

Evaluation of Antimicrobial Activity of Aqueous Extract of “Ocimum Sanctum–Queen of Herb” on Dental Caries Microorganisms: An *In Vitro* Study

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ABSTRACT

Aim: To evaluate the efficacy of three different concentrations of Ocimum sanctum aqueous extract against various caries causing microorganisms, *Streptococcus mutans*, *Streptococcus mitis*, *Streptococcus sanguis*, and *Lactobacillus acidophilus*.

Materials and methods: Aqueous extract of Ocimum sanctum was prepared by the cold extraction method. The extract was diluted with milli Q water, to obtain 3 different concentrations [2%, 3%, and 4%] of the extract. Glycerol 6%v/v and tween-80, 0.1%v/v were also added to get a stable suspension. About 0.2% chlorhexidine was used as a positive control and milli Q water was used as a negative control. The extract, along with the controls, was then subjected to microbiological investigation to determine which concentration among the three different concentrations of extract gave a wider inhibition zone against *Streptococcus mutans*, *Streptococcus mitis*, *Streptococcus sanguis*, and *Lactobacillus acidophilus*. The zones of inhibition were measured in millimeters.

Results: Ocimum sanctum aqueous extract demonstrated maximum antimicrobial activity against microorganisms responsible for dental caries at the 4% concentration level although 3% and 2% were also effective. Maximum activity was seen against *S. mutans* and *S. sanguis* with 4% extract.

Conclusion: Ocimum sanctum aqueous extract was effective against all caries causing the microorganisms.

Clinical significance: Dental caries is still a major oral disease in children which affects their quality of life. It is important to come up with an alternative oral hygiene aid which is easily available and with lesser side effects and maximum benefits by acting against caries causing microbes. Thus aqueous extract of commonly available tulsi is studied to know its effect on caries causing microorganisms.

Keywords: Caries, Children, Chlorhexidine, Mouthwash.

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INTRODUCTION

Dental caries is still considered as the most important global oral health burden and 60 to 90% of the children are affected, due to negligence, lack of prevention awareness, costly dental care services, scarce, or no dental care services, especially in the rural areas.^{1,2} Along with the complex etiology of dental caries many other indirect factors gets involved and complicate the understanding of disease process. Role of microorganisms is very vital in its etiology.³ Mainly the dental caries caused by the microorganisms such as *Streptococcus mutans*, *Lactobacillus acidophilus*, *Streptococcus mitis*, and *Streptococcus sanguis*.⁴ Reduction in the levels of these microorganisms in the oral cavity would greatly contribute in prevention of dental caries. Over the time many chemical agents, mouth rinses, varnishes and gels are used by dental professionals in management of dental caries. These therapeutic agents are usually unaffordable to mass population in developing countries. As there is always a risk of ingestion of mouth washes and gels in very young children, synthetic gels and mouth washes with chlorhexidine better be avoided. So it is necessary to innovate new strategies which may be effective and feasible in managing the disease.

Plants are of the important sources of medicine. They can be used safely and effectively in treating many oral diseases and also they are economical and easily available. Many plants in our surroundings are known for their medicinal value, among which Tulsi or Holy Basil also botanically called as *Ocimum sanctum* found and worshipped in most of the Indian homes.⁵

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The name *Tulsi* in Sanskrit means “*The incomparable one.*” This herb has wide range of antimicrobial substances and is used to treat a number of illnesses such as diabetes, arthritis, bronchitis, skin diseases, etc.^{5,6}

Thus we can also explore the use and benefits of easily available local herb Tulsi to prevent and control commonly occurring oral disease such as dental caries. In our previous ethanolic extract of Tulsi has shown promising antibacterial activity against caries causing bacteria.⁷ But when it comes to the use of mouth rinses in children aqueous based ones are safer than alcoholic based as there high chance of ingestion in small children. Hence in our

present study we have used three different concentrations of aqueous extract of *Ocimum sanctum* [Krishna Tulsi] against different microorganisms responsible for dental caries.

MATERIALS AND METHODS

Collection of Plant Material [*Ocimum Sanctum*]

The collected *Krishna Tulsi* [*Ocimum sanctum*] plant was verified at Mangalore University, Botany department. The collected leaves [200 gm] were dried under sunlight and powdered after thorough cleaning with tap water and sterilized distilled water.

Aqueous Extract of *Ocimum Sanctum* Preparation and HPTLC [High-performance Liquid Chromatography] Analysis

Ocimum Sanctum Aqueous Extract (OSAE)

Twenty-five grams of dried *Ocimum sanctum* powdered leaf [25 gm] was macerated with 250 mL of distilled water [with 10% of chloroform] in stoppered conical flask for 7 days with intermittent shaking. After a period of 7 days, contents in the flask was filtered using Whatman paper no 1 and concentrated on water bath. The concentrated extract was stored in deep freezer at -5°C for 12 hours and later lyophilized at -70°C using operon FDU-7006 freeze dryer.

Different Concentrations of Aqueous Extract of *Ocimum Sanctum* Preparation

10 mL of sterile milli Q water, glycerol 6% v/v, and Tween 80–0.1% v/v was used to dissolve 1 gm of Tulsi extract and the volume was made upto 100 mL and 10 % concentration of the extract was obtained. Sterile distilled water was used to prepare further dilutions [4%, 3%, and 2%]. The positive control used was 0.2 % chlorhexidine and milli Q water was used as a negative control.

Microbiological Analysis

Pure strains of *S.mitis* ATCC 6249, *S.mutans* ATCC 25,175, *S.sanguis* ATCC 10,556, *L. acidophilus* ATCC 4358 [HiMedia].

Culture Media Used

Brain heart infusion agar [HiMedia] for *Streptococcus* species and Lactobacillus MRS Agar [HiMedia] for *Lactobacillus acidophilus* were used.

The selected microorganisms were lyophilized and then were inoculated into mixture of Brain heart infusion broth [1 mL] and Thioglycollate broth [1 mL]. These broth were incubated at 37°C in Anaerobic chamber for a period of 48 hours. *Streptococci* and *Lactobacilli* were subcultured in Brain heart infusion agar and Lactobacillus MRS Agar, respectively.

Standard inoculums of each bacterium were prepared to match McFarland' turbidity standard tube 0.5. Brain heart infusion agar and Lactobacillus MRS Agar were used to make Lawn cultures. Wells of 8 mm diameter were cut in the media. Twenty five micro liters of various concentrations [2%, 3%, and 4%] of aqueous extract of *Ocimum sanctum* and 0.2% chlorhexidine were transferred into these wells. The plates were incubated in anaerobic chamber at 37°C for 48 hours. Plates were removed from anaerobic chamber and zones of inhibition were measured.⁸

RESULTS

The observational assessment of data showed that all the bacterial strains were resistant to sterile milli Q water, glycerol 6% v/v,

and Tween 80–0.1% v/v. 4 % extract of *Krishna Tulsi* showed a wider zone of inhibition when compared to 3% and 2% extract. Antimicrobial activity was maximum against *S. mutans* and *S. sanguis* with 4% extract of *Krishna Tulsi*. A wider inhibitory zone of 26 mm for *S. mutans* was shown by the positive control chlorhexidine [0.2%]. The zones of inhibition of chlorhexidine [0.2%] for *L. acidophilus*, *S. sanguis* and *S. mitis* were 24 mm, 23 mm, and 20 mm, respectively [Fig. 1].

DISCUSSION

One of the most common oral disease in humans is dental caries. It can be controlled by various preventive and therapeutic measures.⁹ Ayurvedic medicines can be used as alternative for the prevention of dental caries.¹⁰ In Ayurveda Tulsi is proven to have good source of antioxidants, antimicrobial, antifungal, antidiabetic, analgesic, cardio-protective agent, which is nontoxic and widely available throughout India.^{11,12,13}

The therapeutic and antimicrobial effect of Tulsi is mainly due to the presence of active component Eugenol [1-hydroxy-2-methoxy-4-allyl benzene] followed by ursolic acid and carvacrol.¹⁴ In our previous study alcoholic extract of Tulsi was made and it showed good inhibitory effect on caries causing microorganisms.⁷ It is not advisable to use mouth rinses below 6 years of age as children tend to swallow large amount of it and also mouth washes with alcohols are generally not indicated in children. Hence the need for water based mouth rinses which are safe for children. According to a previous study conducted by Jain et al. to determine the antimicrobial activity of six Indian plant extracts [aqueous and alcoholic] against *Streptococcus mutans*, showed inhibitory effect of extracts of *Ocimum sanctum* against *streptococcus mutans*.¹⁵ Thus the present study was conducted to know the efficacy of aqueous extract of *Ocimum sanctum* as an antimicrobial agent against different caries causing microorganisms such as *Lactobacillus acidophilus*, *Streptococcus mutans*, *Streptococcus sanguis*, and *Streptococcus mitis*.

In the present study, the leaves of *Krishna Tulsi* were used. The extract was diluted using solvent Sterile milliQ water, glycerol 6%, Tween 80–0.1% and further dilutions were done by using sterile distilled water [2%, 3%, and 4%].

In the present study [Fig. 1], the different concentrations of aqueous extract of *Ocimum sanctum* showed inhibitory zones against selected dental caries causing microorganisms [*Streptococcus mutans*, *Streptococcus Sanguis*, *Streptococcus mitis* and *Lactobacillus acidophilus*]. Four percent extract showed maximum antimicrobial activity compared to 3% and 2% extract. Even though in this study Chlorhexidine showed more antimicrobial activity compared to *Ocimum sanctum* aqueous extract, the side effects of chlorhexidine [antimicrobial resistance, staining, altered taste sensation, etc.] limits its long term usage.^{16,17,18} Among the other medicinal herbs which are effective in treating oral diseases Tulsi is widely available and accepted by people with various cultural backgrounds. Hence, Tulsi may be used for long term treatment of oral disease such as dental caries with minimal side effects.

Although this study results show a remarkable antimicrobial activity of aqueous extract of *Krishna Tulsi* on wide range of caries causing bacteria, the clinical recommendation of aqueous extract of *Ocimum sanctum* in prevention of dental caries among children and others may require further *in vivo* studies.

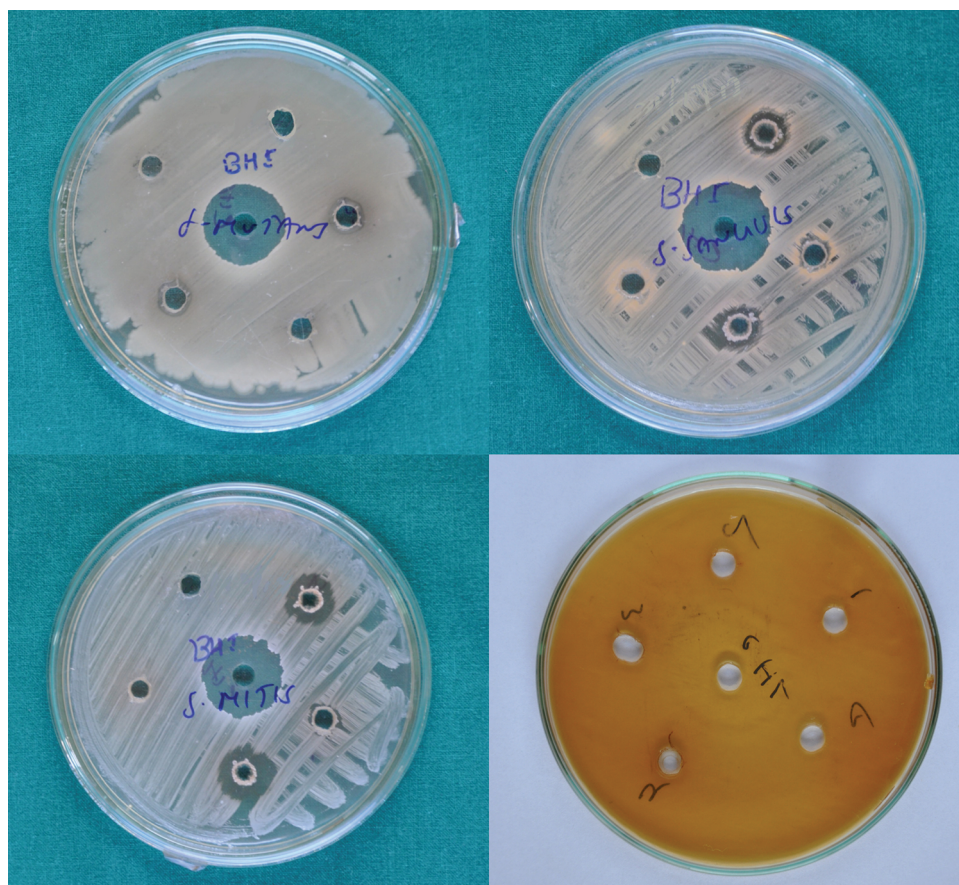


Fig. 1: Different concentrations of aqueous extract of *Ocimum sanctum* showed inhibitory zones against selected dental caries causing microorganisms [*Streptococcus mutans*, *Streptococcus Sanguis*, *Streptococcus mitis* and *Lactobacillus acidophilus*]

CONCLUSION

Aqueous extract of *Ocimum sanctum* in different concentrations was effective in inhibiting the growth of dental caries causing microorganisms. This study result may encourage further use of aqueous extract of *Ocimum sanctum* as a mouth rinse among wide population especially in children due to less side effects and as safe alternative to chlorhexidine in prevention of dental caries.

CLINICAL SIGNIFICANCE

Dental caries is still a major oral disease in children which affects their quality of life. It is important to come up with an alternative oral hygiene aid which is easily available and with lesser side effects and maximum benefits by acting against caries causing microbes. Thus aqueous extract of commonly available Tulsi is studied to know its effect on caries causing microorganisms.

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