Phytochemical and Antimicrobial Activity Screening of Stem Extracts of *Tinospora cordifolia*

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DOI 10.51129/ujpah-2020-29-2(6)

Abstract-*Tinospora cordifolia* is a popular medicinal plant which is used in several traditional medicines to cure various diseases. The common names are Amrita and Guduchi, belonging to the family of Menispermaceae. The aim of the study was to study the stem extract of traditional medicinal plant, *T. cordifolia* for qualitative estimation of phytoconsituents and subsequently to determine its antibacterial activity against two test microorganisms *Escherichia coli* and *Pseudomonas aeruginosa* to authenticate its use in traditional medicines. Stems were air-dried and coarsely powdered samples were subjected to Soxhlet extraction using diverse solvents (Hexane, chloroform, ethyl acetate and methanol and water). Freshly prepared extracts were exposed to standard phytochemical analysis for qualitative estimation of phytoconsituents. The antibacterial activity of the stem extract of *T. cordifolia* was determined by agar well diffusion method. Phytochemical analysis revealed the presence of several phytochemicals *viz.*, alkaloids, flavonoids, steroids, phenol, tannins, steroid, terpenoids, saponins and sugars. The methanolic extract displayed the presence of highest number of phytochemical compounds. It may be due to the higher solubility of active components in this solvent as compared to other solvents. The results revealed that the methanolic extract exhibit the effective antibacterial activity against the tested bacterial species . The studies justify the use of *T. cordifolia* in traditional medicines. The investigation further proposed that the metabolites present in leaf tissue of *T. cordifolia* can be potential source of novel natural antibacterial and antioxidant agents and can be prospective applications in food industry as an antioxidant.

Keywords: Antibacterial activity, Phytochemical, *Tinospora cordifolia,* Quantitative phytochemical

Introduction

Trees and plants are of supreme importance for life of humans since ancient times. Man depended on them for his physical needs such as sources for food, shelter, clothing, medicine, ornaments tools and for spiritual needs. India is a country rich in indigenous herbal resources which grow on their varied topography and under changing agro-climatic conditions permitting the growth of almost 20,000 plant species, of which about 2,500 are of medicinal value [1,2]. Peoples are now showing interest in plant derived drugs mainly due to the current widespread belief that 'Green Medicine' is safe and more dependable than the costly synthetic drugs which have adverse side effects. Medicinal plants are mostly locally available relatively cheaper and there is every virtue in exploiting such local and traditional remedies when they have been tested and proven to be non-toxic, safe, inexpensive and culturally acceptable to the community [3]. *Tinospora cordifolia* is a shady climbing shrub belonging to the family Manispermaceae, is found in the tropical areas of India, Pakistan, Sri Lanka, Burma, Africa, Australia and China.[4] The phytochemical constituents of *T. cordifolia* consists of alkaloids, sesquiterpenoid, polysaccharides, steroids, glycosides, different types of fatty acids and essential oils[4,5]. In this perspective, the plant is considered to be a nectar plant and has been called as amrita in Sanskrit in recognition of its detoxifying, rejuvenating, and immune boosting properties[6]. Experimental studies conducted on *T. cordifolia* have shown that it has significant therapeutic effects on diabetes and its associated Complications, hepatotoxicity, different types of infections, gastrointestinal related complications and different types of cancers.[7] Also, traditionally it has been reported that this plant extract has been used for the treatment of fever [4,8].

The aim of this work was to determine the phytochemical constituents of the stem extract of *T. cordifolia* and also its antibacterial activity against *Escherichia coli* and *Pseudomonas aeruginosa.*

Material and Methods

Plant Collection and Processing

Stem*s* of *Tinospora cordifolia* were collected from Ranipokhari region of Dehradun(Uttarakhand) in the month of November.The botanical identity of plant was identified taxonomically . The plant material were cultivated types of 6-7 meter in height. About 20 plants both male and female were taken for study. The collected stems were cut into pieces with a sharp knife and were first washed with distilled water and disinfected for 30 minutes by immersion in a 2 % solution of sodium hypochlorite. The plant material was then rinsed with distilled water to remove residual hypochlorite. Finally the plant material was dried under shade at room temperature for 20 days. After drying plant sample was blended, pulverized and stored at 4°C. The whole experiment was conducted using a single source of dried powder of selected plant species.

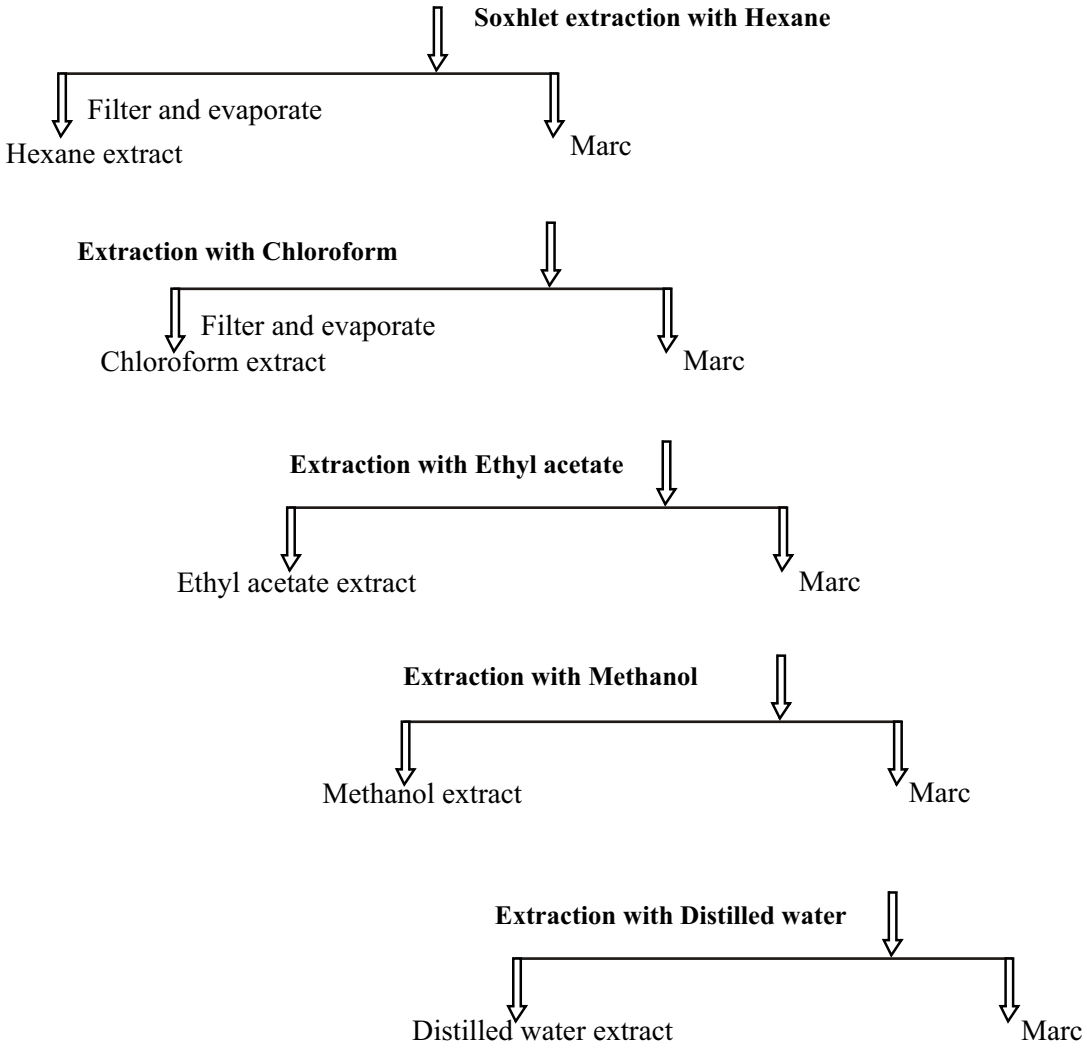
Preparation of extracts

Successive solvent extraction scheme was used for preparation of different extracts. The 500g powder of *Tinospora cordifolia* stem was used for extraction with hexane using Soxhlet's extractor. Different solvents were used for the extraction purpose in a specific sequence based on increasing polarity. Different solvents were used for dissolving different components present in the plant material, based on their different component present in the plant material, based on their different polarity. Solvents in order of increasing polarity were used.

Hexane < Chloroform < Ethyl acetate < Methanol < Distilled water

The 500g plant material was exhaustively extracted with 1000ml each of hexane, chloroform, ethyl acetate, methanol and distilled water, respectively using a soxhlet continuous extraction for 1 week. The final extract was concentrated and dried out.

Figure-1 Scheme of extraction



Marc: residue left after extraction

Determination of extractive value

The percentage extractive values (w/w) was calculated as:

$$\text{Extraction value (\%)} = \frac{\text{Weight of dried extract}}{\text{Weight of plant material}} \times 100$$

Phytochemical Screening

All the five extracts (Hexane, Chloroform, ethyl acetate, methanol, distilled water) were subjected to preliminary phytochemical screening for the detection of various phytoconstituents such as alkaloid, terpenoid, tannin, saponin, flavonoids, amino acids and carbohydrate. Phytochemical analysis was carried out according to following standard procedures.

1. Detection of Alkaloids: Small portion of the solvent free extract was stirred with a few drops of dil. HCL and filtered. The filtrate was then tested for the colour test to detect the presence of alkaloid in 60ml distilled water 5.0g of potassium iodide in 20ml distilled water, 20ml of distilled water) giving cream of ppt.

Hager's reagent: Test solution with Hager's reagent (saturated aq. solution of picric acid i.e. 1.0% w/w solution of picric acid in hot water) gave yellow ppt.

Wagner's reagent: Test solution with Wagner's reagent (1.27g of iodine and 2g of potassium iodide in 5ml of water and 100ml distilled water) gave reddish brown ppt.

2. Detection of Flavonoids

Alkaline reagent test: Extracts were treated with a few drops of sodium hydroxide solution. Formation of intense yellow colour, which became colourless on the addition of dilute acid, indicates the presence of flavonoids.

3. Test for Carbohydrates

Molish's test: To the 2-3 ml of extract, few drops of 95% alpha-napthol solution in alcohol were added. After shaking, conc. H_2SO_4 was added from the sides of the test tube. Appearance of violet ring at the junction of two layers indicated the positive test for reducing sugar.

Fehling's test: To1 ml Fehling A and 1 ml Fehling B reagent 1 ml of extract was added and boiled for about 10min. Formation of brick red color precipitate indicated the presence of carbohydrate[9].

Benedict's solution test: Equal volume of Benedict's reagent and extract were mixed in test tube. Heated in boiling water bath for 5 min. Appearance of red coloured solution indicates the positive test for reducing sugar.

4. Test for steroids

Liebermann-Burchard Reaction: Mixed 2ml of extract with chloroform. Added 1-2 ml of acetic anhydride and 2 drops of conc. Sulphuric acid from the sides of test tube. Development of green colour reveals the positive test for steroid moiety.

Salkowiski reaction: 2ml of crude extract was dissolved in 2ml of chloroform to this added 2ml of con. H_2SO_4 sidewise, red color ring was produced[10].

5. Test for phenolic components and tanins

Small quality of test solution dissolved in water and subjected to the following tests to detect the presence of phenolic compounds and tannins.

Dil. $FeCl_3$ solution (5%) test: Test solution with few drops of ferric chloride solution showed intense green color[11-14].

Vanillin HCl acid test solution: Test solution with vanillin reagent (1gm vanillin in 10 ml concentrated HCl) gave red color.

6. Test for saponins Froth test: 2 ml of crude extract was mixed with 2ml of distilled water in a test tube, the solution was warmed and shaken vigorously; formation of stable foam indicated the presence of saponin

7. Test for protein and amino acids

Ninhydrin solution test: Heated 3ml of extract and 3 drops of 5% Ninhydrin solution in boiling water bath for 10 min. The development of violet or purple colour showedthe presence of amino acids.[15].

Biuret test: To 3ml of aqueous extract added 4% NaOH and few drops of 1% $CuSo_4$ solution. Violet or pink colour is formed, if proteins are present.

Thin layer chromatography profiling of various extracts

Nowadays, TLC is employed as an important tool for the qualitative and quantitative phytochemical analysis of herbal drugs and formulations[16-18]. The TLC profiling was performed as per described by Biradar et al., 2013[19]. Various extracts of stem of *Tinospora cordifolia* were obtained by sequential Soxhlet extraction with different solvents of increasing polarity. 100gm of powdered sample were sequentially extracted in a Soxhlet extractor using 800 ml of hexane, chloroform, ethyl acetate, methanol and distilled water, The extraction was done until solvent in soxhlet became colourless. The extracts were then subjected to distillation for preparation of crude extracts in respective solvents. The TLC plates were prepared by using Silica gel 'G'

as 30 gm of silica gel was weighed and made to a homogenous suspension with 60 ml distilled water for two minutes, this suspension was distributed over the plate which was air dried until the transparency of the layer disappeared. The plates were dried in hot air oven at 110°C for 30 mins and then stored in a dry atmosphere and used whenever required. Samples were prepared by diluting the crude extracts of hexane, chloroform, ethyl acetate, methanol and distilled water, with respective solvent and then applied usually 1-10µl volumes to the origins of a TLC plate 2cm above its bottom with the help of capillary tubes. After the application of the sample on the plate, the plates were kept in TLC glass chamber (solvent saturated) then mobile phase was allowed to move through adsorbent phase up to 3/4th of the plate. TLC was performed for alkaloids, flavanoids, tannins and phenols, solvent system (Table-2)

Assessment of antibacterial activity

Antibacterial activity of *T. cordifolia* stem extract was carried out against *Escherichia coli* and *Pseudomonas aeruginosa, accourdingto the* method of Wang *et al*., (2012)[20]. Aqueous, methanolic, ethyl acetate, chloroform and hexane extracts were used as test compound and ampicillin was used as reference standard. The Petri dishes were thoroughly washed and sterilized in hot air oven at 160ºC for one hour. The sterile plates were previously labelled with description and date. To each petri plate, 15 to 20 mL of liquefied sterilized nutrient agar was transferred and allowed to cool and set. The bacterial cultures were activated prior to the experiment. Soft agar containing target cultures (200 µL) were overlaid. The wells were made with the help of sterile well borer (6 mm). The test solution (0.1ml) was added to the respective bores .Finally, petri plates were kept for proper diffusion and overnight incubated at 37°C for 24 hours. At the end of the incubation period, zone of inhibition was observed and measured using a scale[21].

Results and Discussion

Extraction Value and Color of Plant Extract

The plant material was exhaustively extracted with each of Hexane, chloroform, ethyl acetate and methanol respectively using a Soxhlet continuous extraction apparatus for 1 week. The final extracts were concentrated and dried whose colour, nature and %age yield has been shown in Table-1.

Table -1 The percentage extractive values (w/w) along with color of the extract and nature of the residue is presented below

S .No.	Solvent	Colour of the extract	Natural of residue	Extraction value%(w/w)
1	Hexane	Green	Solid	8.3
2	Chloroform	Brown	Solid	25.23
3	Ethyl acetate	Brown	Semi solid	11.44
4	Methanol	Yellow	Solid	10.34
5	Distill water	Dark brown	Semi solid	11.50

The percentage extractive values (w/w) for Hexane, Chloroform, Ethyl acetate, Methanol and Distilled water was found to be 8.3, 25.23, 11.44, 10.34 and 11.50 respectively (Table-1)

Phytochemical Screening

Phytochemical screening of the sequential extract of *Tinospora cordifolia* revealed the presence of various bioactive components. The test for alkaloid has given positive result whereas saponin and protein test showed negative result for all the four extracts taken under study. Carbohydrates were present only in chloroform extract, amino acid in aqueous extract and flavonoid in ethyl acetate extract.

The result of phytochemical test is presented in Table -2.

Table-2 Shows qualitative phytochemical analysis of extracts of *Tinospora cordifolia*

Phytochemical Test	Hexane Extract	Chloroform Extract	Ethyl acetate Extract	Methanol Extract
Test for flavonoids				
Alkaline reagent test	+	-	+	-
Test for Alkaloids				
Mayer's test	+	+	+	+
Hager's test	+	+	+	+
Wager's test	+	+	+	+
Test for carbohydrates				
Fehling test	+	+	+	+
Molish test	-	+	-	+
Benedict's test	-	+	-	+
Test for phenolic compounds and tannins				
Dil. $Fecl_3$-test	+	+	-	+
Vanillin - HCl Test	_	_	+	+
Test for steroids				
Salkowiski test	-	-	+	+
Test for saponins				
Froth Test	-	-	-	-
Test for proteins				
Ninhydrin	-	-	-	-

(-) A sign indicates absence of constituent in the respective screening test;
(+) sign indicates the presence of a constituent in the respective screening test.

These results are in confirmation with earlier studies done for this plant[22,23]. Flavonoids have extensive biological properties that promote human health and help in reduction of risk of diseases due to their antioxidant, anticancer, anti-inflammatory and anti-microbial properties[24]. Tannins are basically cytotoxic agents. They act as free radical scavengers thus they can be useful in treatment of various degenerative diseases like cancer, atherosclerosis, and aging process[25]. Alkaloids are being used in life saving drugs for some critical disorders like cancer, heart failure, blood pressure due to their wide range of pharmacological activities[26]. Saponins have been considered as bioactive antibacterial agent but also act as anti-tumour agents by inducing apoptosis [27]. Preliminary screening of phytochemicals is a valuable step, in the detection of the bioactive principles present in medicinal plants and subsequently, may lead to drug discovery and development. From the above results, it can be noted that successful extraction of biologically active compounds from plant are largely dependent on the type of solvent used during extraction. In this study, different solventswere used. This study, therefore, validates the hypothesis that variations in solvents used will affect the presence of bioactive compounds of an extract[28].

Thin Layer Chromatographic studies (TLC)

Thin layer chromatography was carried out on silica gel G plates made manually in laboratory. The samples were loaded 2 cm above from the bottom of the plates with the help of micropipettes to uniformly apply the samples and allowed to dry. The plates were developed in a chromatography chamber using different solvent systems according to the extract.For detection of flavonoids solvent system consisted of ethyl acetate: formic acid: water (40: 5: 5) likewise for the detection of alkaloids the solvent system consisted of methanol: ammonia (20: 3) respectively. For the identification boric acid and oxalic acid mixture were used for flavonoids and Mayer's reagent was used.The plates were air dried and then kept in hot air oven at 100 °C for 5-6 minutes and then were observed and visualized under visible light followed by spraying with 10% H_2SO_4 and then again the plates were visualized under UV . The retention factor (Rf values) for each active compound was calculated .

Table - 3 R_f value of different extract of *Tinospora cordifolia* stems extract

S. No	*T.cordifolia* Extracts	Solvent System Used	No of spot	R_f value
1	Hexane	Chloroform:Methanol (20:7)	2	0.1,0.3
2	Chloroform	Toluene:Chloroform:Methanol(4.5:5:0.5)	4	0.1,0.2,0.4,0.7
3	Ethyl acetate	Cyclohexane:Formic acid (6:1)	3	0.2,0.6, 0.8
4	Methanol	Hexane: Ethyl acetate (9:1)	1	0.8
5	Distill water	Butanol:Aceticacid:Water (4:5:1)	1	0.4

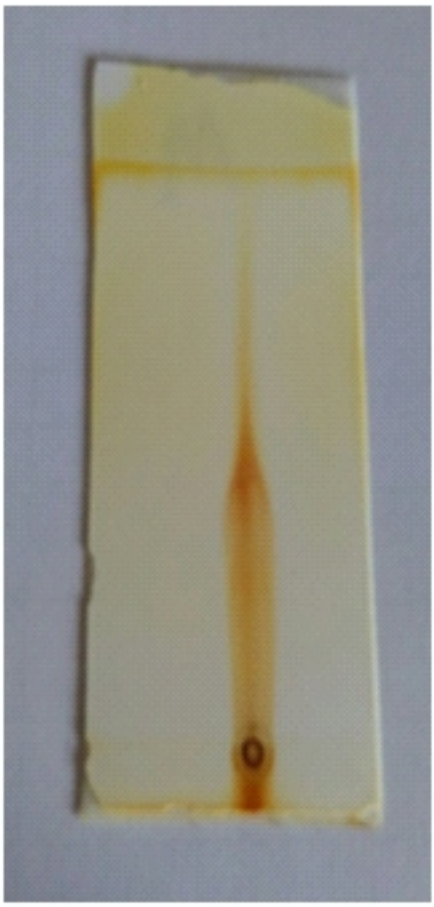


Figure-3
TLC of Methanol extract

Figure- 4
TLC Of chloroform extract

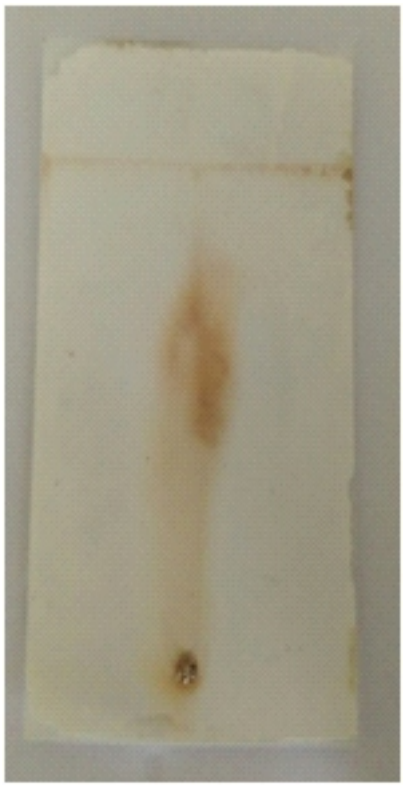


Figure-5
TLC of Ethyl acetate

Antibacterial activity of *T. cordifolia* was recorded against *E. coli* and *Pseudomonas aeruginosa*. All the four extracts showed antibacterial activity against the two target bacterial species under study, although to a varying degree, as shown in Table-4. The zone of inhibition also differed for the same extract against different bacterial species. The activity was measured in terms of zones of inhibition in diameter (mm) for methanolic, ethanolic, chloroform, hexane and acetone extracts of Stem tissue (Table-4).

The results revealed that the methanolic extract exhibit the effective antibacterial activity against the tested bacterial species.Antimicrobial activity of the *T. cordifolia* with different solvents on different micro-organism showed good antifungal and antibacterial activity[29]. Available reports tend to show that secondary metabolites such as alkaloids, flavonoids, tannins, and other compounds of phenolic nature are responsible for the antimicrobial activities in higher plants[30].

Table-4 Anti- microbial activity of stems extract of *Tinospora cordifolia* agains different bacteria strains:

Organism used		Zone of inhibition(mm)												
		Hexane Concentration			Chloroform Concentration			Ethyl acetate Concentration			Methanol Concentration			Ampicillin
		A	B	C	A	B	C	A	B	C	A	B	C	25(µg/ml)
1.	*E. coli*	8.7	10	11.6	8.5	9.1	10.3	10	12	13.6	14.2	15	17.5	25.8
2.	*Pseudomonas aeruginosa*	5	6.5	7.0	6	6.4	7.4	11	11.5	12	11.2	12.1	13	20.4

A: Dose 50 mg/ml B: Dose 75 mg/ml C: Dose 100 mg/ml

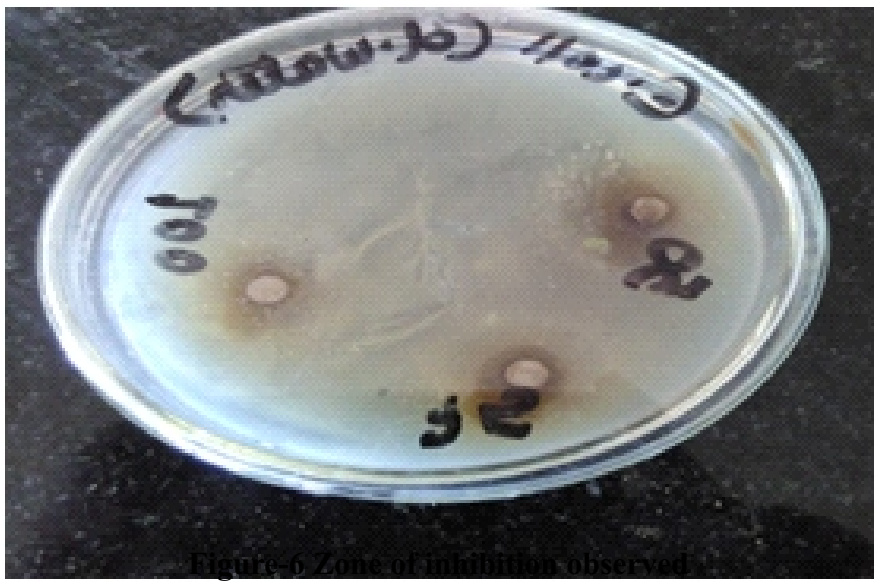


Figure-6 Zone of inhibition observed against *Escherichia coli*(Distil water extract)

Conclusion

Herbal drugs are an integral part of the Indian system of medicine (Ayurveda) which is an ancient and mainstream system. India has one of the richest plants medical traditions in the world. There are estimated to be around 25,000 effective plants based formulations, used in folk medicines and known to rural communities in India. Medicinal plants play a central role not only as traditional medicines, but also as trade commodities.

In the present work phytochemical and antimicrobial investigation of *Tinospora cordifolia* was performed. Successive solvent extraction was done using soxhlet. Preliminary phytochemical screening of *T. cordifolia* gave valuable information about the different phytoconstituents present in the plant. It showed the presence of alkaloids, carbohydrates, flavonoids, phenols, tannins and amino acids. This will help the future investigators concerning the selection of the particular extract for further investigation of isolating the active principles. It gave idea about different phytochemicals which have been found to possess a wide range of activities. *T. cordifolia* stem extracts exhibited marked dose dependent antimicrobial activity in vitro against the two bacteria *E. coli* and *Pseudomonas aeruginosa* which can be used as a good therapeutic approach for infectious disease management and therapy. Methanolic extract was found to be more potent against both the group of bacteria. *T. cordifolia stem* has shown different types of phytochemicals. Methanolic extract of *T. cordifolia* stem exhibited better antioxidant potential also. Further purification, quantification and antioxidant potential of the active compounds would be our priority in future studies. Both in vitro and in vivo studies are recommended for therapeutic applications in modern medicine.

Declaration

Current research work is not funded by any funding organisation/Agency.

Conflict of Interest

There is no any conflict of Interest among any author.

Acknowledgment

The authors are highly thankful to the Department of Biochemistry and Biotechnology, Sardar Bhagwan Singh University,Balawala, Dehradun(Uttarakhand) for providing all help and required facilities to carry out the research.

References

1. Pandey, M.; Debnath, M. and Gupta, S. Review on Phytomedicine: An ancient approach turning into future. Potential source of therapeutics. *Journal of Pharmacognosy and Phytotherapy,* 2011, 3(3):27-37.

2. Farnsworth, N.R. and Fabricant, D.S. The value of plants used in Traditional Medicine for Drug Discovery. *Environ Health Perspective,* 2001, 109(1):69-75.

3. Bannerman, R.; Burton, J. and Wen-Chieh, C. The role of traditional medicine in primary health care, in traditional medicine and health care coverage- A reader for health administrators and practitioners. The WHO, 1983, Geneva, Switzerland.

4 . Panchabhai, T.S.; Kulkarni, U.P. and Rege, N.N.Validation of therapeutic claims of *Tinospora cordifolia,* a review. *Phytotherapy Research,* 2008, 22: 425-441.

5. Patel, M.B. and Mishra, S. Hypoglycemic activity of alkaloidal fraction of *Tinospora cordifolia. Phytomedicine,* 2011,18:1045-1052.

6. Preeti, S. *Tinospora cordifolia* (Amrita)-a miracle herb and lifeline too many diseases. *International Journal of Medicinal and Aromatic Plants,* 2011,1(2):57-61.

7. Mishra, S.P. and Jain, A.P. Preliminary Phytochemical Screening and In *Vitro* Antioxidant Activity of Hydroalcoholic Extract of Tinospora Cordifolia. *International Journal of Pharmaceutics & Drug Research,* 2019, 7 (2): 53-59.

8. Hussain, L.; Muhammad, S.H.; Noor-Ul, A.; Rehman, K. and Ibrahim, Md. The Analgesic, Anti-Inflammatory and Anti Pyretic Activities of *Tinospora cordifolia*. *Adv. Clin Exp. Med.,* 2015, 24(6): 957-964.

9. Joseph, B.S.; Kumbhare, P.H. and Kale, M.C. Preliminary phytochemical screening of selected medicinal plants. *Int. Res. J. of Science & Engineering,* 2013, 1(2):55-62.

10. Singh, K.L. and Bag, G. Phytochemical analysis and determination of total phenolics contents in water extracts of three species of Hedychium. *International Journal of Pharm. Tech. Research,* 2013, 5(4):1516-21.

11. Bhandary, S.K.; Kumari, N.S.; Bhat,V.S.; Sharmila, K.P. and Bekal, M.P. Preliminary phytochemical screening of various extracts of Punicagranatum peels whole fruit and seed. *Nitte University Journal of Health and Science,* 2012, 2(4):34-38.

12. Abebe, H.; Gebre, T. and Haile, A. Phytochemical investigation on the roots of SolanumIncanum, Hadiya zone, Ethiopia. *Journal of medicinal plants studies,* 2014, 2(2):83-93.

13. Yadav, R.; Khare, R.K. and Singhal, A. Qualitative phytochemical screening of some selected medicinal plants of Shivpur District. *Int. J. Life. Sci. Scientifi. Res.,* 2017, 3(1): 844-7.

14. Shanthi, K. and Sengottuvel, R. Qualitative and quantitative phytochemical analysis of *Moringa-concanensis Nimbo*. *Int. J. Curr. Microbiol App. Sci.,* 2016, 5(1):633-40.

15. Tiwari ,P.; Kumar, B.; Kaur, M.; Kaur, G. and Kaur, H. Phytochemical screening and extraction: A review. *Internationale Pharmaceutica Sciencia,* 2011, 1(1):98-106.

16. Singh, S.; Khatoon, S.; Singh, H.; Behera, S.K.; Khare, P.B. and Rawat A.K. A report on pharmacognostical evaluation of four *Adiantum* species, Pteridophyta, for their authentication and quality control. Rev. Bras Farmacogn *Braz J. Pharmacogn.,* 2013;23(2) :207-216.

17. Arya, K.R.; Dubey, P. and Khatoon, S. Development of quality control parameters of ingredients of a folk remedy for piles and their comparative chemo profiling with homeopathic drugs. *JSIR.,* 2019, 68 :385-392.

18. Khatoon, S.; Singh, H. and Goel, A.K. Use of HPTLC to Establish the Chemotype of a Parasitic Plant, *Dendrophthoefalcata* (Linn.f.) Etting. (Loranthaceae), Growing on Different Substrates. *J. Planar Chromatogr.,* 2011, 24(1):60-65.

19. Biradar, R.S. and Rachetti, D.B. Extraction of some secondary metabolites & thin layer chromatography from different parts of Centella asiatica L. *American Journal of Life Sciences,* 2013, 1(6): 243-247.

20. Wang, S.Y.; Grant, I.R.; Friedman, M.; Elliot, C.T. and Situ, C. Antibacterial activities of naturally occurring compounds against Mycobacterium aviumsubsp. paratuberculosis. *Appl. Environ. Microbiol.,* 2008, 74(3):5986-90.

21. Bargah, R.K. Preliminary test of phytochemical screening of crude ethanolic and aqueous extract of Moringapterygosperma Gaertn. *Journal of pharmacognacy and phytochemistry,* 2015, 4(1): 7-9.

22. Singh, S.; Khatoon, S.; Singh, H.; Behera, S.K.; Khare, P.B. and Rawat A.K. A report on pharmacognostical evaluation of four Adiantum species, Pteridophyta, for their authentication and quality control, Rev. Bras Farmacogn *Braz J. Pharmacogn.,* 2013, 23(2) :207-216.

23. Arya, K.R.; Dubey, P. and Khatoon, S. Development of quality control parameters of ingredients of a folk remedy for piles and their comparative chemo profiling with homeopathic drugs, *JSIR.,* (2009), 68 :385-392.

24. Kumar, S. and Pander, A.K. Chemistry and biological activities of flavonoids: An overview. *Sci. World J.,* 2013, 1-16.

25. Chung, K.T.; Wong, T.Y.; Wei, C.I.; Huang, Y.W. and Lin, Y. Tannins and human health: A review. Crit. Rev. *Food Sci. Nutr.,* 1998, 38(6):421-464.

26. Kaur, R. and Arora, S. Alkaloids-Important therapeutic secondary metabolites of plant origin. *J Crit Rev.,* 2015, 2(3): 1-8.

27. Desai, S.D.; Desai, G.D. and Kaur, H. Saponins and their biological activities. *Pharma Times.* 2009, 41(3):13-16.

28. Nayak, S.S. And Singhari, A.K. Antimicrobial activity of roots of *Coculus hirsutus. Anc. Sci. Life,* 2003, 22(3):168-72.

29. Abhijeet, R. and Mokat, D. On vegetative propagation through stem cuttings in medicinally lucrative *Tinospora* species, *J. Pharmacogn. Phytochem.,* 2018, 5(2):2313-2318.

30. Mishra, A.; Kumar, S.; Bhargava, A.; Sharma, B. and Pandey, A.K. Studies on *in vitro* antioxidant and antistaphylococcal activities of some important medicinal plants. *Cellular and Molecular Biology*, 2011, 57(1):16-25.