental biofilm" exhibits unique properties and differs from the characters expressed by similar organisms developing in liquid (planktonic) regions. Hence there is a renewed interest in microbial communities across all sectors of medical microbiology, industries, and also in the environment.[3] Dental biofilms have voids and channels in their structure, which can be best studied and identified by "confocal microscopy." The gradients of biofilm determine microbial development and also their organization in the thickest biomass areas over small distances with basic specifications. The dental plaque composition differs and exterminates on anatomical aspects and is formed on the pit and fissures, smooth aspects of the tooth, gingival crevices, and also on dentures.

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Dental plaque assembles preferably at stationary areas, and different stages of development are appreciated; they are as follows: (1) Absorption of bacterial molecules and host to the tooth aspects[4–6]; (2) Transport of oral pathogens to the tooth aspects[7–9]; (3) Adherence of late colony to previously attached early colony[10–12]; (4) Reproduction of the attached bacteria[13–18]; (5) Active detachment.[19,20] In this context, periodontitis is caused primarily

by anaerobic bacteria microbes such as *Porphyromonas gingivalis, Peptostreptococcus micros,* and *Prevotella intermedia,* as well as by facultative anaerobic bacteria. Periodontal pathology is a group of diseases that affect more than one periodontal tissue. Decreasing the bacteria levels in the oral region is one of the principles for the protection of periodontal disease and also for the prevention of periodontal disease. CHX is efficient against both gram-positive and gram-negative pathogens together, with pathogens including aerobes, anaerobes, fungi, and yeasts. CHX works by a principle called the "pincushion effect," which is explained in two ways. At one end, CHX molecules will be attached to tooth portions and on the other end, there is bacterial membrane interrelation.[21] This principle determines the lack of efficiency of other antimicrobials. They lack a big, firm molecule with two charged ends. Dental plaque agents include chemical and mechanical plaque control agents. Chemical plaque-controlled agents include CHX, CHX gluconate, triclosan, quaternary ammonium compounds, and sanguinarine. Mechanical plaque control agents include powered toothbrushes, interdental aids, interdental brushes, interdental floss, and chewing sticks. CHX is the most vigorous anti-plaque agent and is thus regarded as a "gold standard" antiplaque agent.[22] It can act against the efficiency of other anti-plaque agents with minimal side effects. Its efficiency is assigned to its bactericidal and bacteriostatic properties. CHX is also related to substantivity within the oral cavity (mouth). Nowadays, CHX is a commonly used chemotherapeutic agent against pathogens causing periodontal disease. CHX is studied enormously and compared with other antimicrobial agents, and hence, it is considered a gold standard. Therefore, CHX is frequently used as a positive control for evaluating the antimicrobial activity of other agents. They are used against periodontal complexes. However, its extensive, continued use leads to side effects. CHX side effects are in the form of teeth stains. It also alters the taste sensation. Thus, its use should be limited. Therefore, it is necessary to have a standby that is an equivalently efficient antimicrobial agent. It is used against periodontal complexes causing periodontal diseases. The possible approach is to find out the antimicrobial activity of traditional plants.

In India, *O. sanctum* L. (*Tulsi*) is used as a strong foundation in Ayurveda that belongs to the family Lamiaceae. It is an aromatic herbal ancient plant with India as its native place and is also considered a worldwide cultivated plant. *O. sanctum* L. (*Tulsi*) is extremely determined as the "queen of the herbs" because of its spiritual properties and restorative properties.[21] *O. sanctum* L. is grown for essential oils and also for its traditional and medicinal purposes.

In India, *O. sanctum* L. (*Tulsi*) is used as a foundation of ayurvedic treatment, and the different components of plants are used extremely in the therapy of various systemic diseases, like upper respiratory infections, bronchial diseases (bronchitis), skin infections, malaria, etc. Antimicrobial activity of *O. sanctum* L. (*Tulsi*) against pathogens such as *Candida albicans, Staphylococcus aureus, Klebsiella, Escherichia coli,* and *Proteus* are also documented. Therefore, the main aim of this present *in vitro* study is to evaluate the antimicrobial property of *O. sanctum* L. (*Tulsi*) plant extract against anaerobic oral pathogens.

Materials and Methods

Study Setting

The present *in vitro* study to evaluate the antimicrobial property of *O. sanctum* L. (*Tulsi*) plant extract on anaerobic oral microbes was conducted at Saveetha Dental College & Hospital, Chennai, India.

Preparation of *Tulsi* Extract

A dried extract of *O. sanctum* L. (*Tulsi*) was ordered from Green Chem Organic chemicals. In that 250 gm of *tulsi* powder was taken and soaked in 1000 mL of ethyl alcohol for 48 hours and was filtered in Whatman filter paper. Filter liquid was evaporated and the supercritical fluid was stored at 4°C until use.

Preparation of *O. sanctum* L. (*Tulsi*) Gel

Carbopol 940, which is the main ingredient is soaked in purified water, and added to this was 0.2% sodium benzoate. Both solutions are soaked overnight. A hydroxypropyl methylcellulose (HPMC) solution was added to the prepared mix. Propylene glycol was added to it. A total of 2 mL of super critical fluid (SCF) (homogenized) was added, and triethanolamine was mixed with that solution as drops. Check for pH 6–6.5. The gel was stored at ambient temperature. The prepared *O. sanctum* L. (*Tulsi*) gel was intact in proper consistency for a phase of 6 months. Little pH changes were recognized during that period and then it was rectified.

Evaluation of Properties of *O. sanctum* L. (*Tulsi*) Gel

The prepared gel was evaluated for pH, viscosity assessment, and cytotoxicity. pH was 6.5, which was suitable for oral usage. The viscosity test showed the flow of the gel was adequate enough to use it as an oral gel. A cytotoxicity test was done using shrimp nauplii; results showed even in higher concentrations prepared gel was not toxic and suitable for intraoral use.

Anaerobic Microculture

Subgingival plaque samples were obtained from the deepest pocket of patients diagnosed with chronic periodontitis after obtaining informed consent. The plaque was obtained using sterile Gracey curettes and the plaque sample was immediately transferred into an Eppendorf tube containing thioglycollate broth containing thioglycollate broth. Thioglycollate in the medium consumes oxygen and permits the growth of obligate anaerobes. Anaerobic gas chambers with anaerobic gas packs were used to create an optimistic condition for the growth of the anaerobic microcolonies. The sample was incubated in anaerobic conditions at 37° for 24 hours. This allowed the growth of the microorganism within the broth. The gels, both test and control, were diluted with 1 mL of distilled water into five different concentrations (50, 100, 150, 200, and 250 µL) as per. After 24 hours, 20 µL of the cultured broth was subcultured into the test (*Tulsi* gel) and control group gels (CHX gel). A total of 20 µL of the individual sample was spread into the solidified BHI agar medium in a Petri dish using L-rod; the Petri dishes were incubated at 37° for 24 hours under anaerobic conditions. Anaerobic gas packs were placed in an anaerobic jar to create an anaerobic condition. After 24 hours, the number of colonies formed was observed and noted. The colonies formed were counted manually.

Results

From the results obtained, it is seen that the colonies in the test group were around 200 at 50 µL and the number of colonies formed decreased as low as three colonies at a concentration of 250 µL. The number of colonies formed at 50 µL concentration in the *O. sanctum* L. group was 200, followed by 40 colonies at 100 µL concentration; the colony count was reduced to 15, 7, and 3 at 150, 200, and 250 µL concentrations, respectively. The colonies formed in the control group were zero at 50, 100, 150, 200, and 250 µL, respectively. Whereas, the control group with CHX showed no colonies at all with five concentrations.

Results are shown in Tables 1 and 2 and Figures 1 to 3.

Discussion

Extracts of plants have been used to treat illness since ancient times, and in Ayurveda, Siddha has detailed explanations about the potential usage of medicinal herbs. Various herbs have been used to treat illnesses arising due to bacterial, fungal, and viral origin. Phytochemicals present in the herbs have been shown to inhibit the growth of microbial species present in the oral cavity. They also inhibit their efficiency to proliferate in the host tissues.

Table 1: The table depicts the ingredients for the preparation of 2% *O. sanctum* L. gel, HPMC, and SCF

Ingredients	*Quantity*
Carbopol 940	2 gm
Polymer (HPMC)	2 gm
Tulsi SCF extract	2 mL
Sodium benzoate	0.2 mL
Propylene glycol	5 mL
Triethanolamine	q.s.
Distilled water	q.s. to make 100 mL

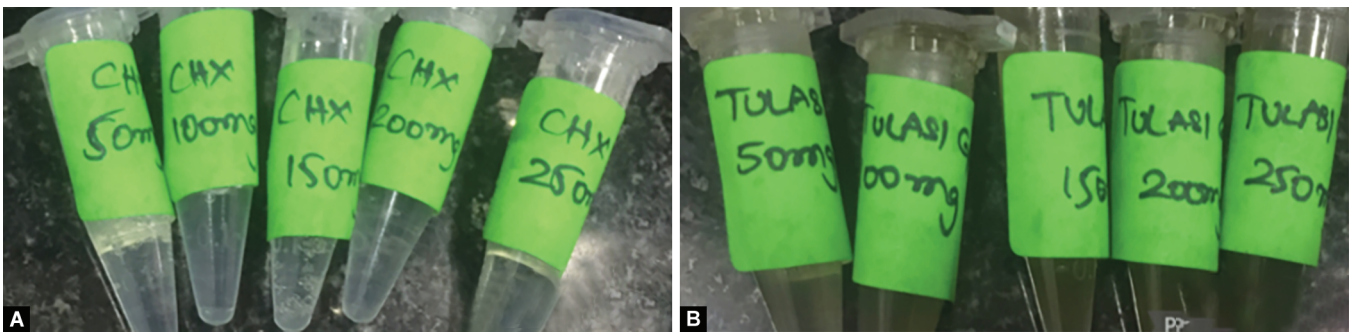
q.s.; quantity sufficient

The variations in the efficiency were shown in *in vitro* studies. The antimicrobial properties of various herbal extracts have been discussed previously. They were categorized as strong, medium, or weak. The main feature of herbal extracts and their parts is their hydrophobicity. Hydrophobicity is used to divide the lipids of the cell membranes of bacteria. They also enable them to partition the mitochondria. They disseminate the cell organization and make them more penetrable thereby killing them.[23]

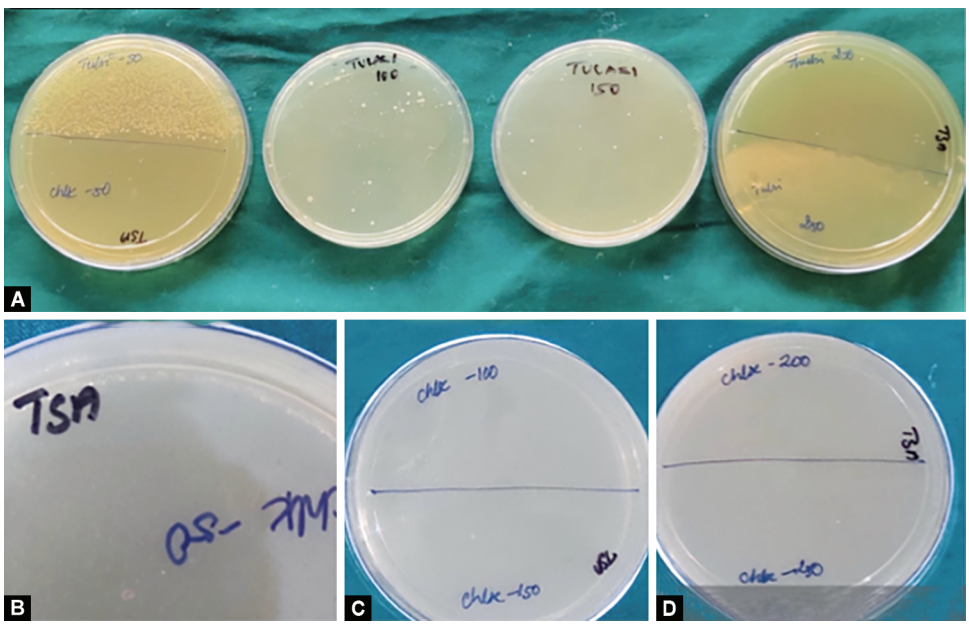
The rationale of the current study was to evaluate the antimicrobial property of *O. sanctum* L. (*Tulsi*) extract against anaerobic oral microbes. *O. sanctum* L. was selected for the study because it has anti-inflammatory, antioxidant, and antimicrobial

Table 2: Indicates colony count of anaerobic oral microbes with *O. sanctum* L. gel and CHX gel at different concentrations

Concentration	*O. sanctum* L. *gel*	*CHX gel*
50 µg	200	0
100 µg	40	0
150 µg	15	0
200 µg	7	0
250 µg	3	0



Figs 1A and B: The images show the CHX gel and *Tulsi* gel mixed with 1 mL of distilled water to get 5, 10, 15, 20, and 25% concentrations



Figs 2A to D: The images show (A) The colony count of anaerobic oral microbes with *O. sanctum* L. gel at different concentrations 5,10,15, 20 and 25% at the Petri plate; (B) The colony count of anaerobic oral microbes with CHX gel at 5%; (C) The colony count of anaerobic oral microbes with CHX gel at 10,15% concentrations; (D) The colony count of anaerobic oral microbes with CHX gel at 20 and 25% concentrations

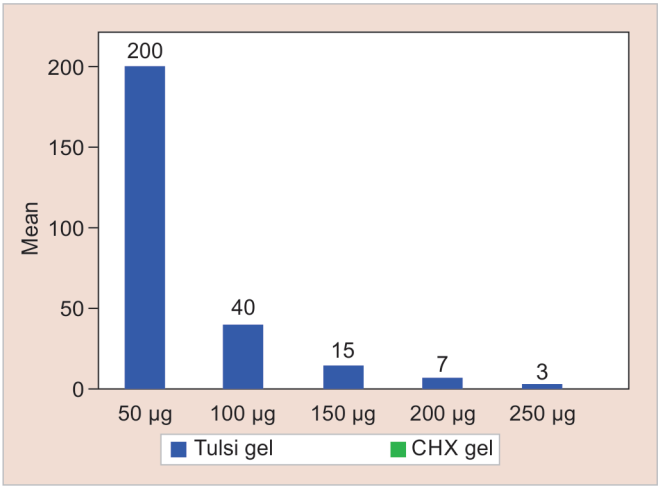


Fig. 3: The graph indicates the colony count of anaerobic oral microbes with *O. sanctum* L. (*Tulsi*) gel and CHX gel at five different concentrations

properties. *O. sanctum* L. has shown promising actions toward the treatment of periodontal diseases by having anti-inflammatory, antioxidant action with less toxicity. Cytotoxicity test showed *O. sanctum* L. *in situ* gel was less toxic compared to antimicrobials and highly biocompatible. *O. sanctum* L. gel showed potent antioxidant and anti-inflammatory effects comparable to a standard drug in an *in vitro* study.[21] It is widely used in the treatment of several systemic diseases because of its antimicrobial property.[24] Hence *O. sanctum* L. was chosen for the study. In our study, *O. sanctum* L. (*Tulsi*) demonstrated effective antimicrobial activity against anaerobic oral microbes at 20 and 25%. Higher concentrations of *O. sanctum* L. gel demonstrated its potential use as an efficient and alternative mode of medicine in the treatment of periodontal conditions compared to conventional treatment. CHX gel showed no growth of microbes when compared to *O. sanctum* L. (*Tulsi*) gel, which showed its superiority over other antimicrobial agents, but it has its own side effects and does not warrant long-term usage.

Jayanti et al.,[25] demonstrated a triple-blinded randomized control trial to detect the efficiency of 4% w/v mouthwash. *O. sanctum* L. (*Tulsi*) and 0.12% CHX were used as mouthwash. The results revealed that *O. sanctum* L. (*Tulsi*) is effective at reducing gingivitis. As a result of the efficiency of CHX, they had a lower plaque level. Jayanti et al.[25] stated that the maximum antimicrobial property of *O. sanctum* L. was discovered at an 8% concentration against *Aggregatibacter actinomycetemcomitans* and *P. gingivalis*. As a result, *O. sanctum* L. (*Tulsi*) was chosen as an alternative to mechanical treatment. It is also used to stop periodontitis from progressing. It can also be used to treat periodontitis.

Prabhakar et al.,[26] evaluated the efficiency of *O. sanctum* L. (*Tulsi*) as an intracanal medicament in primary molars and is compared to sodium hypochlorite. He determined that *O. sanctum* L. (*Tulsi*) had better therapeutic effects when compared to sodium hypochlorite. Its effect was due to eugenol. Eugenol was the main ingredient and it is less toxic than sodium hypochlorite. Mallikarjun et al.,[27] evaluated the antimicrobial activity of *O. sanctum* L. (*Tulsi*)—*in vitro* study against periodontal microbial complexes, like *Aa. comitans, P. intermedia*, and *P. gingivalis* with doxycycline. Doxycycline is used as a standard. He concluded *O. sanctum* L. (*Tulsi*) at 5 and 10% concentrations exhibited a maximum zone of inhibition against *Aa. comitans*. They exhibited smaller zones of inhibition against *P. intermedia* and *P. gingivalis*. Hence it was proposed that *O. sanctum* L. (*Tulsi*) could be used as an effective and alternative mode of therapy to conventional periodontal therapy.

Mirje et al.[28] conducted a study on the anti-inflammatory properties of *O. sanctum* L. (*Tulsi*). He used *O. sanctum* L. as two types; type I: only *O. sanctum* L. (*Tulsi*); type II: used along with indomethacin. It was assessed using carrageenan. Carrageenan was injected into the rat paw, which resulted in edema. Aqueous (liquid) extract of *O. sanctum* L. (200 mg/kg) or *O. sanctum* L. (400 mg/kg) was administered alone. It was also used along with indomethacin (25 mg/kg). Two separate groups of rats were used. Plethysmometer was used to determine the volume of the rat paw; then it was compared with the control. *O. sanctum* L. (*Tulsi*) showed a better reduction of edema when compared to indomethacin. Indomethacin is an anti-inflammatory drug that is standard. *O. sanctum* L. (*Tulsi*) along with indomethacin showed better anti-inflammatory properties. The anti-inflammatory property of *O. sanctum* L. (*Tulsi*) is because of the inhibition of dual pathways that are cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism. Test groups exhibited significant ($p < 0.05$) anti-inflammatory activity in carrageenan-induced rat paw edema.

Ocimum sanctum L. (*Tulsi*) contains many phytochemical constituents such as oleanolic acid, rosmarinic acid, ursolic acid, eugenol, linalool, carvacrol, β-elemene, β-caryophyllene, and germacrene. *O. sanctum* L. (*Tulsi*) was considered to have diuretic stimulant properties. Recent studies suggest that *Tulsi* may be a cyclooxygenase-2 inhibitor, like many modern painkillers, due to its high concentration of eugenol.[29]

In our study, the colony count of anaerobic oral microbes was assessed with *O. sanctum* L. gel and CHX gel at different concentrations of 5, 10, 15, 20, and 25% at the Petri plate. Results showed that the maximum number of microbes were present in the lowest concentration of *Tulsi* gel which was 5%. The colony count drastically reduced at 10% concentration and at 25% concentration it showed excellent antimicrobial activity which was comparable to CHX. The study results were in correlation with a study done by Parida et al.,[30] used ethanolic extract of *Tulsi*. It was obtained from the leaves of *O. sanctum* L. (*Tulsi*). The extract was prepared by using an apparatus named "Soxhlet" apparatus. He used both the agar well diffusion method and broth dilution method to assess the antimicrobial activity with 400 µg/mL of *O. sanctum* L. (*Tulsi*). He used this concentration of 400 µg/mL against common oral pathogens such as *P. intermedia, Streptococcus mutans, C. albicans, Lactobacillus acidophilus, Streptococcus mitis,* and *Peptostreptococcus*. The zone of inhibition was found against *S. mutans* and *S. mitis* (7.33 mm for each zone of inhibition). It was also found to be efficient against *P. intermedia* and *Peptostreptococcus*. It was also effective against *C. albicans* (zone of inhibition was 10.67 mm). Hence no zone of inhibition was found against *Lactobacillus*. Thus, it was concluded that *O. sanctum* L. (*Tulsi*) has inhibitory properties against oral pathogens.

Hassan et al.[31] demonstrated the use of *O. sanctum* L. (*Tulsi*) in oral and systemic diseases and highlighted the effects of *tulsi* gel, like antidiabetic, wound recuperating movement, radio-defensive impact, cancer prevention agent, antimicrobial, gastroprotective, eye diseases, renal diseases, mental illness, skin disorders, *O. sanctum* L. (*Tulsi*) used in oral diseases, like dental caries, periodontal diseases, candidiasis, oral submucous fibrosis, and ulcer. Yamani et al.,[32] *O. sanctum* L. (*Tulsi*) plant extract was efficient against the growth of *S. aureus, E. coli*, and *Pseudomonas aeruginosa*. Hence *O. sanctum* L. (*Tulsi*) was efficient against both gram-positive and gram-negative bacteria.

Ocimum sanctum L. demonstrated an antimicrobial effect comparable to CHX in 25% concentration in our study and is hence suitable for its use as a local drug delivery agent for the treatment of periodontal disease. CHX, although superior in terms of antimicrobial effect, is not suited for long-term use due to its side effects, and hence the nature-based product is preferred for the management of periodontal diseases.

Conclusion

Ocimun sanctum L. (*Tulsi*) activity showed effective antimicrobial properties against anaerobic oral microbes at 250 µg concentration which was higher compared to CHX. Even though CHX showed a superior antimicrobial effect at lower concentrations itself compared to *O. sanctum* L. gel, nature-based products can be preferred for the treatment of periodontal disease as they have lesser side effects. However, the clinical application of *O. sanctum* L. gel in comparison with CHX should be studied for the final conclusion.

Limitations and Future Directions

The limitation of the study is the antimicrobial effect of *O. sanctum* L. gel was not specific to the serotypes of the organisms. There can be variations with specific serotypes susceptibility to *O. sanctum* L. gel. Future studies should be planned to evaluate the effect of *O. sanctum* L. gel in the biofilm environment to assess its effectiveness.

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