Original Article

Antimicrobial efficiency of *Tinospora cordifolia* and *Ocimum tenuiflorum* against *Streptococcus mutans* and *Candida albicans*

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Abstract

Background: The application of traditional medicinal plants and their products for treatment has been an integral part of our culture and continues to play a key role as many infectious microorganisms are resistant to synthetic drugs. *Tinospora cordifolia* and *Ocimum tenuiflorum* herbs have shown medicinal properties and have gained importance in modern research.

Aims: This study assessed the antimicrobial activities of *Tinospora cordifolia* and *Ocimum tenuiflorum* against *Streptococcus mutans* and *Candida albicans* to conventional medications such as chlorhexidine and nystatin.

Methodology: The *S. mutans* and *C. albicans* were grown and maintained on Columbia agar plates and yeast malt extract agar respectively. An ethanolic extract was made and subjected to rotary evaporation to remove the ethanol. The antimicrobial activity of plant extracts was determined using the Kirby-Bauer disc diffusion method. The standard drugs, 10 µg/disc nystatin and 0.12% chlorhexidine, were used as a positive control. The zone of inhibition was measured after 24 hours.

Results: At a concentration of 3 mgs., the zone of inhibition of 25.6 mm was found with *T. cordifolia,* followed by 15.8 mm with *O. tenuiflorum* against *S. mutans,* and 0.12% chlorhexidine, at 21.7 ± 0.43 mm. A zone of inhibition of 23 mm and 22.9 mm was observed in both *T. cordifolia* and *O. tenuiflorum* against *C. albicans,* respectively. Positive control of nystatin showed 26.1 ± 0.46 mm.

Conclusion: *Tinospora cordifolia* has better antimicrobial activity against *S. mutans* compared to *Ocimum tenuiflorum*. Whereas at higher concentrations, both extracts were effective against *C. albicans.*

Keywords: Antimicrobial activity, *Candida albicans*, *Ocimum tenuiflorum*, *Streptococcus mutans*, *Tinospora cordifolia*

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INTRODUCTION

The oral microbiome, also known as oral microflora, is a collection of microorganisms present in the human mouth. Its composition and activity can change rapidly and vary from person to person.[1] Dental plaque is thought to be the most important etiological element

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in the onset and progression of oral diseases. Some *Streptococcus mutans* and *Candida albicans* have exceptional clinical significance due to their roles as pathogens that cause dental caries, gingivitis, periodontitis, and candidiasis.[2] Plaque removal measures can help to prevent these disorders and are usually managed with toothbrushes, mouth rinses, toothpaste, and gels.[3] One of the most commonly prescribed antimicrobials is chlorhexidine digluconate mouth rinse. It is considered to be a gold standard agent, used in the treatment of infectious disorders[4] as it has bacteriostatic as well as bactericidal properties,[5] against a wide range of bacteria and fungi, including yeasts[6] with its substantivity effects. Antimicrobial use, even when implemented safely, can lead to antimicrobial resistance. Microbes that are resistant to antibiotics are a key risk factor for serious illnesses.[7] Due to the widespread emergence of resistance among pathogens against existing antibiotics, the medical community is in desperate need of new medicines. Traditional plants, on the other hand, have been shown to be a better source of new antibiotics.[8] Medicinal herbs have long been regarded as the most promising source of new antimicrobial medicines. One of the medicinal plants is *Tinospora cordifolia* (Giloy), a big deciduous climbing shrub.[9] *Tinospora* also known as heart leaved moonseed plant in English and Guduchi in Sanskrit, is a member of the Menispermaceae family. Myanmar, Sri Lanka, China, and India are among the countries that grow it.[10] Calcium, phosphorus, copper, manganese, zinc, and iron are all abundant in the plant.[11] Its active ingredients have been shown to be effective against a variety of microbiological diseases, including uropathogens, *E. coli*, and *Staphylococcus aureus*.[12-15] *Ocimum sanctum* or Tulsi, is another medicinal plant that belongs to the Lamiaceae family and is thought to have medicinal properties. It can be found in large quantities in West Africa, India, Africa, Australia, Mediterranean, and Malaysia.[16] It has long been recognized as one of the most useful and comprehensive herbs used in herbal medicine, with health benefits found in almost every part of the plant.[17] Plants have been used to treat a variety of systemic disorders caused by different types of bacteria as well as fungal infections.[16,18,19]Antimicrobial activity of other components of *Tinospora cordifolia* and *Ocimum sanctum* plants such as the bark, stem, and fruits have been assessed by several researchers. However, only a few studies have shown that leaf extracts have antibacterial activity against oral bacteria, particularly *Streptococcus mutans* and *Candida albicans*. Therefore, the current in-vitro study aimed at comparing the antimicrobial efficacy of these traditional herbs against *Streptococcus mutans* and *Candida albicans* to conventional medications such as chlorhexidine and nystatin.

MATERIALS AND METHODS

The *S. mutans* DSM 20523 and *C. albicans* DSM 1386 were purchased from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany). The *S. mutans* and *C. albicans* were grown and maintained on Columbia agar plates and yeast malt (YM) extract agar respectively as recommended by DSMZ. Before the experiment, three to five colonies from an overnight culture were suspended in saline (0.85% NaCl) and adjusted to match the turbidity of a McFarland 0.5 standard. This standard bacterial and fungal suspension were used for disk diffusion assay.

In this study, mature leaves from the plants *T. cordifolia* and *O. tenuiflorum were used.* A method described by Troung *et al*.[20] for making an ethanol extract was followed in the study. In the laboratory, the leaves were washed thoroughly and dried on paper towels at 37 ± 1°C for 24 hours and oven-dried at 60°C. After drying, the plant materials were ground separately in an electric grinder in the laboratory [Figure 1a and b]. Sunlight was avoided to prevent the active components from being destroyed. The 100% ethanol (200 ml) was mixed with 50 g each of powdered plant materials. The mixtures were then kept for 24 hours in a tightly sealed conical flask at room temperature, protected from sunlight, and stirred thoroughly several times a day with sterile glass rods. The mixtures obtained were filtered through Whatman No. 1 filter papers. The extracted liquids were subjected to rotary evaporation to remove the ethanol. The semisolid extracts produced were kept at − 80°C in a freezer overnight and then subjected to freeze-drying for 24 hours at − 60°C at 200 ml vacuum. The extracts were then kept in an airtight container in the refrigerator at 4°C until required.

The antimicrobial activity of plant extracts was determined using the Kirby-Bauer disc diffusion method.[21] The plant extracts (150 mg) were re-dissolved in 1 ml 1%

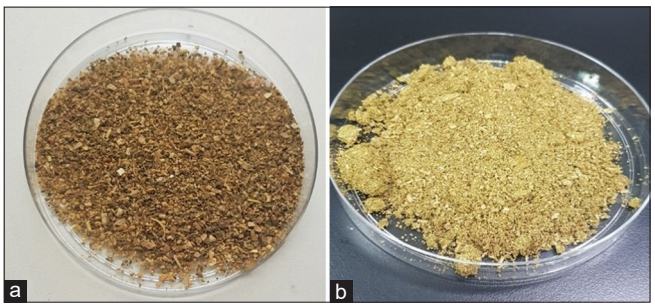

Figure 1: (a and b). *Tinospora cordifolia* leaves powder and *Ocimum tenuiflorum* leaves powder

DMSO (dimethyl sulfoxide), sterilized through a Millipore filter (0.22 μm). The extracts were loaded onto sterile filter paper discs with a diameter of 6 mm to achieve final concentrations of 3 mg/disc and 1.5 mg/disc. The standard drugs of (0.12% chlorhexidine/disc and 10 μg/disc nystatin), antibacterial and antifungal discs, respectively, were used as a positive control. The disc with 1% DMSO was used as a negative control. A sterile cotton swab was dipped into each adjusted bacterial suspension and used to cover the surface of Brain Heart Infusion (BHI) agar uniformly. After that, the disc impregnated with extract (3 mg/ml and 1.5 mg/ml), positive control and negative controls were placed on the surface of the agar plate. After 24 hours of an incubation period at 37°C, the diameter of the inhibitory zone was measured in millimetres using a digital calliper. The same steps were performed for *C. albicans* to determine the zone of inhibition (ZOI) growing on Sabouraud Dextrose Agar (SDA, Oxoid Ltd, Basingstoke, UK). The data was presented as the mean ± SD of three replicates in five determinations. One-way analysis of variance (ANOVA) and Tukey tests was performed by using IBM SPSS Statistics for Windows, Version 27.0. (Armonk, NY: IBM Corp.) to determine significant group differences and means were considered as statistically significant if $p < 0.05$.

RESULTS

A total of two different concentrations of 3 mg/disc and 1.5 mg/disc for *T. cordifolia* and *O. tenuiflorum* extracts were evaluated for both antibacterial and antifungal activity against *S. mutans* and *C. albicans* along with positive and negative control groups.

For the antibacterial activity against *S. mutans,* at a lower concentration of 1.5 mgs, a zone of inhibition of 17.2 mm was seen with *T. cordifolia* and no inhibition zone was detected with *O. tenuiflorum*. Whereas at higher concentration of 3 mgs, maximum zone of inhibition of 25.6 mm was found with *T. cordifolia* followed by 15.8 mm inhibition zone with *O. tenuiflorum* [Figure 2a and b]. The results of positive control group with the standard drug of 0.12% Chlorhexidine (CHX) showed a zone of inhibition of 21.7 mm [Table 1 and Figure 3].

In contrast, for the antifungal activity against *C. albicans*, both the extracts of *T. cordifolia* and *O. tenuiflorum* failed to produce zone of inhibition at lower concentration of 1.5 mgs. At higher concentration of 3 mgs, a maximum amount of zone of inhibition of 23 mm and 22.9 mm was observed in both *T. cordifolia* and *O. tenuiflorum,* respectively, and was almost similar in both the extracts [Figure 2c and d]. However, a zone of inhibition of 21.7 mm was found with 10 μg nystatin which was used as a positive control group [Table 2 and Figure 4].

Zone of inhibition was not detected with 1% DMSO which was used as a negative control for both antibacterial and antifungal activity [Tables 1 and 2].

DISCUSSION

Preventive strategies that target the underlying causes of oral infections are becoming more important as dentistry advances. As a result, plaque accumulation must be controlled, with antibacterial mouth rinses along with mechanical plaque removal in a natural way.[22] Recurrent drug resistance in *Candida* has forced researchers to seek out new antimicrobial drugs that are less harmful and more effective.[23,24]

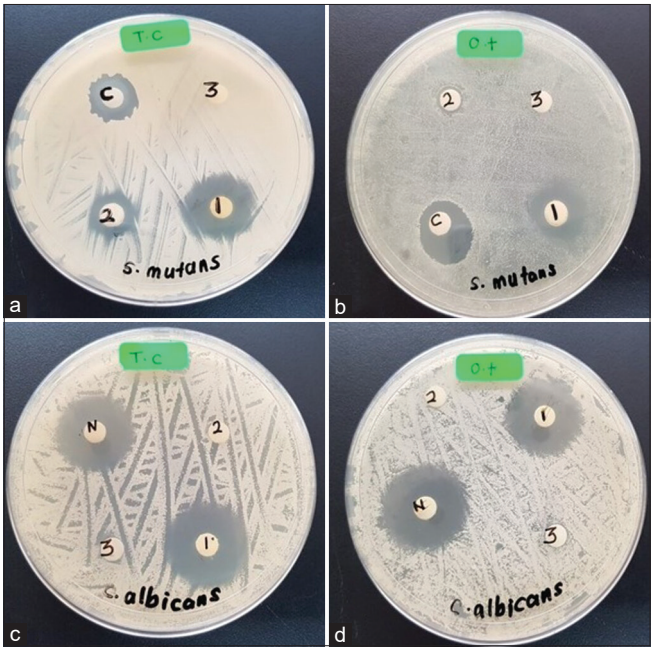

Figure 2: (a and b). The antibacterial activity of *Tinospora cordifolia* and *Ocimum tenuiflorum* against *Streptococcus mutans*. (Disc 1 = 3 mg/disc, Disc 2 = 1.5 mg/disc, Disc 3 = 1% Dimethyl Sulfoxide, Disc C = 0.12% Chlorhexidine) (c and d). The antifungal activity of *Tinospora cordifolia* and *Ocimum tenuiflorum* against *Candida albicans*. (Disc N = Nystatin 10 μg/disc, Disc 1 to 3 are same as Figure a and b)

Table 1: The antimicrobial activity of *Tinospora cordifolia* and *Ocimum tenuiflorum* against *Streptococcus mutans*

Treatment	0.12% Chlorhexidine	*Tinospora cordifolia*		*Ocimum tenuiflorum*		1% Dimethyl Sulfoxide
		3 mg/disc	1.5 mg/disc	3 mg/disc	1.5 mg/disc	
Zone of inhibition (millimeters)	21.7±0.43	25.6±0.48	17.2±0.56	15.8±0.71	Resistant	Resistant

Table 2: The antimicrobial activity of *Tinospora cordifolia* and *Ocimum tenuiflorum* against *Candida albicans*

Treatment	Nystatin	*Tinospora cordifolia*		*Ocimum tenuiflorum*		1% Dimethyl Sulfoxide
		3 mg/disc	1.5 mg/disc	3 mg/disc	1.5 mg/disc	
Zone of inhibition (millimeters)	26.1±0.46	23±0.66	Resistant	22.9±0.57	Resistant	Resistant

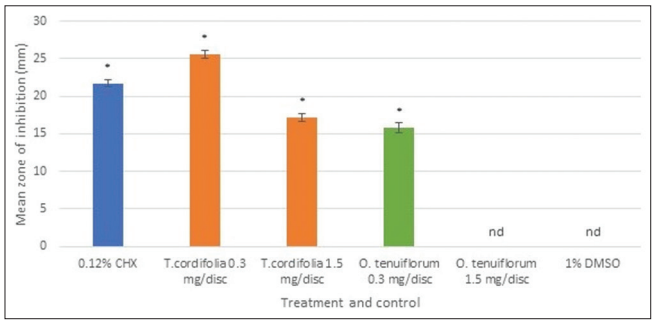

Figure 3: The antibacterial activity of *Tinospora cordifolia* and *Ocimum tenuiflorum* extracts against *Streptococcus mutans*

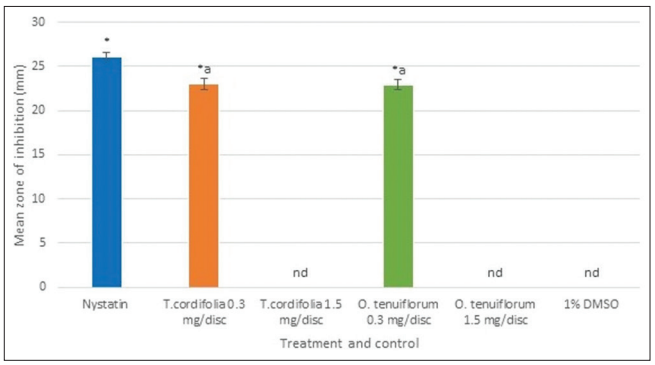

Figure 4: The antifungal activity of *Tinospora cordifolia* and *Ocimum tenuiflorum* extracts against *Candida albicans*.

In both Figures 3 and 4, the values are expressed as mean ± standard deviation. An asterisk indicates the statistically significant difference between treatment groups and negative control (1% Dimethyl Sulfoxide) as measured by Analysis of variance ($p < 0.05$). Means sharing the same letters are not significantly different ($P < 0.05$), according to the Tukey's test. nd = not detected.

Even though, commercially available chemotherapeutic agents are found to be good and widely used,[25,26] traditional medicinal herbs have made significant contributions to human well-being as safer and reasonable alternative sources of antimicrobials.[9] Furthermore, plants are one of the most important sources of secondary metabolites and essential oils.[17] Among them, *T. cordifolia* and *O. tenuiflorum* (Tulsi) are the most widely available, economically feasible, and culturally acceptable plants, with relatively few adverse effects.[12] They have been used to treat inflammation, cough, colds, fever, sore throats, bronchitis, fungal infections, asthma, migraine headaches and other skin problems. Tulsi leaves can also be chewed to treat mouth ulcers and oral infections.[27] While the oral microbiome contains over 700 species, we focused our research on C. albicans and S. mutans as these oral biofilm samples have a high pathogenic potential.[28]

The present study compared two different doses of ethanol extracts of *T. cordifolia* and *O. tenuiflorum* against *S. mutans* and *C. albicans* to standard medications of 0.12% chlorhexidine and 10 g nystatin, as well as a negative control group of 1% DMSO.

Previous studies have used methanolic extracts of *A. Indica*, *O. sanctum, M. Elengi* and *T. cordifolia* with significant antimicrobial activity against *S. mutans*, *E. faecalis* and *S. aureus*.[29] However, few studies on the antimicrobial properties of ethanolic extracts of *T. cordifolia* and *O. tenuiflorum* against oral microorganisms, particularly *S. mutans* and *C. albicans* have been reported.[18,24,27] We chose to employ ethanolic extracts in our study as they're more potent and its active ingredients are more soluble in organic solvents.[30,31] They were found to have antibacterial properties. The disparities in bacterial susceptibility to the extracts could be attributable to differences in cell wall and/or genetic composition, as well as differences in bioactive component composition, concentrations and mode of action.[32]

The present study results showed the zone of inhibition of 15.8 mm with *O. tenuiflorum* against *S. mutans,* whereas another study results with the ethanolic extract of *O. tenuiflorum* showed minimum inhibitory concentration of 25 mg/ml against *S. mutans* and 100 mg/ml with *L. acidophilus*.[33] Greatest zone of inhibition was also observed among 15 different doses of it and showed to have an effective antimicrobial activity on *C. albicans*.[34]

T. cordifolia, O. *tenuiflorum* and *Azadirachta indica* have all been found to have antibacterial and antifungal properties.[12,15,35-38] *T. cordifolia* roots, stems, and leaves have no toxicity when taken orally.[39] They have also been investigated against *Streptococcus mutans* and *Actinobacillus actinomycetemcomitans*.[18,40] The researchers utilized a murine model to test the aqueous extract of *T. cordifolia* against cyclophosphamide-induced immunosuppression and systemic *C. albicans* infection and found that it may be used to re-establish the immune system and cure systemic candidiasis.[41]

In the present study, the larger zones of inhibition exhibited by *Tinospora cordifolia* extract may be due to the presence of variety of active secondary metabolites such as tannins, cardiac glycosides, saponins, and others in plant extracts which are responsible for their antimicrobial activity. This indicates that it has a greater efficacy and contains more active chemicals.[12] Zone of inhibition was also seen better with the higher doses of *O. tenuiflorum*, that could be attributed to the fact that, the bioactive substances found to inhibit microorganisms, and the leaves also contain ascorbic acid and carotene.[42] As a result, these are extremely effective in the treatment of oral disorders as well as in overcoming many of the challenges to dental care such as cost, accessibility, and ease of access.[43] The chemical that is responsible for its beneficial effects is still unknown. The active essential components such as eugenol (1-hydroxy-2 methoxy-4-allylbenzene), Urosolic acid, caryophyllen and Carvacrol have been determined to be substantially responsible for the therapeutic potentials.[44] Although there are three varieties of *Ocimum tenuiflorum* are available in the name of Rama tulsi, Vana tulsi and Krishna tulsi, (variations in their colour of leaves and flowers), in the present study, we have utilized the extract from Rama tulsi (broad leaves with bright green colour). However, studies have shown that, all these plants have similar chemical constituents and common medicinal properties.[45]

In the present study, chlorhexidine showed good antibacterial activity against *S. mutans*, with a zone of inhibition of 21.7 mm when compared to lower doses of *T. cordifolia* extract and both lower and higher doses of *O. tenuiflorum*. But, chlorhexidine's well-known side effects, in the form of discoloration of teeth, altered taste sensation, and the development of resistant pathogens, may limit its long-term use.[40] On the other hand, nystatin was found to be more effective and showed the maximum antifungal properties in the study as compared to both the concentrations of *T. cordifolia* and *O. tenuiflorum*. This might be because nystatin has proven to be more successful fungicidal in most infections and systemic treatment since it binds to the ergosterol of the fungal plasma membrane and produces pores that make it more permeable, causing intracellular potassium loss. Furthermore, autooxidation caused by nystatin induces additional cell injury.[46-48] In the present study, *S. mutans* and *C. albicans* were resistant to 1%DMSO. This suggests that 1%DMSO had no effect on the test strains as it has got inactive solvent property. Nevertheless, *T. cordifolia* showed a better antimicrobial activity against *S. mutans* compared to *O. tenuiflorum*. Both the extracts were effective against *C. albicans* at the higher concentration of 3 mg/disc. As a result, they can be recommended for preventive approach in various formulations such as mouthwashes and tooth paste as these herbs are readily available, easy to use, inexpensive with minimal negative effects. The ethanolic extract of the herbs was employed in the study without involving the phytochemical analysis of their active components. Furthermore, based on our study evaluation, we are unable to speculate on the exact mechanism by which *T.cordifolia* and *O. tenuiflorum* exert their antimicrobial activities. However, further clinical research is needed to evaluate their antimicrobial capabilities and their possible long-term toxicity in the oral environment.

CONCLUSION

The result revealed from the present study suggests that *T.cordifolia* has better antimicrobial activity against *S. mutans* compared to *O. tenuiflorum*. Whereas both the herbs were effective against *C. albicans* at the higher concentration of 3 mg/disc. Hence, these herbal extracts could be used as preventative measures without any adverse effects for the oral infections caused by these organisms.

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Conflicts of interest

There are no conflicts of interest.

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