



In vitro* Antimicrobial Activity of *Ocimum sanctum* (Tulsi) Extract on *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis

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ABSTRACT

Aim: The present study was conducted with an aim to assess the antimicrobial activity of *Ocimum sanctum* (tulsi) extract on *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*.

Materials and methods: 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*. For comparison within the group and between the study groups, one-way analysis of variance (ANOVA) and Tukey's *post hoc* tests were used. A statistical significance level of $p < 0.05$ was established.

Results: The 8% concentration of the tulsi extract showed maximum zone of inhibition against *A. actinomycetemcomitans* and *P. gingivalis* (40.10 ± 0.90 , 33.79 ± 1.82 mm), followed by the 6, 4, and 2% concentrations. The 0.2% chlorhexidine, which was the positive control, had 39.80 ± 1.24 and 32.28 ± 1.28 mm zones of inhibition; dimethylformamide showed 13.55 ± 1.92 and 10.21 ± 2.16 mm zones of inhibition against both the microorganisms. The ANOVA showed highly statistically significant ($p < 0.0001$) results between and within the groups. The antimicrobial activity of tulsi extract at 6 and 8% concentrations, and 0.2% chlorhexidine

against *A. actinomycetemcomitans* showed statistically significant differences between the groups. The concentration of tulsi extract at 8 and 0.2% chlorhexidine on *P. gingivalis* showed statistically significant differences between the groups.

Conclusion: It was concluded that 8% concentration of *O. sanctum* (tulsi) extract showed the maximum antimicrobial activity against *A. actinomycetemcomitans* and *P. gingivalis*. It is thus recommended that this may be useful as an adjunctive to mechanical therapy in the prevention and treatment of periodontal diseases.

Clinical significance: *O. sanctum* (tulsi) is a herb that is abundantly available, easily accessible, economically feasible, and culturally acceptable. Therefore, it is very useful in the management of oral diseases and also for overcoming many barriers that exist for the utilization of dental services, such as affordability, accessibility, availability, and acceptability.

Keywords: *Aggregatibacter actinomycetemcomitans*, Antimicrobial, *Ocimum sanctum*, *Porphyromonas gingivalis*.

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INTRODUCTION

Periodontal disease is a pathological oral disease manifested by inflammation of the supporting structures of the teeth involving complex interactions of specific microorganisms or their groups and the immune response of the host. This can often lead to teeth exfoliation combined with worsening of the systemic health, if enough heed is not given to treat it.¹

Various microorganisms that have been known to be associated with periodontitis are *P. gingivalis*, *Prevotella intermedia*, *A. actinomycetemcomitans*, *Fusobacterium*

nucleatum, etc. However, some predominant subgingival microbial species, such as *P. gingivalis* and *A. actinomycetemcomitans* at elevated proportions have been associated with destructive periodontal disease activity.^{2,3}

Among the periodontal pathogens, the most commonly involved microorganism in the causation of periodontal disease is the gram-negative, facultative anaerobe, and nonmotile bacterium *A. actinomycetemcomitans* present in dental plaque. Therefore, reducing their levels in the oral cavity becomes the very rationale behind the prevention and control of periodontal disease.⁴

A black-pigmented gram-negative anaerobic rod, *P. gingivalis*, has been implicated as a major pathogen in chronic periodontitis. In the scientific literature, recent research employing deoxyribonucleic acid hybridization also indicates the increased prevalence of *P. gingivalis* and other "red complex species" (*P. gingivalis*, *Treponema denticola*, and *Tannerella forsythia*) among the subjects with chronic periodontitis.⁵

In India, *O. sanctum* or tulsi has been a strong pillar of the ayurvedic holistic health system. An aromatic plant belonging to the family Lamiaceae, tulsi is indigenous and native to the Indian subcontinent and widespread as a cultivated plant. It has been famously considered as "The Queen of the herbs" for its restorative and spiritual properties. Apart from being cultivated for essential oils, tulsi is cultivated for religious as well as medicinal purposes too.⁶

The treatment regimen of several systemic diseases, such as upper respiratory infections, bronchitis, skin diseases, and malaria has extensively included various parts of this plant. Moreover, antimicrobial property of tulsi has been tested against microbes, such as *Candida albicans*, *Staphylococcus aureus*, enteric pathogens, *Klebsiella*, *Escherichia coli*, and *Proteus*. Antigonorrhoeal efficacy against multiresistant strains of *Neisseria gonorrhoeae* and clinical isolates of beta-lactamase-producing methicillin-resistant *S. aureus* has been demonstrated by this wonderful herb.⁷ Therefore, the present study was conducted to assess the antimicrobial activity of *O. sanctum* (tulsi) extract against two microorganisms, namely, *A. actinomycetemcomitans* and *P. gingivalis*.

MATERIALS AND METHODS

This was an *in vitro* study, which was conducted with the aim to assess the antimicrobial activity of *O. sanctum* (tulsi) extract on *A. actinomycetemcomitans* and *P. gingivalis*. This study was conducted in Kalinga Institute of Dental Sciences, Bhubaneswar, Odisha, India.

Preparation of Tulsi Extract

Tulsi powder that was commercially available was purchased for conducting the study (Fig. 1). Finely powdered



Fig. 1: Tulsi powder used in the present study

tulsi was measured at 300 gm and macerated with 1 L of 100% ethanol. Whatman filter paper was then used for filtration and a clear filtrate was obtained. Further, a low temperature of <math><60^{\circ}\text{C}</math> was used to reduce the filtrate and a solid residue of tulsi extract was obtained. Overall, 300 gm of tulsi powder dissolved in 1 L of ethanol gave 18 gm of residue (extract), and thus the yield was 6% w/w.

Preparation of Different Concentrations of Tulsi Extract

Dissolution of 1 gm of extract in 10 mL of dimethylformamide was done to obtain a 10% concentration of the tulsi extract. Following this, 1 mL of the extract was transferred to a sterilized test tube and labeled as 10%. Further, dilution was done on the remaining 9 mL of the extract with dimethylformamide, to obtain various concentrations (2, 4, 6, and 8%). A 0.2% chlorhexidine served as the positive control, whereas the negative control was dimethylformamide.

Bacteria and Growth Condition

Porphyromonas gingivalis was isolated using Kanamycin blood agar, the major ingredients of which are Trypticase blood agar base with 5% sheep blood supplemented with yeast extract, hemin, Vitamin K₁, L-cysteine, and in addition, 100 mg/L of Kanamycin.

Aggregatibacter actinomycetemcomitans was isolated using Dentaid agar. Brain heart infusion agar was added with 5 gm of yeast extract, 1.5 gm of sodium fumarate, and 1 gm of sodium formate per liter to prepare the Dentaid agar. Autoclaving of the medium was done for 15 minutes at 121°C. The final pH was estimated to be 7.2 ± 0.2 . The medium was then cooled to 50°C and vancomycin was added to obtain a final concentration of 9 µg/mL.

Porphyromonas gingivalis and *A. actinomycetemcomitans* were subcultured by incubating them at 35 to 37°C for 48 to 72 hours. Inoculation of the agar plates was done, and they were placed in the anaerobic jars, incubated for 48 hours, and reincubated for another 2 to 4 days, so as



Fig. 2: Antimicrobial activity of tulsi extract against *A. actinomycetemcomitans*



Fig. 3: Antimicrobial activity of tulsi extract against *P. gingivalis*

to allow those slow-growing organisms to form colonies. *Porphyromonas gingivalis* formed as black and mucoid colonies, whereas *A. actinomycetemcomitans* formed as tiny, translucent colonies.

Antimicrobial Effect of Extracts against *A. actinomycetemcomitans* and *P. gingivalis*

Well diffusion method was used to test the efficacy of the extracts (Figs 2 and 3). The antimicrobial action was assessed by measuring the zones of inhibition around the wells. Blood agar plates were prepared and colonies that were adjusted to 0.5 McFarland standard were swabbed onto the plates. Further, wells of 8 mm were created using a Cork borer. Freshly prepared extracts measuring 50 μ L were pipetted using micropipette and then added to the wells. Incubation was done for a period of 48 hours, both for aerobic (*A. actinomycetemcomitans*) and anaerobic (*P. gingivalis*) bacteria. The plates were incubated in the McIntosh and Fildes anaerobic jar for the anaerobic organisms, while the aerobic microorganisms were cultured in the incubator at 37°C for 48 hours. Further, the zones around the wells were measured.

Statistical Analysis

The Statistical Package for the Social Sciences software version 20.0 was used to analyze the data. One-way ANOVA and Tukey's *post hoc* tests were used for comparison within the groups and between the study groups. A statistical significance level of $p < 0.05$ was established.

RESULTS

Table 1 summarizes the mean zone of inhibition of the different concentrations of tulsi extract, 0.2% chlorhexidine, and dimethylformamide. An 8% concentration of the tulsi extract showed the maximum zone of inhibition (40.10 ± 0.90 and 33.79 ± 1.82 mm) against

Table 1: Mean zone of inhibition of different concentrations of tulsi extract and 0.2% chlorhexidine and dimethylformamide

Tulsi extract (%)	<i>A. actinomycetemcomitans</i> (mm)	<i>P. gingivalis</i> (mm)
2	16.24 ± 1.01	14.32 ± 1.21
4	26.75 ± 2.57	22.94 ± 1.52
6	33.38 ± 1.86	29.80 ± 1.60
8	40.10 ± 0.90	33.79 ± 1.82
0.2% chlorhexidine	39.80 ± 1.24	32.28 ± 1.28
Dimethylformamide	13.55 ± 1.92	10.21 ± 2.16

Table 2: Comparison of zone of inhibition among extracts using ANOVA

	Sum of squares	df	Mean square	f-value	Significant
Between groups	6942.413	6	1009.228	2348.210	0.0001
Within groups	18.200	48	0.482		
Total	7960.613	54			

A. actinomycetemcomitans and *P. gingivalis*, followed by the 6, 4, and 2% concentrations. Positive control (0.2% chlorhexidine) showed 39.80 ± 1.24 and 32.28 ± 1.28 mm zones of inhibition; dimethylformamide showed 13.55 ± 1.92 and 10.21 ± 2.16 mm zones of inhibition against both the microorganisms.

Table 2 compares the statistical differences in the antimicrobial effects of tulsi between and within the study groups by the ANOVA test, and it was found to be highly significant ($p < 0.0001$).

Table 3 summarizes the comparison of the individual preparations with *A. actinomycetemcomitans*. The individual preparations of tulsi extract at 6 and 8% concentrations as well as 0.2% chlorhexidine showed a highly statistically significant difference between the groups. No statistically significant difference was demonstrated by tulsi extracts at 2 and 4% concentrations as well as dimethylformamide.

Table 4 summarizes the comparison of the individual preparations against *P. gingivalis*. The tulsi extract at 8%

Table 3: Within-group comparison between different concentrations of tulsi extract, 0.2% chlorhexidine, and dimethylformamide with *A. actinomycetemcomitans*

Tulsi extract (%)	<i>A. actinomycetemcomitans</i> (mm)	Standard error	p-value
2	16.24 ± 1.01	0.2812	0.10
4	26.75 ± 2.57	0.1960	0.06
6	33.38 ± 1.86	0.0120	0.01
8	40.10 ± 0.90	0.0110	0.001
0.2% chlorhexidine	39.80 ± 1.24	0.0100	0.001
Dimethylformamide	13.55 ± 1.92	0.2824	0.218

concentration and 0.2% chlorhexidine showed a highly significant difference between the study groups. Tulsi extracts at 2, 4, and 6% concentrations as well as dimethylformamide did not show any significant statistical difference.

DISCUSSION

The rising occurrence of periodontal disease and caries among the people of developing countries, combined with the high expenditure in the treatment of these oral diseases, along with the possible side effects of the conventional antiplaque agents demand for alternative approaches. In the literature, it is being noticed that the natural and plant-derived medicinal extracts that were of great significance in the historical times are regaining their relevance nowadays, worldwide. These herbal extracts along with being therapeutic have no side effects as opposed to the synthetic drugs that are, to a certain degree, unsafe to human beings and their environment. For many thousands of years, plant-derived medicines have been used traditionally and have been the most important healing agents.⁸

Many diseases, including predominant oral diseases, such as periodontitis, have been caused by the combination of microorganisms and inflammatory response. In such situations, medicinal compounds functioning with dual anti-inflammatory and antimicrobial activity may be the desirable therapeutic agents. It should be noted that plant extracts have been increasingly employed for their antibacterial, antifungal, and antiviral activities globally.⁹

Being an infectious disease, periodontal disease is an oral disease that fluctuates in severity from mild gingivitis to advanced conditions, such as loss of connective tissue attachment as well as supporting alveolar bone.¹⁰ A successful treatment of periodontitis calls for the suppression and elimination of the subgingival periodonto pathogens. As the probing depth increases, the vigor and efficaciousness of the nonsurgical mechanical procedures and conventional home care in controlling the pathogenic microbe's decrease. Antimicrobial agents attempt to directly diminish the microflora from the periodontal pocket, functioning as an adjunct to mechanical debridement.¹¹

Table 4: Within-group comparison between different concentrations of tulsi extract, 0.2% chlorhexidine, and dimethylformamide with *P. gingivalis*

Tulsi extract (%)	<i>P. gingivalis</i> (mm)	Standard error	p-value
2	14.32 ± 1.21	0.1040	0.146
4	22.94 ± 1.52	0.1314	0.09
6	29.80 ± 1.60	0.1289	0.06
8	33.79 ± 1.82	0.1876	0.001
0.2% chlorhexidine	32.28 ± 1.28	0.1489	0.001
Dimethylformamide	10.21 ± 2.16	0.1812	0.184

In this study, we assessed the antimicrobial efficacy of tulsi against two major periodontal pathogens, namely, *A. actinomycetemcomitans* and *P. gingivalis*. These microorganisms are most commonly implicated in the initiation and progression of various periodontal diseases, especially aggressive periodontitis.^{12,13} Results of this *in vitro* study showed that at concentrations of 6 and 8%, tulsi inhibited the growth of *A. actinomycetemcomitans* effectively, which was comparable with that of dimethylformamide. Many authors have discussed various mechanisms of action of tulsi previously in the literature. The antimicrobial activity of tulsi was explained by authors Vishwabhan et al¹⁴ by virtue of its essential oil content. Studies conducted in the past also reaffirm the fact that tulsi yields various essential oils that have been responsible for its medicinal uses including antimicrobial, antioxidant, antifungal, and anti-inflammatory activities. This explains the activity of tulsi against the microorganisms discussed hereby.¹⁵ It has been affirmed that tulsi has immunomodulatory effects by strengthening the host's response to infections and increasing the T-helper cells, interferon, and interleukin-4 levels. Singhal et al¹⁶ theorized that the antibacterial activity of tulsi leaf extract can be accredited to its ability to reduce silver ions to silver nanoparticles that have antibacterial properties against both gram-negative and gram-positive bacteria.

In this study, it was found that 0.2% chlorhexidine was less effective for *A. actinomycetemcomitans* and *P. gingivalis*, in comparison with the 8% tulsi extract. However, it should be noted that the well-known side effects of chlorhexidine, i.e., staining of teeth and restorations, alteration of taste sensation, and development of resistant organisms, may limit its long-term use. In the study by Eswar et al,⁷ tulsi showed a high margin of safety with particularly low toxicity, with no known human drug interaction.

This *in vitro* study was chiefly carried out to assay the efficacy of tulsi extract against periodontal pathogens, such as *A. actinomycetemcomitans* and *P. gingivalis*. Similar results have been obtained by Rathod et al,¹⁷ Shah et al,¹⁸ and Prasannabalaji et al,¹⁹ who have all demonstrated the antimicrobial properties of tulsi against various microorganisms. The evidence clearly shows that plant

products can be used effectively in the therapy against periodontitis.

A study conducted by Bansal et al²⁰ to ascertain the efficacy of a tulsi-containing herbal mouthrinse demonstrated that it was a strong plaque inhibitor, even though it was found to be less effective than chlorhexidine gluconate. However, it is recommended to serve as a potent alternative for patients with special needs as in the case of diabetics and xerostomics.

Gupta et al²¹ conducted a triple-blinded randomized controlled trial to test the efficacy of 4% w/v mouthrinse containing tulsi and 0.12% chlorhexidine. It was found that the mouthrinse containing *O. sanctum* was as effective in reducing gingivitis and plaque levels as chlorhexidine. In another study by Mistry et al,²² it was observed that the extracts of plant sources, such as *O. sanctum* (Tulsi), *Aristolochia indica* (neem), *Tinospora cordifolia* (Giloy), and *Mimusops elengi* (bakul) along with the positive control chlorhexidine gluconate demonstrated antimicrobial efficacy against the endodontic pathogens *Enterococcus faecalis*, *S. mutans*, as well as *S. aureus*. A 3 mg concentration of *O. sanctum* was found to be most efficacious against *S. mutans*, whereas chlorhexidine was most active against *S. aureus*. The various obstacles that exist against the utility of dental services, such as accessibility, availability, affordability, and acceptability can be overcome by the effective use of herbs, such as tulsi in the treatment of oral diseases.

CONCLUSION

It was concluded that 8% concentration of *O. sanctum* (tulsi) extract showed the maximal antimicrobial activity against *A. actinomycetemcomitans* and *P. gingivalis*. This herb shows to be definitely useful as an adjunctive to mechanical therapy in the prevention and treatment of periodontal diseases.

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