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Original Research Article

Evaluation of the Antimicrobial Activity of *Ocimum Sanctum L.* (Tulsi) Extract against *Streptococcus Mutans* and *Lactobacillus Acidophilus* - An in Vitro Study

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ABSTRACT

Dental caries is a major public health problem. *Streptococcus mutans* and *Lactobacillus acidophilus* are the main organisms responsible for the initiation and progression of caries. Various antimicrobials have been used against these caries causing organisms but they have many side effects. Hence there is a need for an alternative which is effective as well as economical. *Ocimum sanctum L.* has been used in traditional medicine since ancient times for its diverse healing properties. However there is a paucity of literature regarding its antimicrobial activity against oral microorganisms.

Objective: To determine the antimicrobial effect of *Ocimum sanctum L.* against caries causing microorganisms *Streptococcus mutans* and *Lactobacillus acidophilus.*

Material and Methods: Ethanolic *Ocimum sanctum L.* extract was prepared and Minimum Inhibitory Concentration (MIC) of the extract was determined against *Streptococcus mutans* and *Lactobacillus acidophilus* by serial broth dilution method.

Results: The MIC of ethanolic extract of *Ocimum sanctum L.* against *Streptococcus mutans* and *Lactobacillus acidophilus* was 2.5% (25mg/ml) and 10% (100 mg/ml) respectively.

Conclusion: The ethanolic extract of *Ocimum sanctum L.* showed antimicrobial activity against *Streptococcus mutans* and *Lactobacillus acidophilus.*

Keywords: Antimicrobial, *Ocimum Sanctum L, Streptococcus mutans, Lactobacillus acidophilus.*

INTRODUCTION

Dental caries is defined as an infectious bacterial disease that results in destruction of the calcified tissues of the teeth. The main etiological agents of dental caries are *Streptococcus mutans* and *Lactobacillus acidophilus*. [1] They can easily colonize the tooth surface and initiate acid production by synthesizing extracellular polysaccharides from sucrose foods. [2] The reduction of bacteria associated with caries in the dental plaque is a major preventive strategy. [3] Caries is a global public health problem, whose control requires the introduction of low cost treatments, such as strong prevention strategies, minimally invasive techniques and chemical prevention agents. [4] Nature has been a source of medicinal treatments for thousands of years and plant-based systems continue to play an essential role in the primary health care of 80% of the world's underdeveloped and developing countries. [5] During the last two decades, natural products represented one of the main

sources of new drugs approved by the Food and Drug Administration. [6]

Ocimum sanctum L. (also known as *Ocimum tenuiflorum*, Tulsi) has been used for thousands of years in Ayurveda for its diverse healing properties. Tulsi, 'The Queen of herbs', the legendary 'Incomparable one' of India, is one of the holiest and most cherished of the many healing and health giving herbs of the orient. The sacred basil, Tulsi, is renowned for its religious and spiritual sanctity, as well as for its important role in the traditional Ayurvedic and Unani system of holistic health and herbal medicine of the east. [7]

It is mentioned by Charaka in the Charaka Samhita. Tulsi extracts are used in Ayurvedic remedies for common colds, headaches, stomach disorders, inflammation, heart disease, various forms of poisoning and malaria. [8]

Ocimum sanctum L. has showed antimicrobial activities against various pathogenic microorganisms like *Staphylococcus aureus*, *Bacillus pumius* and *Pseudomonas aeruginosa, Bacillus subtilis, Escherichia coli*. [9,10] However there is paucity of literature regarding the antimicrobial effect of *Ocimum sanctum L.* against caries causing organisms like *Streptococcus mutans* and *Lactobacillus acidophilus*. Hence the aim of the study was to determine the antimicrobial effect of *Ocimum sanctum L.* against *Streptococcus mutans* and *Lactobacillus acidophilus*.

MATERIALS AND METHODS

Collection of plant material

The whole plant of *Ocimum sanctum L.* was obtained from botanical garden of KLE ayurvedic college, Belgaum. The fresh plant material was washed in distilled water, shade dried and ground into coarse powder form.

Preparation of the extract:

50 grams of the powdered sample was weighed and added to 250 ml of ethanol and subjected to cold maceration process. The flask was kept for 5 days with occasional shaking. The sample was then filtered using sterile muslin cloth. The filtrate was again filtered using Whatman No. 1 filter paper. The filtrate was concentrated using rotavapor evaporator and poured into wide glass petriplates and kept for drying in hot air oven at 40^0C till the solvent was completely evaporated. The dried extract (Total yield 2g) was collected and further used to determine its antimicrobial activity.

Preparation of stock solution of *Ocimum sanctum L.* extract

50 mg of the extract was dissolved in 500 µl Dimethyl sulphoxide (an inert solvent) to obtain a stock solution of 10%.

Microorganisms

The following bacterial strains were used in the antimicrobial tests. Gram positive bacteria *Streptococcus mutans* (ATCC 25175) and *Lactobacillus acidophilus* (ATCC 314). The culture media used was Brain Heart Infusion (BHI) Broth.

Preparation of the inoculum

The inoculum was prepared by making a BHI Broth suspension of isolated colonies selected from 18 to 24 hour agar plate. The broth with the test organism was kept for overnight incubation. The overnight broth culture was diluted by matching with 0.5 McFarland Standards to get bacterial concentration of 1.5×10^8 CFU/ml.

Determination of Minimum inhibitory concentration (MIC)

MIC of the extract against *Streptococcus mutans* and *Lactobacillus acidophilus* by serial broth dilution method.

9 dilutions of extract were done with BHI for Minimum inhibitory concentration. In the initial tube only 200 µl of extract was added. For dilutions 200 µl of BHI broth was added into the next 9 tubes separately. In the 2nd tube 200 µl of extract was added which already contains 200 µl of BHI broth. This was considered as 10^{-1} dilution. From 10^{-1} diluted tube 200 µl was transferred to second tube to make 10^{-2} dilution. The serial dilution was repeated up to 10^{-8} dilution for each extract. In each serially diluted tube 200 µl of above culture suspension was

added. From the last tube, 200 µl final solutions were discarded. The last tube contained only the media and culture suspension. The concentrations of 10%, 5%, 2.5%, 1.25%, 0.62%, 0.31%, 0.15%, 0.07%, 0.03% respectively were achieved by serial dilution method. The tubes were kept for incubation for 24 hours at 37^0C in bacteriological incubator and observed for turbidity.

RESULTS

The MIC is the lowest dilution which inhibits microbial growth and it is judged by the lack of turbidity in the tube. The MIC of *Ocimum sanctum L.* against *Streptococcus mutans* and *Lactobacillus acidophilus* was determined by serial broth dilution method. *Ocimum sanctum L.* showed antimicrobial activity against both the organisms. The MIC was 2.5% (25mg/ml) against *Streptococcus mutans* and against *Lactobacillus acidophilus* was 10%(100 mg/ml) as shown in Table 1.

Table 1: MIC of *Ocimum sanctum L.* by serial broth dilution method

Test organisms	10%	5%	2.5%	1.25%	0.62%
Streptococcus mutans	S	S	S	R	R
Lactobacillus acidophilus	S	R	R	R	R

S=Sensitive
R=Resistant

DISCUSSION

The present study was conducted to evaluate the in vitro antimicrobial activity of *Ocimum sanctum L.* against *Streptococcus mutans* and *Lactobacillus acidophilus*.

Ocimum sanctum L. is described as sacred and medicinal plant in ancient literature. Different parts of *Ocimum sanctum L.* (leaves, stem, flower, root, seeds) are known to have several therapeutic potentials and have been used as analgesic, antimicrobial, anticancer, antiasthmatic, antidiabetic, anti-fertility, antispasmodic, hepatoprotective, antiemetic, cardioprotective and antistress agents.

Ocimum sanctum L. fixed oil has shown good antibacterial activity against *Staphylococcus aureus*, *Bacillus pumius* and *Pseudomonas aeruginosa*. Higher content of linoleic acid in *Ocimum sanctum L.* fixed oil could contribute towards its antibacterial activity. [9] Geeta *et al.* [10] studied that the aqueous extract of *O. sanctum L.* (60 mg/kg) show wide zones of inhibition compared to alcoholic extract against *Klebsiella*, *Escherichia coli*, *Proteus*, *Staphylococcus aureus* and *Candida albicans* when studied by agar diffusion method. Alcoholic extract showed wider zone for *Vibrio cholera*. Extract of *Ocimum sanctum L.* caused inhibition of *Neisseria gonorrhoeae* clinical isolates and WHO organization strains. [11]

Ocimum sanctum L. has specific aromatic odour because of the presence of essential or volatile oil, mainly concentrated in the leaf. This aromatic volatile oil mainly contains phenols, terpenes and aldehydes. The oil extracted from seeds is called fixed oil and mainly composed of fatty acids. Besides oil, the plant also contains alkaloids, glycosides, saponins and tannins. These have been associated with the antibacterial activity. [12] It has been suggested that the antimicrobial properties of tannins might be related to their ability to inactivate microbial adhesions, enzymes and cell envelope transport proteins, their complexity with polysaccharides and their ability to modify the morphology of microorganism. Several reports have shown that bioactive compounds isolated from plant extracts have inhibitory effect on pathogens strains. [13-15]

The leaves contain ascorbic acid and carotene as well. [16] Eugenol (1-hydroxy-2 methoxy-4-allylbenzene), the active constituents present in *O. sanctum L.* have been found to be largely responsible for the therapeutic potentials. The other important constituents are carvacrol, methyl eugenol, Urosolic acid and caryophyllene. [17]

Ocimum sanctum L. has two varieties – black (Krishna tulsi) and green (Ram tulsi), their chemical constituents are similar. [18] Krishna tulsi was used for the study. Ethanolic extract was used as they are more powerful. This is due to the better

solubility of active components in organic solvents. [19, 20] The essential oils in tulsi are more soluble in alcohol as compared to distilled water.

In the present study, MIC was determined using serial broth dilution method. The MIC of *Ocimum Sanctum L.* against *Streptococcus mutans* was determined to be 2.5% (25mg/ml).In a previous study conducted by Agarwal P *et al.* [21] and Gupta B *et al.* [22] the MIC against *Streptococcus mutans* was found to be 4% and 6% respectively by cup and plate method.The variation may be due to the fact that the chemical constituents may vary due to edaphic and geographic factors, difference in the microbological method used and variation in the solvent used to prepare the extract.

The minimum inhibitory concentration of *Ocimum sanctum L.* against *Lactobacillus acidophilus* was determined to be 10% (100mg/ml).There were no comparison studies for this result.

The results of the present in vitro study shows that *Ocimum sanctum L.* possesses antibacterial properties and this can be translated into clinical effectiveness. *Ocimum sanctum L.* can thus be used an antibacterial agent for the prevention of dental caries. Furthermore it is easily available, accessible and possesses minimum side effects.

CONCLUSION

The extracts of *Ocimum sanctum L.* were found to be active against the tested micro organisms. *Ocimum sanctum L.* showed maximum antimicrobial activity against *Streptococcus mutans* and *Lactobacillus acidophilus* at 2.5% (25mg/ml) and 10% (100 mg/ml) respectively. Hence it can be employed as a source of herbal antimicrobial for dental caries. However further studies are recommended for assessing its use both in clinical and field setting.

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