Indian Journal of Ecology (2022) 49(1): 38-41
DOI: https://doi.org/10.55362/IJE/2022/3473

Manuscript Number: 3473
NAAS Rating: 5.79

Tinospora cordifolia: A Valuable Plant in Ayurveda

Varsha Marke, Pranali Shete and Vishal Dhundale

Post Graduate Department of Microbiology
Smt Chandibai Himathmal Mansukhani College, Ulhasnagar-421 003, India
E-mail: pranalikale2@gmail.com

Abstract: *Tinospora cordifolia* commonly is a natural herbal shrub that belongs to the moonseed family Menispermaceae. The phytochemical analysis showed the presence of alkaloids, flavonoid, saponins, cardiac glycosides, steroids, carbohydrate and proteins. The aqueous extracts of *Tinospora cordifolia* were evaluated for antibacterial activity against standard pathogens i.e. *Staphylococcus aureus, Escherichia coli, Vibrio cholera, Salmonella typhi, Shigella* at concentrations 5, 10 and 20%. The 20% aqueous extract of *Tinospora cordifolia* showed an optimum zone of inhibition. *Vibrio cholera* MTCC 3906 was highly sensitive to aqueous extract followed by *S. aureus* MTCC 3160*, Salmonella enterica typhi* MTCC 734*, Shigella sonnei, Escherichia coli* MTCC 1018. *Salmonella enterica typhi* MTCC 734 was highly sensitive to methanol extract followed by *Shigella sonnei, Staphylococcus aureus* MTCC 3160*, Vibrio cholera* MTCC 3906*, Escherichia coli* MTCC 1018. These phytochemical data analysis and antibacterial activity of *Tinospora cordifolia* may be useful and may be lead to the improvement and formulation of drugs and discovery of drugs development against tested gastrointestinal tract pathogens.

Keywords: *Tinospora cordifolia,* Gastrointestinal tract pathogens, Antibacterial activity.

Ayurvedic herbs played important role in Ayurvedic treatments from ancient time to this most modern time. One of the plant known for having many medicinal use in traditional system of medicines is *Tinospora cordifolia* which belongs to the Menispermaceae family and is common climbing shrub in India (Shah and Shah 2016), extending from Himalayas down to the Southern Part of India. Root leaves and stem region of this shrub used for medicinal purposes and contain columbin, tinosporin, and tinosporin acid. The other different types of phytochemicals also identified which includes alkaloids, diterpenoids, lactones, steroids, glycosides, aliphatic compounds, polysaccharides, saponins, flavonoids, tannin. The *T, cordifolia* act as immunomodulatory, anti-microbial (Bonvicini et al 2014), anti-oxidant, hepatoprotection, diuretic, anti-neoplastic, anti-hypoglycemic, antipyretic, anti-inflammatory (Li et al 2010, Tiwari et al 2014), anti-stress, anti-hyperglycemic, anti-diabetic, and anti-tuberculosis also been reported (Sharma et al 2015). The stem preparation is used in general debility, dyspepsia, fevers, and urinary infection. The root is a powerful emetic and its aqueous extract is used for visceral obstruction, leprosy, etc. The present study is based on the use of its extract against certain types of pathogenic bacteria for anti-inflammatory activity.

MATERIAL AND METHODS

Collection of plant materials: The *Tinospora cordifolia* was collected from the Karjat Mahamandal Depo. The freshly collected stem region was then subjected to preliminary treatment, which includes washing and cutting appropriate pieces. These pieces were further dried at 54° C in the oven for 2-3 days and converted into powder form, which was further used for extract preparation.

Preparation of extract: For the preparation of hot water extract and methanol extract, hot water and methanol both were separately added to the powder at a ratio of 1:10. Hot water extract was heated in a boiling water bath to make the volume 3/4th of the original volume. The methanol extract was kept shaker at 1500 rpm for 48-72 hours. Both the extract was filtered and dried at 54°C. Then dried extract weight was determined. The extracts were stored in a cool condition to protect them from direct sunlight.

Extract quantification and sterility checking: Extract quantification was done by calculating the percent yield of the extract as:

Percent yield = weight of the extract/ weight of the total mass of the powder X 100

For preliminary sterility testing of extracts, streaking was done on a sterile nutrient agar plate and sterile potato dextrose agar plate. Then the plate was incubated and observed for growth.

Phytochemical Analysis

Alkaloids: 200mg of plant material was dissolved in 10ml of methanol and filtered. To this 2ml filtrate, 1% HCl was added and steamed, to 1ml of this filtrate, 6 drops of Dragendroff reagent were added and observed for orange precipitate.

Tannins: 200mg plant material was mixed with 10 ml of distilled water and then filtered. To this 2ml filtrate, 2ml $FeCl_3$ was added and observed for blush black precipitate.

Flavonoids: 200mg of plant material was heated with 10ml of ethyl acetate over steam and kept for 3 min and then filtered. To 4ml filtrated, 1ml of dilute ammonia solution was added and observed for yellow colouration.

Saponin: 200mg plant material mixed with 10ml of distilled water. The presence of frothing after shaking the tube confirms the presence of saponin.

Steroids (Libermann- Buchard reaction): 200mg plant material was mixed with 10ml of chloroform and filtered. To 2 ml of filtrate, 2 ml of acetic anhydride then conc. H_2SO_4 was added and observed for the development of blue-green rings (Xiong et al 2007).

Cadiac glycosides (Keller - Killani test): 200 mg plant material was mixed with 10ml of ethanol. To 2 ml of this filtrate, 1 ml of glacial acetic acid, $FeCl_3$ and conc. H_2SO_4were added and observed for greenish-blue colouration.

Carbohydrates: Fehling's Test for carbohydrates was performed for both water extract and methanolic extract.

Amino acids: The estimation was done by using Ninhydrin's Test for the presence of amino acids in both the extracts were tested.

Antibacterial study against gastrointestinal tract pathogens: Standard gastrointestinal tract pathogens such as *Escherichia coli* MTCC 1885*, Salmonella enterica typhi* MTCC 734*, Shigella sonnei, Staphylococcus aureus* MTCC 3160and *Vibrio cholera* MTCC 3906were freshly subculture on sterile Nutrient Agar slant before use for antibacterial activity.

The antibacterial activity of methanol extract and water extract of Tinospora cordifolia (stem) were tested by the agar cup method. Different concentrations of both extracts (5, 10 and 20%) were prepared by reconstituting with 25% DMSO (Rose et al 2010). 20 ml of sterile Nutrient Agar was bulk seeded with test culture (0.2 ml of 0.5 OD culture). After solidification of agar the wells were made on this plate using 6mm sterile borer (Shubha and Hiremath, 2010) and then 0.2 ml of extract (5, 10 and 20%) was added. Dimethyl sulphonic acid (25%) was used as control. The antibacterial assay plates were incubated at 37°C for 24hrs. The diameter of the inhibition zone of was measured in mm.

Table 1. Yield of water and methanol extract

Methanol extract	Yield (%)	Hot water extract	Yield (%)
TM1	11.33	TW1	12.93
TM2	11.91	TW2	12.43
TM3	11.77	TW3	11.67
TM4	12.14	TW4	12.39
TM5	11.43	TW5	12.52
TM6	11.72	TW6	12.39

Preparation of syrup: Firstly, three different concentrations of 40, 50 and 60% sugar were prepared in D/W and sterilized using an autoclave (Sivakumar et al 2011). Extract powder was added using aseptic techniques to make the final syrup concentration 10%. These mixtures were homogenized using a vortex mixer. Final syrup formulations were used for antibacterial activity against GI tract pathogens as used above.

Acid and bile stability of extract: For this acid and bile stability of extract, phosphate buffer of 2.5, 4 and 6 pH were prepared to which 0.85% bile was added. In that buffer, the extract was added to make its final concentration 10%. Antibacterial activity of the acid and bile subjected extract were determined at different time intervals (1, 2 and 3hrs) by using the agar cup method.

Anti-Inflammatory activity of extract: The anti-inflammatory activity of the extract was studied by using the inhibition of the albumin denaturation test. The reaction mixture consists of test extract within the concentration range of 200 to 800mcg/ml and 1% aqueous solution of bovine serum albumin. pH of the reaction mixture was adjusted with 1N HCl. The sample extract was incubated at 37°C for 20min and then heated to 51°C for 20 min. After cooling the mixture, turbidity was measured at 660nm. The percent of inhibition of denaturation was calculated using the following equation (Dharmadeva et al 2018):

Percent inhibition = (Abs control – Abs sample) X 100/Abs control

The aqueous extract yield was marginally higher than the methanol extract yield. This could be due to the higher polarity of water than methanol and this plant may contain more polar components. A number of different active principles including alkaloids, flavonoids, carbohydrates, saponins, steroids, cardiac glycosides and amino acids have been identified for observing its medical effect in *T. cordifolia*.

The phytochemical analysis done shows the presence of alkaloids, flavonoids, saponins, cardiac glycosides, steroids, carbohydrates and proteins (Table 2). All the phytochemicals were present except tannin. Amino acids were estimated with the help of Ninhydrin's Test which showed intense dark blue colouration, which could be due to the presence of a high concentration of amino acids. Test for steroids, flavonoids and cardiac glycosides also showed intense dark colouration *i. e.* present in abundant amounts. The larger foam formation points out the presence of a high amount of saponins. Other phytochemical tests gave light colour development which could indicate its intermediate and low presence in the extract. From the phytochemical

screening, the extracts are rich in amino acid, saponin, steroids, flavonoids and cardiac glycosides. Similar results were also obtained by Sivakumar and Dhana Rajan (2011).

When tested by the well diffusion method, the aqueous extracts of leaf extracts of *T.cordifolia* were subjected for antibacterial activity against standard pathogens i.e. *S. aureus, E. coli, V. cholera, S. typhi, Shigella* at concentrations 5, 10 and 20%. as the concentration of extract increases, there was also an increase in the zone of inhibition occurs. It was found that 20% of the extract showed the highest zone of inhibition which has a 25 mm zone of inhibition against *V. cholera* MTCC 3906 was highly sensitive to aqueous extract and followed by *S. aureus* MTCC 3160*, S. enterica typhi* MTCC 734*, Shigella sonnei, E. coli* MTCC 1018 (Fig. 1).

This Figure 2 indicates as the concentration of methanol extract increases, there was also an increase in the zone of inhibition occur. The 20% of the extract showed the highest zone of inhibition and S*. enterica typhi* MTCC 734 was highly sensitive to methanol extract and showed 20 mm zone of inhibition followed by *Shigella sonnei, S. aureus* MTCC 3160*, V. cholera* MTCC 3906*, E. coli* MTCC 1018. Rose et al (2010) revealed that the maximum antibacterial activity of hot and cold methanol extracts was exhibited against *Staphylococcus aureus* when compared with standard drugs. Shanthi and Nelson (2013) observed that maximum inhibitory activity of ethanol extract of leaf of *T. cordifolia* was against *Klebsiella pneumoniae* was followed by *Pseudomonas aeruginosa* while the chloroform extract of leaf showed moderate activity against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* but was less against *E. coli.* Prajwala et al (2019) observed the antibacterial activity of *T. cordifolia* leaf extract of methanolic, ethanolic, chloroform, hexane, aqueous and acetone extract but only the methanolic extract showed the antibacterial activity against the *E. coli.* In the present study, the results of both the extract show good antibacterial activity against GI tract pathogens. In this investigation, the antibacterial activity of water extract syrup and methanol extract syrup was also evaluated against GI tract pathogens. The antibacterial activity was determined for both the extract containing syrup against GI tract pathogens. As the concentration of extract increases, there was an increase in the zone of inhibition. It and 60 and 50% extract showed the highest zone of inhibition.

In the present study acid and bile tolerance of *Tinospora cordifolia* indicated that at different pH values (2.5, 4 and 6

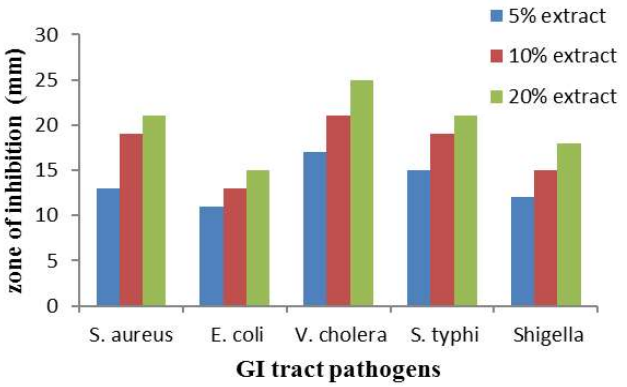

Fig. 1. Antibacterial activity of water extract against GI tract pathogens

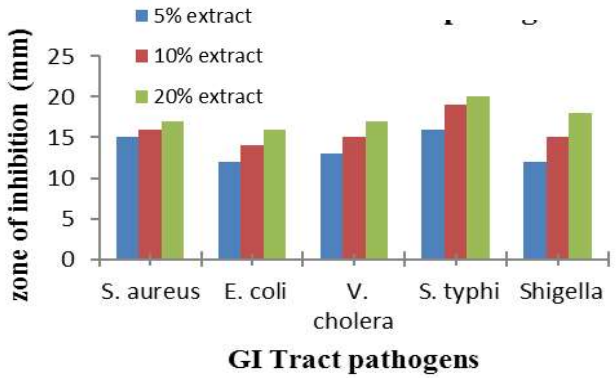

Fig. 2. Antibacterial activity of methanol extract against GI tract pathogens

Table 2. Phytochemical analysis of *Tinospora Cordifolia*

Phytochemicals	Test used	Observations/ colour	Results
Alkaloids	Dragendorff test	Reddish brown precipitate	+
Tannin	Ferric chloride test	Brown colour	-
Flavonoid	Alkaline reagent test	Green colour	++
Saponins	Foam formation	Foam	++
Carbohydrate	Molish test	Blue colour ring	+
Amino acid	Ninhydrin's test	Dark blue colour	++
Cardiac glycosides	Keller – Kiliani test	Greenish blue colour	++
Steroids	Libermann – Burchard test	Blue green ring	++

+Light colour, ++ Intense dark colour, - Negative test

Table 3. Effect of *Tinospora cordifolia* extract on protein denaturation

Concentrations (mcg ml^{-1})	Absorbance (540 nm)	% inhibition at protein denaturation
200	0.20	48
400	0.25	34
600	0.16	59
800	0.13	65
Control	0.38	--

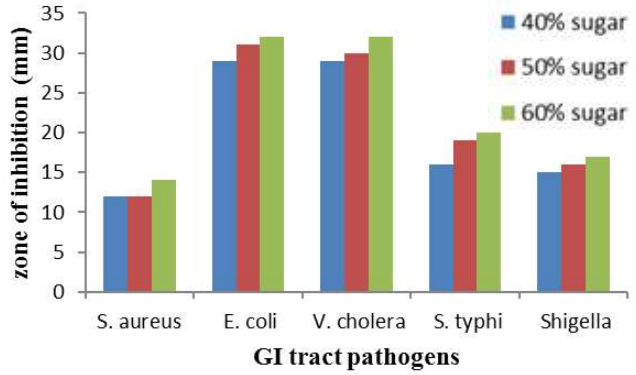

Fig. 3. Antibacterial activity of syrup (Methanol extract) against GI tract pathogens

Fig. 4. Plate showing acid and bile stability of extract at different interval

pH) high bile contain does not affect the activity of extract. The extract showed antibacterial activity against all the pathogens. When the extract activity was determined at different interval the activity of extract remained (Fig. 4). Both extract (water and methanol extract) at 600mcg/ml and 800mcg/ml showed higher inhibitory activity of protein denaturation of bovine serum albumin (Table 3).

CONCLUSION

Tinospora cordifolia may be useful due the presence of quantitative and qualitative alkaloids, flavonoid, saponins, cardiac glycosides, steroids, carbohydrates and proteins that may be lead to the improvement and formulation of drugs and discovery of drugs development against gastrointestinal tract pathogen. The 20% of the extract showed the highest zone of inhibition with 25 mm zone of inhibition against *V. cholera,* MTCC 3906 was highly sensitive to aqueous extract and followed by *S. aureus* MTCC 3160*, S. enterica typhi* MTCC 734*, Shigella sonnei, E. coli* MTCC 1018. The *S. enterica typhi* MTCC 734 highly sensitive to methanol extract and showed 20 mm zone of inhibition followed by *Shigella sonnei, S. aureus* MTCC 3160. From the present investigation, can conclude that *Tinospora cordifolia* has good antibacterial activity against gastrointestinal tract pathogens.

Received 24 July, 2021; Accepted 19 January, 2022

REFERENCES

Bonvicini F, Mandrone M, Antognoni F, Poli F and Gentilomi GA 2014. Ethanolic extracts of *Tinospora cordifolia* and *Alstonia scholaris* show antimicrobial activity towards clinical isolates of methicillin-resistant and carbapenemase-producing bacteria. *Natural Product Research* **28**(18): 1438-1445.

Dharmadeva S, Galgamuwa LS, Prasadinie C and Kumarasinghe N 2018. *In vitro* anti-inflammatory activity of *Ficus racemosa* L. bark using albumin denaturation method. *Ayu* **39**(4): 239-242.

Li RW, Leach DN and Myers SP 2004. Anti-inflammatory activity, cytotoxicity and active compounds of *Tinospora smilacina* Benth. *Phytotherapy Research* **18**(1): 78-83.

Prajwala B, Priyanka S, Raghu N, Gopenath N, Gnanasekaran A, Karthikeyan M, Indumathi R, Ebrahim NK, Pugazhandhi B, Pradeep P, MS Ranjith, Balasubramanian S and Basalingappa KM 2019. In vitro anti-bacterial activity of *Tinospora cordifolia* leaf extract and its phytochemical screening. *Journal of Biomedical Sciences* **5**(2): 10-17.

Rose FM, Noorulla KM, Asma M, Kalaichelvi R, Vadivel K,Thangabalan B and Sinha BN 2010. *In vitro* antibacterial activity of methanolic root extract of *Tinospora cordifolia* (willd). *International Journal of Pharma Research and Development* **5**(2): 1-5.

Shah PA and Shah GB 2016. Preliminary screening of *Tinospora cordifolia* extracts and *Guduchi satva* for anti-rheumatoid activity. *International Journal for Pharmaceutical Research Scholars* **5**(2): 7-13.

Shanthi V and Nelson R 2013. Antibacterial activity of Tinospora cordifolia (Willd) Hook. F. Thoms on urinary tract pathogens. *International Journal of Current Microbiology and Applied Sciences* **2**(6): 190-194.

Sharma H, Galib A and Prajapati PK 2015. Antidiabetic claims of *Tinospora cordifolia* (Willd.) Miers: critical appraisal and role in therapy. *Asian Pacific Journal of Tropical Biomedicine* **5**(1): 68-78.

Shubha HS and Hiremath RS 2010. Evaluation of antimicrobial activity of *Rasaka Bhasma* Ayu. **31**(2): 260-262.

Sivakumar MK, Moideen MM, Varghese R, Shatha A and Dhanapal CK 2011. Formulation and Evaluation of Herbal Syrup containing *Cocos nucifera Linn.* Root *Research Journal of Pharmaceutical, Biological and Chemical Sciences* **2**(4): 53-61.

Sivakumar V and Dhana Rajan MS 2011. Standardization and Characterization of *Tinospora cordifolia* (Willd.) Miers ex Hook. F. & Thoms. Plant stem extract in different solvent fractions. Asian *Journal of Biochemical and Pharmaceutical Research* **4**(1): 105-112.

Tiwari M, Dwivedi UN and Kakkar P 2014. *Tinospora cordifolia* extract modulates COX-2, iNOS, ICAM-1, pro-inflammatory cytokines and redox status in murine model of asthma. *Journal of Ethnopharmacology* **153**(2): 326-337.

Xiong Q, William K, Wilson Æ, Pang J 2007. The Liebermann–Burchard reaction: sulfonation, desaturation, and rearrangement of cholesterol in acid. *Lipids* **42**: 87-96.