ORIGINAL ARTICLE

An *in vitro* study on antimicrobial activity of *Solanum sisymbriifolium* extract on *Streptococcus mutans*

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Abstract

Context: Dental caries is one of the most common chronic infectious diseases of humans. Among the causative microorganism of dental caries, *Streptococcus mutans* is considered to be a pioneer initiator for this disease. Dental caries is prevalent in spite of the widespread use of mechanical and chemical plaque control methods. Considering the current era, research studies focus on incorporating traditional ingredients in controlling of dental caries. This is one such study on *Solanum sisymbriifolium* a known antimicrobial plant. **Aims:** The aim of the study was to determine the minimum zone of inhibition of *S. sisymbriifolium* against *S. mutans*. **Settings and Design:** This was an *in vitro* study. **Materials and Methods:** The well diffusion method using blood agar plates was used to evaluate the antibacterial activity of 5%, 10%, and 50% concentration of *S. sisymbriifolium* extract against *S. mutans* in comparison with 0.2% chlorhexidine gluconate mouth wash. **Statistical Analysis Used:** Results were statistically analyzed using independent sample t test to compare mean or median zone of inhibition between two groups. **Results:** Minimum zone of inhibition for 0.2% Chlorhexidine gluconate mouthwash at 5%, 10%, and 50% are found to be 3.3000 mm, 4.5033 mm, and 7.2767 mm, respectively, similarly for *S. sisymbriifolium* extract are 2.2133 mm, 3.7533 mm, and 5.3633 mm, respectively. **Conclusions:** *S. sisymbriifolium* has antimicrobial activity against *S. mutans*. The zone of inhibition (in mm) was statistically significant at 10% of concentration comparable with other concentrations. The inhibitory effect of 5%, 10%, and 50% concentrations of 0.2% Chlorhexidine gluconate mouthwash is significantly better than corresponding concentrations of *S. sisymbriifolium*. However, the inhibitory effect of *S. sisymbriifolium* is found to increase as the concentration increases.

Key words: *Solanum sisymbriifolium*, Dental Caries, Antimicrobial activity, *Streptococcus mutans*

INTRODUCTION

Dental caries is ancient and is associated with the dental profession. Dental caries is an irreversible microbial disease of the calcified tissues of the teeth, characterized by demineralization of the inorganic portion and destruction of the organic substance of the tooth. It is a complex and dynamic process which involves various factors. The word caries has received its origin from the Latin word meaning decay or rot. Humans' oral microbiome is a home to many microorganisims, bacteria which are proven to cause caries are as follows *Streptococcus salivarius* strain, *Streptococcus mutans, Streptococcus mitis*, *Streptococcus milleri, Streptococcus oralis, Streptococcus sanguaris, Actinomyces naelsundii, Lactobacilus casei* strain, *Peptostreptococcus intermedius,* and many more.[1] The fact is that despite several efforts toward total eradication, this disease is still prevalent. Nevertheless, an ecstatic success of the profession is the global decline in the incidence comparably. Dental caries is not an exception to this compulsion and numerous theories were devised in regards to dental caries eradication.[1]

S. mutans considered as initial colonizers of the biofilm (coating on the surface of tooth) is one of the primary causative agents of dental caries. They are responsible for the adherence of other acid-producing bacterial species

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of the *genera Veillonella, Scardovia, Lactobacillus, and Propionibacterium,* leading to progression of dental caries. Synthetic antimicrobials have been used as effective preventive methods for dental caries, one among them is 0.2% chlorhexidine gluconate which has various side effects such as staining of teeth, oral mucosal erosion, and increased supragingival calculus formation However, in present times, use of natural products is been recommended, as in comparison to synthetic drugs, they possess lesser side effects and are economically viable.[2,3]

Many of plants and their volatile oils have been used traditionally worldwide as a disinfectant and as a preservative in food. This has reignited interest among scientist community for the use of plant and natural extract as substitute for synthetic drugs. The plant essential oils (or volatile oils) are a potential source of biologically active compounds which are obtained from plant parts such as flowers, seeds, leaves, bark, wood, fruits and roots by expression, distillation, and solvent extraction methods. It is proven that essential oils have antibacterial, antifungal, antiviral, insecticidal and antioxidant properties. Various traditional plants such as clove, neem, and turmeric are being researched for their antimicrobial activity for oral health.[2,4]

Solanum sisymbriifolium (sticky nightshade or litchi tomato) belonging to Solanaceae family has also been used traditionally as various remedies in South American countries. According to various studies, chemical constituents of this genus act as anti-inflammatory, antihypertensive, anticonvulsant, antibacterial, and antioxidant agents. Phytochemical studies of this plant extract have revealed the presence of antioxidants, flavonoids, tannins, and secondary metabolites such as terpenes, fatty acids, and alkaloids. These bioactive agents are responsible for the antioxidant and antimicrobial activity of *S. sisymbriifolium* It is found to be effective against *Escherichia coli, Pseudomonas aeruginosa*, *Bacillus subtiis, Bacillus coagulans*, *and Saccharomyces cerevisiae Staphylococcus aureus*.[5,6]

However, there are no documented studies of *S. sisymbriifolium* against *S. mutans*. Therefore, the aim of the present study is to evaluate the antibacterial activity of the flower of *S. sisymbriifolium* essential oil against *S. mutans*.

MATERIALS AND METHODS

This *in vitro* study was conducted after approval by the institutional ethics committee. Plant flower material was obtained from a horticulturist. It was washed and cleaned thoroughly with tap water then with distilled water and air dried in shade for 4 weeks. Then, it was grinded into fine powder using grinder. The powder was weighed into 5 g, 10 g, 50 g, and transferred into respective sterile beaker, later 95 mL, 90 mL, and 50 mL of chloroform was added to the powdered tissue and stored at room temperature for 48 h. After 48 h, the extract was filtered through Whatman No.1 filter paper and 5%, 10%, and 50% choloroform extract of *S. sisymbriifolium* was stored at -20° Celsius until further use.[7,8]

Preparation of Culture Media

The strains of *S. mutans* strain (ATCC 25175) was procured from HIMEDIA. It was cultured in nutrient broth on incubation at 37°C for 24 h and then was streaked onto the blood agar plate using loop for a nutritious growth [Figure 1].[8]

The mouthwash as a positive control was measured into 5 mL, 10 mL, and 50 mL amounts and transferred into labeled bottles, to which 95 mL, 90 mL, and 50 mL of distilled water were added, respectively. The mixture was shaken well.[8] For the negative control, distilled water was used. Separate agar plate was used as there was no space available in the agar plate. As the triplicate method was used, the negative control was performed separately for each set.

Ditch plate method: The solid agar plate (60 numbers) was punched with wells having a diameter of 7 mm and streaking of *S. mutans* was done on the blood agar plates in sterile laminar air flow [Figure 2], later the wells were filled with *S. sisymbriifolium* extracts of various concentration. Separate plates for *S. sisymbriifolium* extract and 0.2% chlorhexidine gluconate were used, with different concentrations.

The plates were then incubated at 37°C for 48 h. After incubation, the zone of inhibition of *S. sisymbriifolium* [Figure 3a] *and Chlorhexidine* [Figure 3b] was measured in millimeters using vernier callipers.[7,8]

RESULTS

Statistics

The Statistical Package for the Social Sciences (version 19) was used for data analysis. Data were presented as mean ± standard

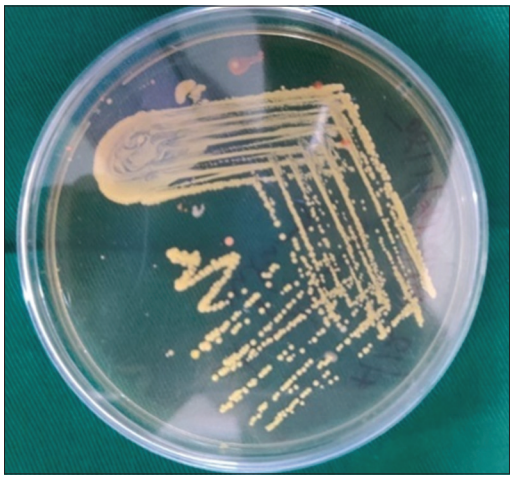


Figure 1: The strains of *Streptococcus mutans* were cultured onto the blood agar plate using loop for a nutritious growth

Figure 2: The solid agar plate was punched with wells having a diameter of 7 mm and streaking of *Streptococcus mutans* was done on the blood agar plates in sterile laminar air flow

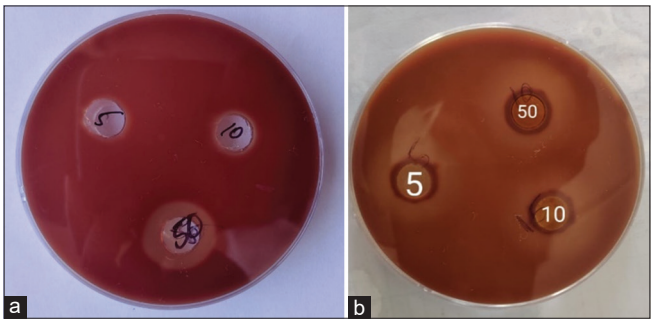


Figure 3: Zone of inhibition of *Solanum sisymbriifolium* (a) *and Chlorhexidine* (b) was measured in millimetre

deviation of the mean zone of inhibition. Data obtained were organized in an excel sheet and subjected to statistical analysis. As minimum zone of inhibition (5%, 10%, and 50%) is not normally distributed; a comparison of the zone of inhibition of *S. mutans* by *S. sisymbriifolium* and 0.2% chlorhexidine was done using non-parametric test, and Mann–Whitney U test. Comparison of the mean zone of inhibition (in mm) in between groups was done using independent t test. Zone of inhibition was analyzed using mean of all the readings obtained and the level of significance at <0.05 was considered statistically significant.

Detailed Analysis of Results

The anticariogenic activity of different concentrations of *S. sisymbriifolium* extract against bacterial strain was assessed quantitatively by measuring the diameter of the zone of inhibition. Following readings are recorded after incubation of plates at 37° for 48 h.

5% Concentration

The mean zone of inhibition at 5% concentration of *S. sisymbriifolium* extract and 0.2% chlorhexidine against *S. mutans* was found to be 2.1233 with standard deviation of 0.2967 and 3.3000 with standard deviation of 0.1619, respectively.

10% Concentration

The mean zone of inhibition at 10% concentration of *S. sisymbriifolium* extract and 0.2% chlorhexidine against *S. mutans* was found to be 3.7533 with standard deviation of 0.1408 and 4.5033 with standard deviation of 0.1129, respectively.

50% Concentration

The mean zone of inhibition at 50% concentration of *S. sisymbriifolium* extract and 0.2% chlorhexidine against *S. mutans* was found to be 5.3633 with standard deviation of 4537 and 7.2767 with standard deviation of 0.6961, respectively.

The inhibitory effect of 5%, 10%, and 50% *S. sisymbriifolium* is significantly lower than the corresponding concentration of 0.2% chlorhexidine mouthwash, respectively.

The mean zone of inhibition at 5%, 10%, and 50% concentrations of *S. sisymbriifolium* was found to be 2.1233 mm, 3.7533 mm, and 5.3633 mm, respectively. The mean zone of inhibition at 5%, 10%, and 50% concentrations of 0.2% chlorhexidine mouthwash was found to be 3.3000 mm, 4.5033 mm, and 7.2767 mm, respectively [Table 1].

The inhibitory effect of *S. sisymbriifolium* is significantly lower than 5%, 10%, and 50% chlorhexidine gluconate mouthwash ($P < 0.001$) [Table 2]. However, within the group, this inhibitory effect of *S. sisymbriifolium* is found to increase as its concentration increases. ($P < 0.001$) [Table 2 and Graph 1].

DISCUSSION

Dental caries is the most common microbe-mediated oral disease with accepted etiology based on a four-factor theory that includes oral microorganisms, oral environment, host, and time. *S. mutans* being primary initiators of dental biofilm formation, the most common preventive measures against dental caries are use of chemical agents such as chlorhexidine as antimicrobials.[2] Recent research has shown the presence of bioactive compounds of plants which can have anticariogenic property similar to chemical agents but without their harmful side effects.[8]

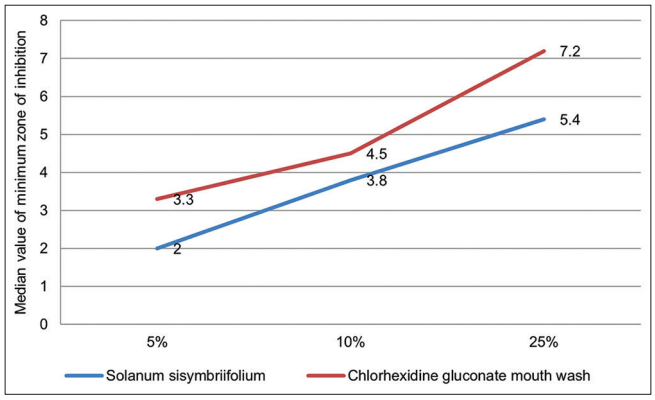
S. sisymbriifolium plant extract has been used as edible vegetable and as traditional medicine in Central South America, though not much research has been conducted on its biological activities. Various studies have demonstrated differences in the essential oil composition of fruits, flowers, and leaves of *S. sisymbriifolium*.[5] Screening analysis studies

Table 1: Descriptive of minimum zone of inhibition across *Solanum sisymbriifolium* and Chlorhexidine gluconate mouthwash groups

Minimum zone of inhibition	*Solanum sisymbriifolium* (n=30)					Chlorhexidine gluconate mouthwash (*n*=30)				
	Q1	Median	Q3	Minimum	Maximum	Q1	Median	Q3	Minimum	Maximum
5%	1.90	2.00	2.30	1.70	2.90	3.20	3.30	3.40	3.00	3.50
10%	3.70	3.80	3.90	3.40	4.00	4.40	4.50	4.60	4.40	4.80
50%	5.20	5.40	5.60	4.30	6.50	6.60	7.20	8.10	6.40	8.40

Table 2: Comparison of minimum zone of inhibition at 5%, 10%, and 25% across the groups

Statistics	Minimum Zone of inhibition		
	5%	10%	50%
Mann–Whitney U	0	0	0
Z	−6.68	−6.72	−6.60
P-value	<0.001	<0.001	<0.001



Graph 1: A graph portraying median value of the minimum zone of inhibition at 5%, 10%, and 50% concentration of *Solanum sisymbriifolium* plant extract and Chlorhexidine gluconate mouthwash.

have proven aerial parts of *S. sisymbriifolium*, especially the flower extracts essential oil have antimicrobial activities comparable to the synthetic drug ampicillin. This effectiveness has been attributed to the presence of high number of aldehydes and sesquiterpenes, which are proven to have antimicrobial activity comparable to previous studies conducted on ginger and curcumin which have similar metabolites.[9]

In vitro antibacterial activity of *S. sisymbriifolium* evaluated for ethanolic, ethyl acetate, and hexane extracts against Gram-positive and Gram-negative bacteria, showed significant zone of inhibition against tested bacterial strains[10] Studies have shown, cold percolation method of extraction using chloroform also has effective antimicrobial activity.[11] Hence, in this present study, the chloroform extraction of *S. sisymbriifolium* was done using same method.

Unlike the previous Argentine and Bangladeshi studies which were not successful in demonstrating the antimicrobial activity of this species, the findings of the present study support the antimicrobial activity *S. sisymbriifolium* like few other Indian studies that have demonstrated the antibacterial property of aerial part of *S. sisymbriifolium*.[6,10,12,13]

However, this study is the first to demonstrate the antimicrobial activity of *S. sisymbriifolium* flower extract against *S. mutans* ($P < 0.001$).

Although this inhibitory effect is significantly lower than corresponding 5%, 10%, and 50% of 0.2% chlorhexidine gluconate mouthwash, findings of this study support the antimicrobial activity *S. sisymbriifolium* flower extract against *S. mutans*.

CONCLUSION

This is a preliminary study of *S. sisymbriifolium* flower extract with antimicrobial potential against *S. mutans*. The results which are favorable suggest the possibility of incorporating it for the preparation of various dental therapeutic agents in the near future using pure form of the extract.

Limitation and Future Research

Flowers of the plant were obtained and were visually authenticated by a horticulturist. While no specimen voucher is available, the photographic records are available in the institution. This was a preliminary study using a simple form of extract. The efficacy of this plant can be explored using advanced extraction methods like high-performance liquid chromatography. Furthermore, *in vitro/in vivo* experimental studies can be planned for dental setup.

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