

Original Article

Assessment of antimicrobial activity of different concentrations of *Tinospora cordifolia* against *Streptococcus mutans*: An *in vitro* study

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ABSTRACT

Background: The antimicrobial property of *Tinospora cordifolia* has been tested against a variety of microorganisms in the literature. The present study aimed to assess the antimicrobial activity of different concentrations of commercially available *T. cordifolia* powder against *Streptococcus mutans*.

Materials and Methods: An *in vitro* study was undertaken in which extract of *T. cordifolia* was obtained using 100% ethanol by maceration. Seven different concentrations were prepared and tested against *S. mutans* in brain–heart infusion agar medium. Plates were incubated aerobically at 37°C for 48 h, and zone of inhibition was measured using Vernier caliper. 0.2% chlorhexidine and dimethylformamide were used as positive and negative controls respectively. The data were analysed by descriptive analytic tests.

Results: The maximum antibacterial activity of *T. cordifolia* was observed with a volume of 40 µl at 2% concentration with a zone of inhibition of 19 mm. A 30 µl volume of 0.2% chlorhexidine showed a zone of inhibition of 28 mm, and no zone of inhibition was observed with dimethylformamide.

Conclusion: *Tinospora* exhibited antimicrobial activity against *S. mutans*. However, it needs to be confirmed further with *in vivo* studies.

Key Words: Antimicrobial, *Streptococcus mutans*, *Tinospora*

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INTRODUCTION

The use of plants for medicinal purposes is as old as human civilization itself. Medicinal plants have been used for curing diseases in different traditional systems of medicine such as Ayurveda, Siddha, European, Tibetan, and Unani.^[1] Herbal medicine is still the mainstay of treatment in about 75%–80% of people in many developing countries for their primary health care because of better cultural acceptability and compatibility with the human body and fewer side effects.^[2]

One such immensely valuable plant regarding its constituents and pharmacology is *Tinospora cordifolia*

of the *Menispermaceae* family, commonly called as Guduchi in Sanskrit.^[3] It is a deciduous climbing shrub with small greenish flowers, having enormous medicinal value in all its parts such as leaves, stem, and also the root.^[4] It is a Rasayana (rejuvenator) and anti-aging medicine in Ayurveda, used to improve the immune system and the body resistance against infections.^[5] It has also been found that *Tinospora* has antispasmodic,^[6] antipyretic,^[7] anti-inflammatory,^[8] anticomplementary,^[9] and immunomodulatory activities.^[10] In addition to it,

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Tinospora has been found to exhibit antidiabetic,^[11] hepatoprotective,^[12] anticancer,^[13] and antioxidant properties^[14] as well. It has been listed as an insecticide, an antifungal agent, and an antibacterial agent.^[15,16] Antimicrobial features have been found in its root, stem, and leaf extracts on pathogenic microorganisms.^[17] Ethanolic and aqueous extracts of *T. cordifolia* have been successfully tested against various bacteria such as *Staphylococcus epidermidis*, *Escherichia coli*, *Aspergillus niger*, *Candida albicans*, and *Staphylococcus aureus*.^[18,19] Unfortunately, no much research has been done to explore its antimicrobial properties against oral organisms. Hence, the present *in vitro* study was undertaken to assess the antimicrobial properties of *Tinospora cordifolia* against *Streptococcus mutans*, the most common organism associated with dental plaque and caries.

MATERIALS AND METHODS

Study design

An *in vitro* study was designed to assess the antimicrobial activity of different concentrations of commercially available *Tinospora* powder against *S. mutans*. The study was approved by the Institutional Ethical Committee (SRDCHRC/146/11).

Preparation of *Tinospora cordifolia* extract

Tinospora powder was first sieved to obtain uniform sized particles. 300 g of finely sieved powder of *T. cordifolia* was macerated with 1 L of 100% ethanol. It was then subjected to filtration with Whatman filter paper (No. 1) to obtain a clear filtrate. This filtrate was reduced at 80°C temperature in a water bath for 48 h to get a solid residue of *T. cordifolia* extract. From 300 g of *Tinospora* powder dissolved in 1 L of ethanol, 1.6 g of residue (extract) was obtained.

Preparation of seven different concentrations of *Tinospora cordifolia* extract

One gram of extract was dissolved in 10 ml of dimethylformamide to obtain a 10% concentration of extract. One milliliter of the extract was transferred to a sterilized test tube and labeled as 10%. The remaining 9 ml of the extract was diluted further with dimethylformamide to obtain seven different concentrations (2%, 3%, 4%, 5%, 6%, 7%, and 8%). In this study, 0.2% chlorhexidine was used as positive control, and dimethylformamide was used as negative control. The method for obtaining different concentrations was adopted from a study by Agarwal *et al.*^[2]

Bacterial sample

Pure strains of *S. mutans* (MTCC 890) obtained from Microbial Type Culture Collection and Gene Bank, Chandigarh, were used in the present study.

Culture media and methods

Glass petri dishes and brain–heart infusion agar were used for culture and to determine the zones of inhibition. 20 ml of freshly prepared, autoclaved, brain–heart infusion agar media was transferred to each petri dish. Four circular wells to accommodate four different volumes (10 µl, 20 µl, 30 µl, and 40 µl) of the *T. cordifolia* extract were cut in the agar plates. Nine such petri dishes were prepared and labeled, seven of the seven different concentrations of *Tinospora* extract, one for the positive control (chlorhexidine), and one for the negative control (dimethylformamide). The sterile environment around the Petri dishes was maintained using split lamps. *S. mutans* (MTCC 890) strains were mixed with saline and swabbed to each agar plate using sterile wooden tongue blades. The plates were then incubated aerobically at 37°C for 48 h. After 48 h, the zone of inhibition was measured using a Vernier caliper. Data were tabulated and expressed as descriptive as this observational study did not require any inferential analysis.

RESULTS

A total of seven different concentrations of *T. cordifolia* extract along with positive and negative control were assessed for antimicrobial activity against *S. mutans*.

At 2% concentration, a maximum zone of inhibition of 19 mm was seen with a volume of 40 µl followed by 9 mm with 30 µl. On the contrary, no inhibition zone was found with secondary volumes such as 10 µl and 20 µl. At 4% and 5% concentrations, a maximum area of inhibition of 5 mm was observed at a volume of 40 µl while lesser quantities failed to produce any zone of inhibition. At 7% and 8% concentrations, a maximum area of inhibition of 2 mm was observed at a volume of 40 µl while no zone of inhibition was seen with lesser volumes. At 3% and 6% concentrations, no area of inhibition was observed with any of the volumes [Table 1].

The results with 0.2% chlorhexidine which was used as a positive control showed a maximum zone of inhibition of 28 mm with 30 µl followed by 20 mm with a volume of 40 µl, 8 mm with 20 µl, and the least of 7 mm with 10 µl, respectively [Table 2].

No zone of inhibition was seen with any of the volumes of dimethylformamide, which was used as a negative control, indicating total lack of antimicrobial activity [Table 3].

DISCUSSION

The enormous heritage of vast natural, time-tested medicinal resources is worth exploring the possibility of developing efficient, economically viable, and clinically acceptable antimicrobials for human application. One among them is *T. cordifolia*, an indispensable medicinal plant, referred to in Ayurveda as “Amruth” or the “Nectar of Immortality” in recognition of its ability to impart youthfulness, vitality, and longevity. Preclinical and clinical pharmacological studies affirm the importance of its therapeutic efficacy and hence have placed it as a novel candidate to be used as the primary drug in the treatment of different ailments.^[3]

Previously, plants such as Tulsi (*Ocimum sanctum*) and *Stevia rebaudiana* have been tested for their antimicrobial activity on *S. mutans* with

positive outcomes.^[2,20] However, studies regarding antimicrobial properties of *Tinospora* against oral microorganisms, especially *S. mutans*, are lacking though studies have evaluated its antimicrobial activity against *E. coli*, *S. aureus*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*.^[21,22] Some studies found that the maximum antimicrobial property was exhibited against *S. aureus*.^[23]

In the present study, out of seven different concentrations and volumes tested against *S. mutans*, maximum inhibition of 19 mm was found with 40 µl at 2% concentration of the extract. Lesser amounts of the same strength failed to elicit the same results. It was also found that the zone of inhibition decreased with the increasing concentration of the extract of *Tinospora*. Furthermore, a volume of <30 µl of extract in all concentrations except for 2% failed to elicit any zone of inhibition. This indicates that the antimicrobial activity of *Tinospora* is effective at a lower concentration and higher volume with a maximum antibacterial activity as seen with 40 µl at 2% concentration. However, further studies are required to confirm the same. A study by Vermani et al. on antimicrobial properties of crude *Tinospora* extract against five dental pathogens showed similar results. The zone of inhibition against *S. mutans* was 10 mm, 11 mm, 19 mm, and 18 mm with ether, chloroform, methanol, and aqueous extracts of *Tinospora*, respectively.^[24]

The antimicrobial property of *Tinospora* against *Streptococcus mutans* could be attributed to the secondary metabolites and the phytochemicals present in it such as quinones, polyphenols, alkaloids, flavonoids, tannins, coumarins, terpenoids, lectins, and polypeptides.^[25,26] While quinones and flavonoids bind to adhesins form complexes with cell wall and inactivate bacterial enzymes, terpenoids, polyphenols, and tannins cause membrane disruption and form metal ion complexes, thus inactivating the bacteria.^[27]

In the present study, chlorhexidine was found to be more effective and exhibited better antimicrobial properties against *S. mutans* with a zone of inhibition of 28 mm, as compared to *T. cordifolia* extract. However, the well-known side effect of chlorhexidine, i.e., staining of teeth and restorations, alteration of taste sensation, and development of resistant microorganisms, may limit the long-term use of chlorhexidine.^[2] On the contrary, *Tinospora* is abundantly available, easily accessible, economically

Table 1: Zones of inhibition at different concentrations and volumes of *Tinospora* extract

Concentrations (%)	Volumes			
	10 µL	20 µL	30 µL	40 µL
	Zones of inhibition			
2	Resistant	Resistant	9 mm	19 mm
3	Resistant	Resistant	Resistant	Resistant
4	Resistant	Resistant	Resistant	5 mm
5	Resistant	Resistant	Resistant	5 mm
6	Resistant	Resistant	Resistant	Resistant
7	Resistant	2 mm	Resistant	Resistant
8	Resistant	Resistant	2 mm	Resistant

Table 2: Zones of inhibition with different volumes of 0.2% chlorhexidine (positive control)

Concentrations	Volumes			
	10 µL	20 µL	30 µL	40 µL
	Zones of inhibition			
Chlorhexidine (0.2%)	7 mm	8 mm	28 mm	20 mm

Table 3: Zones of inhibition with different volumes of dimethylformamide (negative control)

Volumes	Dimethylformamide			
	10 µL	20 µL	30 µL	40 µL
Zones of inhibition	Resistant	Resistant	Resistant	Resistant

feasible, and culturally acceptable and may possess minimal side-effects. Hence, it can be recommended for long-term use to prevent plaque formation by *S. mutans* and in turn decrease the risk of caries and periodontal disease as plaque is a common etiological factor for both.

In the present study, dimethylformamide was resistant to *S. mutans*. This could be attributed to its property of being an inert solvent. It was used in the present study to dilute the *Tinospora* extract and to neutralize the effect of alcohol so that the results could be solely attributed to *Tinospora*.

CONCLUSION

According to the present study, *Tinospora* exhibited antimicrobial properties against *S. mutans* with the maximum activity at 2% concentration. It could be formulated as mouthwash and used to prevent plaque in patients at high risk for caries and gingivitis. However, more research is required to test its antimicrobial properties in the oral environment through *in vivo* studies and also the adverse effects if any on oral mucosal cells and teeth.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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