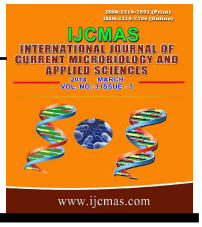
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 3 (2014) pp. 224-234**
http://www.ijcmas.com



Original Research Article

Phytochemical analysis and assessment of *in vitro* antibacterial activity of *Tinospora cordifolia*

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K e y w o r d s

Tinospora cordifolia; antibacterial activity; phytochemical analysis; zone of inhibition.

A B S T R A C T

The main aim of the present study was to find out antibacterial property of *Tinospora cordifolia* belonging to the family Menispermaceae. *Tinospora cordifolia* is an important herb of tropical India in Ayurvedic system of medicines. It has been traditionally used for treatment of diabetes, jaundice, chronic diarrhOea, cancer, dermatological diseases, general debility, and asthma. Tinospora cordifolia was collected from the garden during winter and was identified by the botanical department of Maharaja Sayajirao University, Vadodara. The work was performed using ethanolic and hydromethanolic extracts of the stem. A preliminary phytochemical screening was also performed for qualitative determination of the phytoconstituents. Antibacterial assay was carried out using different strains of bacteria like *Escherichia coli* (MTCC No.40), *Staphylococcus aureus* (MTCC No.87), *Proteus vulgaris* (MTCC No.742), *Pseudomonas aeruginosa* (MTCC No.424), *Bacillus subtilis* (MTCC No.441), *Staphylococcus epidermidis* (MTCC No.9041), and *Micrococcus luteus* (MTCC No.106), using cup plate method. The results obtained were compared against standard antibiotic streptomycin. The crude ethanol extract of *T. cordifolia* showed activity against tested bacteria. The ethanolic extract exhibit effective antibacterial activity against all the organisms ,except for E.coli, Proteus vulgaris and Pseudomonas aeruginosa while the hydromethanolic extract exhibits inhibition zone on limited species such like *Staphylococcus aureus* (2mm), *Bacillus subtilis* (3mm), *Micrococcus luteus* (2mm), *Staphylococcus epidermidis* (4mm).

Introduction

Tinospora cordifolia (*T.cordifolia*) which is also known as Giloe belongs to the family *Menispermaceae*. It is an important medicinal plant used in Ayurvedic system of medicine. The stem of the plant is greyish brown-black in colour and bitter in taste. The stems of *T.Cordifolia* are rather succulent with long filiform fleshy aerial

roots from the branches. The stem is soft wooded, dry, cylindrical and 5 mm to 25 mm in diameter. Traditionally, the plant has been in used as an anti-spasmodic, anti-inflammatory, jaundice, diabetes, seminal weakness, urinary tract infections, fever, general debility, skin diseases, and expectorant, carminative, digestive, anti-stress and aphrodisiac. Piles problem can be controlled by eating this plant mixed with milk or water and thus, preventing the bleeding and constipation (Kirtikar KR et al., 1987). The stem is bitter stomachic, diuretic (Nayampalli SS et al., 1988), stimulates bile secretion, causes constipation, allays (satisfies) thirst, burning sensation, vomiting, enriches the blood and cures jaundice. The extract of its stem is useful in skin diseases (Ayer KN et al., 1963; Raghunathan K et al., 1982). The root and stem of *T.Cordifolia* are prescribed in combination with other drugs as an antidote in snake bite and scorpion sting (Nadkarni KM et al., 1976; Zhao TF et al., 1991). Oral administration of an aqueous T. cordifolia root extract to alloxan diabetic rats caused a significant reduction in blood glucose and brain lipids (Dhaliwal KS et al., 1999). *T. cordifolia* is reported to benefit the immune system in a variety of ways (Kapil A et al., 1997). The hepatoprotective action of *T.Cordifolia* was reported in one of the experiment in which goats treated with *T. cordifolia* have shown significant clinical and hemato-biochemical improvement in CCl_4 induced hepatopathy. Extract of *T. cordifolia* has also exhibited in vitro inactivating property against Hepatitis B and E surface antigen (Mehrotra R et al., 2000). The aqueous extract of *T.cordifolia* exerted a significant anti-inflammatory effect on cotton pellet granuloma and formalin induced arthritis models (Jana U et al., 1999). In a clinical evaluation, a compound preparation 'Rumalaya' containing *T.Cordifolia* was reported to significantly reduce the pain in patients suffering from Rheumatoid Arthritis.

Phytochemical profile

Numerous constituents belonging to different chemical classes such as alkaloid, terpenoid, lactone, glycoside, steroid, phenolics, aliphatic compounds, lignan, and polysaccharide have been isolated and characterized from different parts of *T.Cordifolia* in Table 1. Leaves are rich in protein, calcium, and phosphorus (Singh SS et al., 2003; Sinha K., 2004). Methanol extract of leaves is rich in flavanoids, alkaloids and glycosides (Soni HP et al., 2011). A post harvest experiment has revealed that mechanical drying of the herb at 40°C provides the highest alkaloid (tinosporin) content (0.045%). However, the content decreases (0.033%) with drying at 60°C or in direct sunlight. Further, the dried stem bits packed in polyethylene lined gunny bag retain the highest alkaloid content (0.042%) as compared to storage under ambient conditions (Padmapriya S et al., 2009). These findings suggest that tinosporin may be either photosensitive and/or thermo labile. The phytochemical profile of *T.Cordifolia* are summarised in Table 1.

Biological activities

Ayurvedic literature quotes *guduchi* as a constituent of several compound preparations, used in general debility, dyspepsia, fever and urinary diseases. It has multiple actions like; stem is a bitter stomachic; stimulates bile secretion; causes constipation; tonic; allays thirst, fever, burning sensation, prevents vomiting; diuretic; enriches the blood; cures jaundice; useful in skin diseases; the juice is useful in diabetes, vaginal and

urethral discharges, low fevers, and enlarged spleen (Ayurveda). The roots and stems are prescribed in combination with other drugs as an antidote to snake bite and scorpion sting. An infusion of the powdered stem is used as an alternative and tonic and an aphrodisiac (Singla A et al., 2010). The activities of *T.Cordifolia* are summarised in Table 2.

Materials and Methods

Plant collection and authentication

Fresh stems of *Tinospora cordifolia* (figure 1) were collected from the garden and were identified by the Botanical Department, Maharaja Sayajirao University, Vadodara.

Bacterial strains and culture conditions

In this present study the test microorganisms used (bacteria: *Escherichia coli* (MTCC No.40), *Staphylococcus aureus* (MTCC No.87), *Proteus vulgaris* (MTCC No.742), *Pseudomonas aeruginosa* (MTCC No.424), *Bacillus subtilis* (MTCC No.441), *Staphylococcus epidermidis* (MTCC No.9041), and *Micrococcus luteus* (MTCC No.106), were procured from MTCC Chandigarh.

Extraction procedure

Grinding of selected plant materials

The plant material was dried at 37^{o}C for 72 hours. Exposure to sunlight was avoided to prevent the loss of active constituents. After drying the plant material was cut into pieces. The powdered plant material was taken for extraction procedure.

Preparation of ethanolic extract

Stems of the plant were washed thoroughly with distilled water and shade-dried. Ethanolic extract of the dried stems of *T. cordifolia* was prepared by maceration method using 50 ml ethanol. The extraction was done at room temperature for 7 days.

Preparation of hydro methanolic extract

The plant material (matured stems) was collected and shade dried. These dried stems were then cut into pieces. Then it was extracted by hydromethanol (1:1) using maceration method (Khan R et al., 2011). The cut pieces of plant material were placed in a stoppered container with the solvent and were allowed to stand at room temperature for 7 days with frequent agitation until the soluble matter was dissolved. The mixture was strained, the marc (the damp solid material) was pressed, and the extract was taken for the study.

Storage conditions

The extract was stored in a cool condition protected from direct sunlight.

Qualitative phytochemical analysis

The hydromethanolic and ethanolic extracts of *Tinospora cordifolia* were subjected to different chemical tests for the detection of phytoconstituents such as carbohydrates, glycosides, alkaloids, proteins, amino acids, tannins, phenolics, saponins, flavonoids, triterpenoids, steroids, fixed oils, gums and mucilage.

Tests for carbohydrates

The carbohydrates were tested by using Benedict's test, Fehling's test, Molisch test and Barfoed's test. (Kokate CK., 1994)

Tests for alkaloids

The alkaloids were detected using Dragondroff's test, Wagner's test, Mayer's test and Hager's test. (Ansari SH., 2006).

Tests for proteins and amino acids

The Biuret test, Xanthoproteic test, Lead Acetate test and Ninhydrin test were used for the analysis of proteins and amino acids (Ansari SH., 2006).

Tests for tannins and phenolics

Test for tannins and phenolics were performed by adding 2-3 drops of ferric chloride to 1ml of extract and the formation of a dark blue or greenish black colour product shows the presence of tannins. (Mukherjee PK., 2002).

Test for flavonoids

Flavonoids were detected by means of Shinoda Test. (Kokate CK., 1994)

Test for triterpenoids

Test for triterpenoids was done by dissolving two or three granules of tin metal in 2 ml thionyl chloride solution and then, adding 1 ml of the extract into the test tube. The formation of a pink colour indicates the presence of triterpenoids. (IP. 1996)

Tests for steroids

The steroids were identified by using Lieberman Burchard test, Salkowski test and Liebermann's reaction. (Ansari SH., 2006; Mukherjee PK., 2002).

Test for saponins

The procedure adopted for the identification of saponins was to take 1 ml of extract which is diluted with 20 ml distilled water and then shaken in a graduated cylinder for 15 minutes. A 1 cm layer of foam indicates the presence of saponins. (Ansari SH., 2006).

Tests for fixed oils

The fixed oils were tested by means of Spot test and Saponification test. (Kokate CK., 1994).

Tests for glycosides

Tests like Legal test, Baijet test, Borntrager's test and Keller Killiani Test were used for the analysis of glycosides. (Kokate CK., 1994; IP., 1996).

Test for gums and mucilages

Test for gums were performed by hydrolyzing the 1 ml of extract using dil. HCl (3ml). Then Fehling's solution was added drop by drop till the appearance of red. (Ansari SH., 2006) .

Test for mucilage were carried out by treating 1 ml of extract with 2 ml of ruthenium red solution to get red coloured solution. (Ansari SH., 2006).

Antibacterial activity

The antibacterial activity of the hydromethanolic and ethanolic extracts of *T. cordifolia* was determined by agar-cup plate method. Bacteria were first cultured in a nutrient broth for 24 h before use.

Figure.1 *T .cordifolia*

Young stem

Heart shaped Leaves

Table.1 Phytochemical Profile of *T.cordifolia*

Chemical class	Phytoconstituents	Plant part
Alkaloids (Singh SS et al., 2003; Sinha K et al., 2004)	Berberine, Tembeterine,	Stem
	Choline,Tinosporin,isocolumbin tetrahydropalmitine,jatrorrhizine	Root
	Palmitine	Stem and root
Glycosides (Singh SS et al., 2003; Gagan VD et al., 1994; Wazir V et al., 1995 ; Gagan VD et al., 1996; Maurya R et al.,1997; Ghosal S et al.,1997)	18-norclerodane glucoside, furanoid diterpene glucoside, cordiofolioside A,cordiofolioside B, palmatosides C, palmatosides $P_{1,}$ cordiofolioside C,cordiofolioside D, cordiofolioside E	Stem
Diterpenoid lactones (Singh SS et al., 2003; Maurya R et al.,1997; Maurya R et al., 1989; Swaminathan K et al., 1989)	Clerodane derivatives, tinosporon, tinosporides, jaiterine,columbin	Whole plant
Sesquiterpenoids (Maurya R et al.,1998)	Tinocordifolin	Stem
Steroids (Singh SS et al., 2003)	β –sitosterol, δ-sitosterol, 20β-hydroxy ecdysone, ecdysterone, makisterone A, giloinsterone	Aerial parts Stem
Aliphatic compounds (Singh SS et al., 2003; Thippeswamy G et al., 2008)	Octacosanol, heptacosanol, nonacosan-15-one	Whole plant
Miscellaneous compounds (Singh SS et al., 2003; Hanuman JB et al., 1986)	Tinosporidine, cordifol, cordifelone, N-trans-feruloyl tyramine as diacetate, giloin, gilonin, tinosporic acid	Root

Table.2 Biological activities of *T.cordifolia.*

Phytoconstituent	Biological activities
Berberine	Antimicrobial (Cernakova M et al., 2002); Antiproliferative (Tungpradit R et al., 2011); Antiplasmodial,Antiamooebic (Wright CW et al., 2000); Antifungal (Vollekova A et al.,2003); Antiphotooxidative (Kim JP et al.,2000)
Columbin	Schizonticidal (Patel JP et al., 2010)
Cordifolioside A	Immunomodulatory (Kapil A et al.,1997; Sudhakaran DS et al., 2006)
Cordioside	Immunomodulatory (Sudhakaran DS et al., 2006]; Schizonticidal[Patel JP et al., 2010)
ECD	Anticancer (Muniyappan D et al.,2009)
Ecdysterone	Anabolic (Syrov VN et al., 1976); Immunomodulatory(Chiang HC et al.,1979)
Isocolumbin	Antiinflammatory(Moody JO et al., 2005) ;Antimicrobial (Yuan SHI et al., 2010); Antimalarial (Roja G et al 2005)
Jatorrhizin	Antimicrobial(Yuan SHI et al., 2010); Antimalarial (Roja G et al 2005)
Magnoflorine	Cytotoxic (Kokorus ZRD et al 2006); Antioxidant(Hung TM et al., 2007)
Palmatine	Antiplasmodial, Antiamoebic, Cytotoxic (Wright CW et al., 2000); Antifungal (Vollekova A et al.,2003); Antiphotooxidative(Kim JP et al.,2000); Antimicrobial (Yuan SHI et al., 2010)
Syringin	Immunomodulatory(Kapil A et al.,1997); Hypotensive (Ahmad M et al.,1995); Anti-inflammatory(Choi J et al., 2004)
Tembetarine	Antinociceptive(Nishiyama Y et al.,2010)
Tinocordifolin, Tinosporide	Schizonticidal (Patel JP et al., 2010)
Tinocordifolioside	Schizonticidal (Patel JP et al., 2010); Antihyperlipidemic (Thahera DP et al.,2011)
Tinosporaside (Cordiol)	Immunomodulatory (Kapil A et al.,1997)

Table.3 Qualitative Phytochemical analysis *of T.cordifolia*

S.No.	Phytoconstituent	Hydroalcoholic extract	Ethanolic extract
1.	**Alkaloids**		
	Dragondroff's test	+	+
	Wagner's test	+	+
	Mayer's test		
	Hager's test		
2.	**Carbohydrates**		
	Molisch Test	+	+
	Benedicts Test	+	+
3.	**Glycosides**		
	Legal test	-	-
	Baljet test	+	+
4.	**Steroids**		
	Lieberman Burchard Test	+	-
5.	**Proteins & Amino acids**	-	-
	Biuret test		
	Xanthoproteic test	+	-
	Lead Acetate test	-	-
6.	**Fixed oils and Fats**		
	Spot test	-	-
	Saponification test	-	-
7.	**Tannins & Phenolics**	-	-
	Ferric Chloride test	-	-
	Potassium dichromate test		
8.	**Saponins**		
	Foam test	-	-
9.	**Flavonoids**		
	Shinoda test	-	-
10.	**Gums**	-	-
11.	**Mucilages**		
	Ruthenium Red Test	-	-

Table.4 Antibacterial activity of *T.Cordifolia* and standard (Streptomycin)

Tested bacteria	Standard	Diameter of zone of inhibition	
	Streptomycin	Ethanolic extract	Hydromethanolic extract
E. coli	6 mm	-	-
S. aureus	6 mm	-	2mm
P. vulgaris	7 mm	-	-
P. aeruginosa	6 mm	-	-
B. subtilis	7 mm	2mm	3mm
S. epidermidis	6mm	6mm	4mm
M. luteus	7 mm	5mm	2mm

The standardized cell suspensions were spread on an agar media (Merck). Sterile 6 mm diameter of cork borer was used to bore wells into the agar plate. Approximately 200µl T. *cordifolia* (TC1 & TC2) extract were introduced into the wells separately, allowed to stand at room temperature for about 2 h and then incubated at 37°C. The plates were observed for zones of inhibition after 24 h and compared with standard Streptomycin.

Results and Discussion

Qualitative phytochemical analysis

The present study reveals that *T.cordifolia* plant shows the presence of phytochemical constituents like alkaloids, flavonoids, carbohydrates, glycosides, proteins, saponins, tannins, terpenoids and anthraquinones in different solvent extracts as shown in Table 3.

Antibacterial Activity

Antibacterial activity of *T.cordifolia* was seen against to the several organisms namely *Escherichia coli* (MTCC No.40), *Staphylococcus aureus* (MTCC No.87), *Proteus vulgaris* (MTCC No.742), *Pseudomonas aeruginosa* (MTCC No.424), *Bacillus subtilis* (MTCC No.441), *Staphylococcus epidermidis* (MTCC No.9041), and *Micrococcus luteus* (MTCC No.106). The ethanolic extract exhibit effective antibacterial activity against all the organisms ,except for E.coli, Proteus vulgaris and Pseudomonas aeruginosa while the hydromethanolic extract exhibits inhibition zone on limited species such like *Staphylococcus aureus* (2mm), *Bacillus subtilis*(3mm), *Micrococcus luteus* (2mm), *Staphylococcus epidermidis* (4mm) as shown in Table 4.

Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms. They often have pharmacological effects and are used as medications. (Kokate CK.,1994). Most phytochemicals have antioxidant activity and protect our cells against oxidative damage and reduce the risk of developing certain types of cancer. The antibacterial activity was screened because of their great medicinal properties towards the pathogenic organisms. The stem extract of *T.cordifolia* was screened for antibacterial activity and was found to have so against pathogenic strains. The present study demonstrates that the plant *T.cordifolia* is a rich source of alkaloids, glycosides, carbohydrates and steroids .

The antibacterial activity of Tinospora cordifolia was clearly shown in the present study against organisms namely *Staphylococcus aureus, Bacillus subtilis, Micrococcus luteus, Staphylococcus epidermidis.*

Acknowledgement

The authors thank the management of Pioneer Pharmacy Degree College, Vadodara, for all their support and encouragement in carrying out the study.

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