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## Screening of Antibacterial Activity of *Tinospora cordifolia* Miers. Extracts Against Dental Pathogens

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### ABSTRACT

Plants are considered potent candidate for safe and efficient therapeutic agents. In present study, crude extracts of *Tinospora cordifolia* was investigated for its ability to inhibit the growth of dental (bacterial) pathogens i.e., *Staphylococcus aureus* (MTCC 1144), *Streptococcus mutans* (MTCC 890), *Streptococcus salivarius* (MTCC 1938), *Lactobacillus acidophilus* (MTCC 447), *Streptococcus sanguinis* (ATCC 10556) and their isolates. Collected stems of *T. cordifolia* were properly washed and shade dried at room temperature, crushed and extracted in petroleum ether (PET), chloroform (CHCl<sub>3</sub>), methanol (MeOH) and aqueous (H<sub>2</sub>O) by using Soxhlet apparatus. The antimicrobial activity of extracts was examined by agar well diffusion method at 200 mg mL<sup>-1</sup> sample concentration. Phytochemical analysis was done for plant extract. The result of antibacterial activity was found that MeOH extract of *T. cordifolia* was most effective against all tested bacterial pathogens. Maximum antibacterial activity was observed against *S. sanguinis* (23 mm) and lowest activity against *S. salivarius* (17 mm). The phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins, phenols, saponins, glycosides, amino acids and steroids which might be accountable for its antimicrobial potential. The results validate the traditional uses of *T. cordifolia* in treatment of dental diseases.

**Key words:** Alkaloids, antibacterial, dental pathogens, *Tinospora cordifolia*

### INTRODUCTION

The biology of microorganisms is very complex. Microorganisms are able to carry out their life processes with enormous potential to endure in their environmental situations. Day by day, microbial susceptibility in term of synthetic drugs becomes extremely critical. Therefore, it is necessary to search an alternative way for managing infectious diseases with efficient cure and very low or no side effects. Secondary metabolites produced by medicinal plants are a broad source of bioactive substances that effect on a wide range of antibiotic resistant microorganisms (Nimri *et al.*, 1999).

Mouth is very hectic place with millions of bacteria are harboured, may be harmful or harmless. Dental infection is one of the most familiar disease, requires the occurrence of dental plaque. Dental plaque is a dense mass of bacteria that adhere tight to the tooth surface. A large number of residents i.e., *Actinomyces*, *Camphylobacter*, *Fusobacterium*, *Haemophilus*, *Lactobacillus*, *Prevotella*, *Porphyromonas*, *Streptococcus* and *Veillonella* are occupied in root caries and peridental

infections (Kononen *et al.*, 1992, 1994; Marsh, 1992; Schupbach *et al.*, 1995). The ability of these microorganisms to produce extracellular polysaccharides is probably the single most important feature in plaque development.

*Tinospora cordifolia* Miers. belongs to family Menispermaceae. It is commonly known as Giloe or Amrita (Hindi), Guduchi (Sanskrit) and Tinospora (English), respectively (The Ayurvedic Pharmacopoeia of India, 1999; Srinivasan *et al.*, 2008). It is a climbing shrub. The leaves are petioled, membranous and cordate with broad sinus. The flowers are small, yellow or greenish yellow, unisexual leafless in axillary and terminal racemes or racemose penicles. The plant is valued as immunomodulator (Jagetia and Rao, 2006a), anticancerous (Kavitha *et al.*, 2011), antidiarrhoeal (Mathew and Kuttan, 1997), antioxidant (Sivakumar *et al.*, 2010; Premanath and Lakshmidevi, 2010; Wani *et al.*, 2011), aphrodisiac (Tiwari *et al.*, 2011), antihelminthic (Nagaprashanthi *et al.*, 2012; Jain *et al.*, 2010), antipsychotic (Stanely *et al.*, 2000) and hypoglycaemic (Sinha *et al.*, 2004). Various crude extracts of *T. cordifolia* were studied against enteric bacteria, respiratory tract pathogens, peritonitis infection and bacteraemia (Thatte *et al.*, 1992).

Hence, this study is a conscientious attempt to find out antibacterial potential of *T. cordifolia* stem extracts against dental pathogens.

## MATERIALS AND METHODS

**Plant material:** Plants were collected from local market, Haridwar and authenticated at Botanical Survey of India, Northern Regional Center, Dehradun where a herbarium voucher specimen was deposited. Collected plant material was washed in running fresh water and dried under shade at room temperature. Stems were crushed to small pieces using pestle and mortar and then powdered in an electric grinder.

**Preparation of extract:** Plant extracts were prepared by immersing 200 gm of dried powdered material in 600 mL of solvents i.e., petroleum ether (PET), chloroform (CHCl<sub>3</sub>), methanol (MeOH) and aqueous (H<sub>2</sub>O), loaded in Soxhlet assembly and extracted for 72 h through successive method (Ahmad *et al.*, 1998). Plant extracts were filtered through Whatman No. 1 filter paper and crude extracts obtained by removing solvent in vacuum evaporator at 30°C. Residues were stored at 4°C until further use. The yield of PET extract was 1.241±0.23 g, CHCl<sub>3</sub> extract 1.489±0.10 g, MeOH extract 6.868±0.18 g and H<sub>2</sub>O extract 5.415±0.14 g, respectively. Extracts were dissolved in dimethyl sulfoxide (DMSO) to a final concentration of 200 mg mL<sup>-1</sup> for agar well diffusion method.

**Test microorganisms:** The strains of dental infection related bacteria used in this study were *Staphylococcus aureus* (MTCC 1144), *Streptococcus mutans* (MTCC 890), *Streptococcus salivarius* (MTCC 1938), *Lactobacillus acidophilus* (MTCC 447) and *Streptococcus sanguinis* (ATCC 10556), respectively. The standard bacterial strains were procured from Institute of Microbial Technology (IMTECH), Chandigarh. The isolated strain was identified according to published guidelines (Burnett *et al.*, 1994). All the bacterial strains were grown and maintained on nutrient agar slants at 4°C.

**Preparation of inoculums:** Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of cells from stock cultures to test tubes of Mueller-Hinton Broth (MHB) for bacteria that were incubated without agitation for 24 h at 37°C.

**Antibacterial testing:** The agar well diffusion method was used to evaluate the antibacterial activity (Ahmad *et al.*, 1998). This method depends upon the diffusion of the tested material to such an extent that growth of the added microorganism is prevented entirely in a zone around the hole containing a solution of tested material (Ahmad *et al.*, 1998; Navneet *et al.*, 2005). One hundred microliters of diluted inoculum of  $10^5$  CFU mL<sup>-1</sup> (IP, 1996) of 24 h old cultures of test organisms were mixed in Mueller Hinton Agar (MHA) medium and shaken. The media was poured (25-30 mL) in sterilized petri dishes (20×90 mm). Plates were allowed to solidify for 5-10 min. A cork borer (8 mm diameter) was used to punch wells in medium and filled with 45 µL of 200 mg mL<sup>-1</sup> final concentration of extracts. DMSO was used as negative control. Each extract was assayed in triplicate and the mean values were observed. The plates were incubated at 37°C for 24 h. The antibacterial activity was interpreted from the size of the diameter of zone of inhibition measured in millimeters (mm) as observed from clear zones surrounding the wells.

**Phytochemical screening:** Major phytochemicals, in the crude extracts of *T. cordifolia* were subjected to phytochemical analysis to determine the presence of bioactive components by using standard qualitative methods (Trease and Evans, 1996).

**Test for alkaloids:** Test solution was acidified with acetic acid and a drop of Mayer's reagent was added. A white precipitate indicated the presence of alkaloids.

**Test for flavonoids:** On addition of conc. HCl in methanolic extract of material, a red colour appeared which indicated the presence of flavonoids.

**Test for glycosides:** Plant extract was filtered and sugar was removed by fermentation with baker's yeast. The acid was removed by precipitation with Ba(OH)<sub>2</sub>. The remaining extract contained the glycosides. The hydrolysis of solution was done with conc. H<sub>2</sub>SO<sub>4</sub> and after hydrolysis the presence of sugars was determined with help of Fehling's solution.

**Test for steroids:** The extract mixed with 3 mL CHCl<sub>3</sub> and 2 mL conc. H<sub>2</sub>SO<sub>4</sub> was poured from side of test tube and colour of the ring at junction of two layers was noted. A red colour showed the presence of steroids.

**Test for saponins:** Extracts were diluted with distilled water to 20 mL and this was shaken in a graduated cylinder for 15 min. Formation of 1 cm layer of foam indicates the presence of saponins.

**Test for tannins:** Extract was added in 1% ferric chloride and observed the colour. Bluish black colour appeared which disappeared on addition of dilute H<sub>2</sub>SO<sub>4</sub> follow a yellow brown precipitate indicates the presence of tannins.

## RESULTS AND DISCUSSION

The result of antibacterial activity was found that MeOH extract of *T. cordifolia* was most effective against all tested bacterial pathogens followed by H<sub>2</sub>O, CHCl<sub>3</sub> and PET. The isolated pathogens were more sensitive to crude extract than designated cultures. Maximum antibacterial activity was observed against *S. sanguinis* (23 mm) and lowest activity was detected against *S. salivarius* (17 mm). Moderate antibacterial activity was noted against *S. aureus* (21 mm),

*L. acidophilus* (21 mm) and *S. mutans* (19 mm), respectively (Table 1). It can be interpreted that the antibacterial activity against test microorganisms is due to presence of secondary metabolites in plant extracts. Samy and Ignacimuthu (2000) reported good antimicrobial activity of various extracts (hexane, dichloromethane, ethyl acetate, diethyl ether and methanol) of leaves against *B. subtilis*, *E. coli*, *P. vulgaris* and *S. aureus*. Samy (2005) also reported antibacterial activity of methanolic extract of stem against *E. aerogenes*, *P. vulgaris* and *P. mirabilis*. PET extract of the stem has inhibited in vitro growth of *M. tuberculosis* (Singh *et al.*, 2003).

The phytochemical screening of *T. cordifolia* extracts has shown that plant contains flavonoids, glycosides, alkaloids, steroids, terpenes, saponin and tannin which are very important constituent when looking for pharmacologically active phytochemicals in the plant (Table 2). According to Gopi *et al.* (2004), a variety of constituents (alkaloids, diterpenoids, lactones, glycosides, steroids, sesquiterpenoids, phenolics, aliphatic compounds and polysaccharides) have been isolated from *T. cordifolia* and their structures were elucidated. Alkaloids like berberine (Padhya, 1986; Rao *et al.*, 2008; Srinivasan *et al.*, 2008), palmatine, tembetarine, magnoflorine, choline, tinosporin, columbin, isocolumbin, tetrahydropalamatine have been isolated from extracts of stem and roots of the plant (Bisset and Nwaiwu, 1983; Sarma *et al.*, 1998; Gupta, *et al.*, 2003; Singh *et al.*, 2003). Various glycosides were isolated from the stem (Sipahimalani *et al.*, 1994) like tinosporaside (Khan *et al.*, 1989), furanoid diterpene glucoside (C<sub>26</sub>H<sub>34</sub>O<sub>11</sub>) (Bhatt and Sabata, 1989), tinocordioside (Maurya *et al.*, 1995), tinocordifolioside (Maurya *et al.*, 1997), cordifolioside A, B, C, D and E (Gangan *et al.*, 1994, 1995), cordiol, syringin, cordioside (Wazir *et al.*, 1995), palmatosides C and E (Singh *et al.*, 2003). Gopi *et al.* (2004) isolated amritosides A, B, C and D as their acetates from stem. A sesquiterpene, tinocordifolin was isolated from stem (Maurya and Handa, 1998). Iqbal *et al.* (2005) and Bhatt *et al.* (1988) isolated a clerodane diterpenoid tinospnone and

Table 1: The percentage of potency of *T. cordifolia* extracts against dental pathogens

Pathogens	Diameters of inhibition zone (mm)			
	Petroleum ether	Chloroform	Methanol	Aqueous
<i>S. aureus</i>	11	12	21	19
<i>S. aureus</i> MTCC 1144	10	13	19	17
<i>S. mutans</i>	10	11	19	18
<i>S. mutans</i> MTCC 890	10	10	18	16
<i>S. sanguinis</i>	14	17	23	21
<i>S. sanguinis</i> ATCC 10556	12	14	22	19
<i>S. salivarius</i>	9	9	17	14
<i>S. salivarius</i> MTCC 1938	9	9	15	13
<i>L. acidophilus</i>	10	10	21	17
<i>L. acidophilus</i> MTCC 447	9	9	19	15

\*Values are means of three replicates, Cork borer diameter: 8 mm

Table 2: Phytochemical screening of crude extracts of *T. cordifolia*

Solvents	Alkaloids	Flavonoids	Glycosides	Steroids/terpenoids	Saponins	Tannins/phenols	Amino acids
Petroleum ether	-	-	-	+	-	-	-
Chloroform	+	-	-	+	-	-	-
Methanol	+	+	+	+	-	+	-
Water	+	-	+	-	-	+	+

+: Present, -: Absent

(C<sub>20</sub>H<sub>22</sub>O<sub>8</sub>) from the stem of the plant. Hanuman *et al.* (1986) isolated a diterpenoid furanolactone (C<sub>20</sub>H<sub>22</sub>O<sub>8</sub>) from stem. Ecdysterone, makisterone,  $\beta$ -sitosterol, 20  $\beta$ -hydroxyecdysone and giloinsterol steroids have been reported from the stems of the plant (Gupta *et al.*, 2003; Singh *et al.*, 2003). Other than these octacosanol, cordifol, heptacosanol, cordifellone, giloin, giloinisin, tinosporic acid and tinosporaside are also reported from the plant (Kaushik and Dhiman, 2000; Gupta *et al.*, 2003; Jagetia and Rao, 2006b).

## CONCLUSION

On the basis of results, it is concluded that stems of *T. cordifolia* has good antibacterial activity against selected dental pathogens. This study supports the traditional use of *T. cordifolia* and indicated that it contains some major bioactive compounds inhibiting the growth of microorganisms there by proving very effective source of derived drugs.

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