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Research Article

Phytochemical and Biological Analysis of *Tinospora cordifolia*

Gagandeep Kaur[1], Pranav Kumar Prabhakar[2], Uma Ranjan Lal[3], Ashish Suttee[1*]

[1]*Department of Pharmacognosy and Phytochemistry.*
[2]*Department of Paramedical Sciences Lovely School of Pharmaceutical Sciences, Lovely Professional University, Punjab.*
[3]*BITS, Ranchi.*

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ABSTRACT
The aim of this work was to do phytochemical extraction and screening and also evaluation of their antibacterial and antioxidant properties for *Tinospora cordifolia*. The crude drug (Stem part) was successively extracted by Soxhlet assembly using various solvents. Preliminary phytochemical screening of different extracts was carried out using several colour and precipitate chemical reagents as per described methods. Gram +ve bacteria (*Staphylococcus aureus*) and Gram–ve bacteria (*Escherichia coli*) using the agar wells dilution method. Preliminary phytochemical screening of *T. cordifolia* showed showed the presence of carbohydrates, glycosides, flavonoids, phenols, tannins and amino acids in the crude drug. *T. cordifolia* stem extracts exhibited marked dose dependent antimicrobial activity *in vitro* against both gram positive and gram negative bacteria and 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. The *T. cordifolia* stem has shown different types of phytochemicals. Methanolic extract of *T. cordifolia* stem exhibited better antioxidant potential also. Further studies on isolation of phyto-constituents and both *in vitro* and *in vivo* evaluation of pharmacological activities of isolated bioactive constituents of the crude drug are recommended as future works.

Keyword: *Tinospora cordifolia;* phytochemicals; antibacterial; antioxidant; phenolics; alkaloids.

INTRODUCTION

Trees and plants are of paramount importance for human life, not only in the present time, but also in the remote past as well. The early man depended on them for his physical needs such as sources for food, shelter, clothing, medicine, ornaments, tools and for spiritual needs like magic or ritualistic practices. Medicinal plants are generally locally available which are 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[1]. The genus *Tinospora* has been widely investigated by a number of workers and reported to contain a number of phytochemicals with marked therapeutic activity. The plant family Menispermaceae consists of about 70 genera and 450 species that are found in tropical lowland regions. These are generally climbing or twining, rarely shrubs. Leaves are alternate or lobed, flowers, small chimes, seeds usually hooked or uniform. This family is a rich source of alkaloid and terpenes[2]. The therapeutic activity of the plant is mentioned both in Ayurveda and traditional system of medicines. The plant is distributed throughout the tropical region of India up to 1,200 m above sea level from Kumaon to Assam, in north extending through West Bengal, Bihar, Deccan, Konkan, Karnataka and Kerala. It is a fairly common plant of deciduous and dry forests, growing over hedges and small trees. It prefers wide range of soil, acid to alkaline and it needs moderate level of soil moisture[3]. The drug Guduchi or Amrita consists of dried pieces of mature stem of *Tinospora cordifolia.* Roots and leaves are also medicinal. It is one such plant which is widely used in Indian System of Medicine (ISM) and used in medicines since times immemorial[4]. In Hindi, the plant is commonly known as Giloya, which is a Hindu mythological term that *refers to the heavenly elixir that have saved celestial beings from old age and kept them eternally young*[4]. The starch obtained from the stem known as "Guduchi-satva" is highly nutritive, digestive and used in many diseases. During the last two decades, the drug has been subjected to extensive phytochemical, pharmacological and clinical investigation. It is widely used in folk and Ayurvedic systems of medicine. The term Amrita is attributed to its ability to impart youthfulness, vitality and longevity to the consumer. Guduchi is widely used in Ayurvedic system of medicine "*Rasayanas*" to the immune system and body resistance against infections. In modern medicine it is used for general weakness[5]. According to Patanjali yogapith this plant is very effective in preventing swine flu that has been declared epidemic worldwide. There are a number of reports available for the use of these plants as Antiallergic, Anti-Inflammatory and Immunosuppressive, Immunomodulatory, Anticancer, Hypoglycemic, Antihelminthic, and many others[6-14]. A variety of constituents have been isolated from this plant. The

**Author for Correspondence*

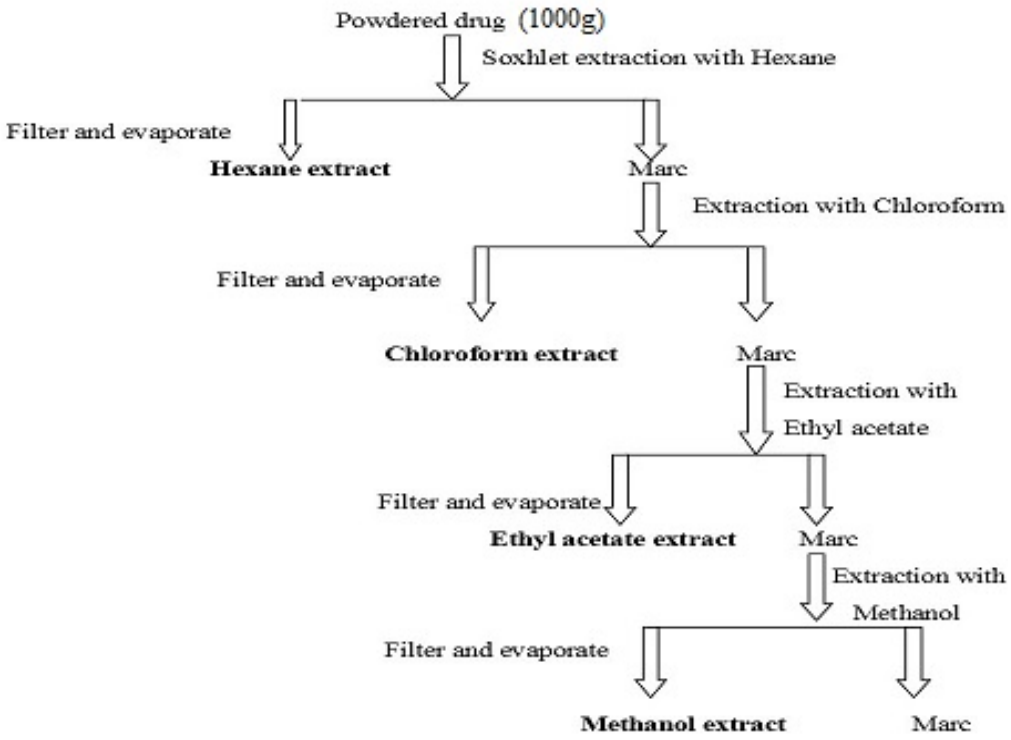


Figure 1: Scheme of extraction[15].

Table 1: Color, nature and percent yield of *Tinospora cordifolia*.

S. No.	Extraction	Solvent	Color	Nature	%yield (w/w)
1.	Sequential	Hexane	Dark brown	Semisolid	0.62
2.		Chloroform	Yellowish green	Solid	1.5
3.		Ethyl acetate	Dark brown	Semisolid	0.58
4.		Methanol	Dark brown	Semisolid	0.68

chemical constituents reported from the shrub belong to different classes such as alkaloids, glycosides, diterpenoid lactones, steroids, sesquiterpenoid, phenolics, aliphatic and polysaccharides. The leaves of this plant are rich in protein (11.2%) and are fairly rich in calcium and phosphorus.

MATERIAL AND METHODS

The dried stems of Tinospora cordifolia were purchased from the local market of Amritsar. The plant materials were dried under shade and ground to a coarse powder. All chemicals, reagents and solvents used in quantitative analysis and chemical investigation were of analytical grade and Lab grade procured from E. Merck, SD Fine and CDH Chemicals.

Microbial strains used in this work were procured from (namely *Streptococcus aureus* and *E. coli*) IMTECH, Chandigarh, India. Bacteria were sub-cultured from the stock maintained in nutrient agar at 37ºC.The bacterial strains were grown on MacConkey agar plates at 37º C and maintained on nutrient agar slants.

Extraction of plant material

The plant material (1000g) was exhaustively extracted with 2.5 L 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[15-21] (figure 1).

Chromatographies studies

Samples for the TLC study were prepared by dissolving completely dried ethyl acetate and methanol extract in small quantity of methanol[22]. TLC plate pre-coated with silica gel 60 F_{254} thickness 0.2 mm was used as the stationary phase. The mobile phase i.e Ethyl acetate: Acetic acid: Formic acid: Water (10:1.1:1.1:2.3), was saturated for 30 minutes in a TLC chamber. After activation of TLC plate, the sample was spotted using capillary tube and the plate was dried for few min. Then the plate was kept in the TLC chamber containing saturated mobile phase and allowed to run up to three forth of the plate. After development, plate was removed and air dried. The plate was examined with the help of nonspecific and specific chromatographic methods and the Rf value was calculated[16-21,25].

Quantitative chemical examination of extracts (Preliminary phytochemical evaluation)

Different extracts of plant material obtained were subjected to various chemical tests to detect the chemical constituents present in them.

Detection of alkaloids

Extracts were dissolved individually in dilute

Table 2: Qualitative chemical examination of extracts.

S. No	Chemical Constituents	Tests	Hexane Extract	$CHCl_3$ Extract	EtOAc Extract	MeOH Extract
1.	Alkaloids	Mayer's test	+	+	+	+
		Wagner's test	+	+	+	+
		Dragendorff's	+	+	+	+
		Hager's test	+	+	+	+
2.	Carbohydrates	Molisch's test	+	-	+	+
		Benedict's	+	-	+	+
		Fehling's test	+	+	+	+
3.	Glycosides	Modified Borntrager's	+	+	+	+
		Legal's test	-	-	+	+
4.	Saponins	Froth test	-	+	+	+
5.	Phytosterol	Salkowski's	-	-	+	-
6.	Resins	Acetone-water	+	+	+	+
7.	Phenols	Ferric chloride	+	+	+	+
8.	Fixed oil	Stain test	+	-	+	+
9.	Tannins	Ferric chloride	+	+	-	+
10.	Diterpenes	Copper acetate test	+	+	+	+
11.	Protein and amino acid	Xanthoprotectic test	-	-	-	+
		Ninhydrin test	+	-	+	+
12.	Flavonoids	Alkaline reagent	+	+	+	+
		Lead acetate	-	-	+	+
		Zinc Hcl test	-	+	-	-

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

hydrochloric acid and filtered. The filtrates were used to test for the presence of alkaloids. The alkaloid detection was performed by different tests like Mayer's test (Filtrates were treated with Mayer's reagent (saturated solution of potassium mercuric iodide) and formation of a yellow cream precipitate indicates the presence of alkaloids), Wagner's reagent (Filtrates were treated with Wagner' reagent (saturated solution of iodine in potassium iodide) and formation of brown/reddish brown precipitate indicates the presence of alkaloids), Dragendorff's reagent (Filtrates were treated with Dragendorff's reagent (saturated solution of potassium bismuth iodide) and formation of a red precipitate indicates the presence of alkaloids) and Hager's reagent (Filtrates were treated with Hager's reagent (saturated solution of picric acid) and formation of a yellow colored precipitate indicates the presence of alkaloids).

Detection of flavonoids

Alkaline reagent test

Extracts were treated with a few drops of sodium hydroxide solution. Formation of intense yellow color, which become colorless on the addition of dilute acid, indicates the presence of flavonoids.

Lead acetate test

Extracts were treated with a few drops of lead acetate solution. Formation of yellow colored precipitates indicates the presence of flavonoids.

Zinc- hydrochloric acid reduction test

To the crude extracts, a pinch of zinc dust and small amount of concentrated hydrochloric acid were added. Appearance of magenta color after few minutes indicates the presence of flavonoids.

Detection of carbohydrates

Extracts were dissolved individually in 5ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates via various qualitative tests like Molisch test, Benedict test, Fehling's test according to the standard principle.

Detection of glycosides

Extracts were hydrolyzed with dilute hydrochloric acid and then were subjected to tests for glycosides by different tests such as Modified Borntrager's test and Legal's test.

Detection of saponins

The saponins were identified by Froth test. Extracts were diluted with distilled water to 20ml and were shaken in graduate cylinders for 15 min. Formation of foam of height of 1 cm indicates the presence of saponins.

Detection of fixed oils & fats (Stain test)

Small quantities of extracts were pressed between two filter papers. An oily stain on filter paper indicates the presence of fixed oil.

Detection of phenols (Ferric chloride test)

Extracts were treated with a few drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

Detection of tannins (Gelatin test)

Gelatin solution (1%) containing sodium chloride was added to the extracts. Formation of white precipitates indicates the presence of tannins.

Detection of proteins and amino acids

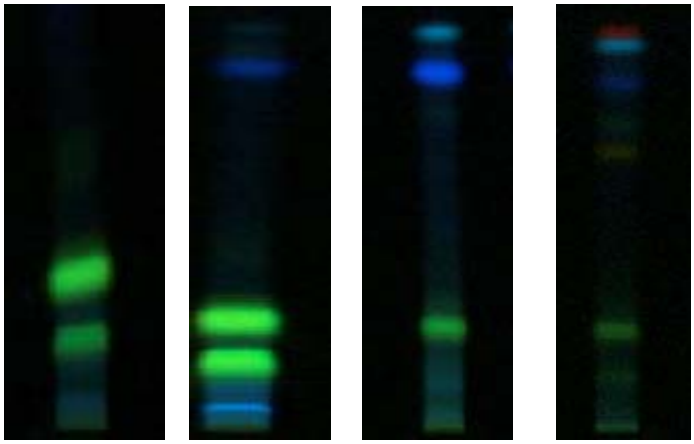
The amino acids, protein and peptides, and aromatic amino acids were detected by the Ninhydrine, Burt and Xanthoproteic test through standard procedure.

Detection of resins (Acetone-water test)

Extracts were treated with acetone. Small amount of

Table 3: TLC with a solvent system (Ethyl acetate: acetic acid: formic acid: water 10:1.1:1.1:2.3) (366 nm): Derivatising agent-Natural product – polyethylene glycol reagent (NP-PEG reagent).

S. No.	Extract	The distance travelled by solute (cm)	The distance travelled by solute (cm)	R_f value	Color of spot
1.	Hexane	10.3	1.5	0.14	Light blue
			3.5	0.33	Light green
			5.3	0.51	Dark green
2.	Chloroform	10.5	2	0.19	Light blue
			2.5	0.23	Blue
			3.4	0.32	Light green
			4.7	0.44	Dark green
			7.3	0.69	Light green
			7.8	0.74	Blue
			8.2	0.78	Light blue
3.	Ethyl acetate	10.7	0.7	0.06	Green
			1.2	0.11	Green
			4.5	0.42	Yellowish green
			5.7	0.53	Green
			7.3	0.70	Light green
			8.1	0.75	Blue
			8.9	0.81	Light Blue
4.	Methanol	10.5	0.5	0.05	Green
			1	0.1	Light green
			3.2	0.3	Green
			5.8	0.55	Light yellow
			7.6	0.72	Yellow
			8	0.76	Light blue
			8.7	0.83	Blue
			9.2	0.87	Light red



Hexane Chloroform Ethyl acetate Methanol

Figure 2: TLC plates of solvent system were dipped in NP reagent.

water was added and shaken. The appearance of turbidity indicates the presence of resins.

Detection of diterpenes (Copper acetate test)

Extracts were dissolved in water and treated with a few drops of copper acetate solution. Formation of emerald color indicates presence of diterpenes.

Detection of phytosterol (Salkowski's Test)

Extracts were treated with chloroform and filtered. The filtrates were treated with a few drops of conc. Sulfuric acid, shaken and allowed to stand. Appearance of golden yellow color indicates the presence of triterpenes[16-21,27].

Antibacterial Activity

Preliminary screening was carried out for antibacterial activity and the doses were decided on screening results. The Petri dishes were thoroughly washed and sterilized in hot air oven at 160ºC for one hour. 30 ml of sterile nutrient agar medium was poured into sterile Petri dishes for solidifying. Bores were made on the medium using sterile borer. The test solution (0.1ml) was added to the respective bores and the Ampicillin (0.1ml) at a concentration of 25 µg/ml was taken as a standard reference. The Petri dishes were kept in the refrigerator at 4ºC for 45 min. For diffusion to take place. After diffusion, the Petri dishes were incubated at 37ºC for 24

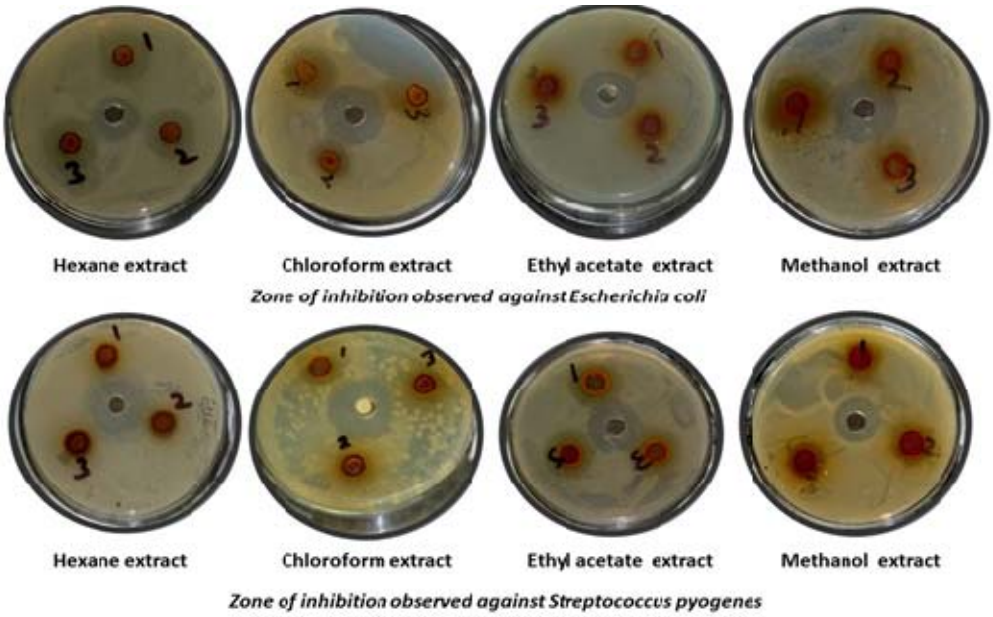


Figure 3: Showing zone of inhibition after the various treatment with plant's extracts.

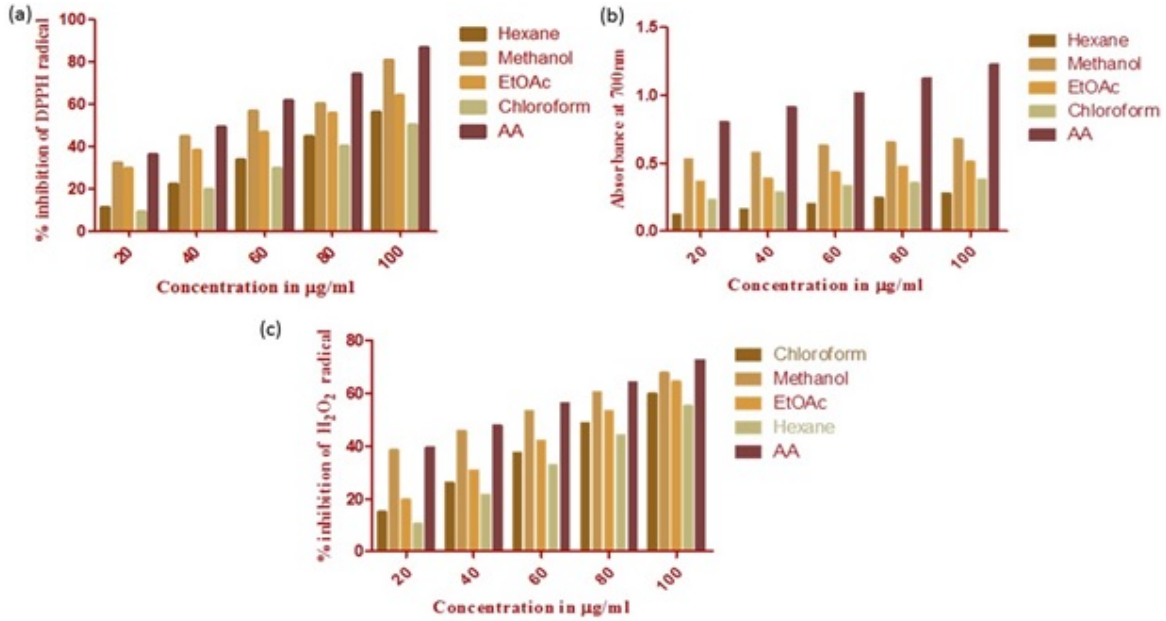


Figure 4: Different plants extracts and their mechanism of free radical chelation. (a) DPPH dew radical scavenging assay (b) Reducing power assay (c) H2O2 scavenging assay.

hours and zone of inhibition was observed and measured using a scale. Antibacterial activity of all the extracts was carried out against two microorganisms (*E. Coli, Staphylococcus aureus*). The same media was used both for subculturing and for antibacterial activity. All the reading was taken in triplicate and Mean±SEM was calculated[17,21,23].

Determination of TPC

The total phenolic content of the extracts was measured by the modified Folin- Ciocalteu method. An aliquot of the extract was mixed with the 5ml of Folin-Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4ml (75g/l) of sodium carbonate. The tubes were vortexed for 15 Sec. And allowed to stand for 30 min at 40ºC for color development. Absorbance was then measured at 765 nm using UV-VIS Spectrophotometer. Total phenolic content was measured as µg/g Tannic acid equivalent[17,21,24].

Determination of Flavonoid Content

The total soluble flavonoid content was estimated by aluminium chloride colorimetric method for extracts. 0.5 ml of stock solution (1g/ml of extract), 1.5 ml of methanol, 0.1 ml of potassium acetate (1M) was added to

Table 4: Evaluation of antibacterial activity of different extracts of *Tinospora cordifolia.*

Treatment	Dose (mg/ml)	Zone of inhibition(mm)	(Mean±SEM)
		Gram -ve bacteria	Gram +ve bacteria
		Escherichia coli	*Staphylococcus aureus*
Hexane	100	11.6±0.62*	9.6±0.99**
	150	12.6±0.81**	11.6±0.37*
	200	15.6±0.57**	13±0.53**
Chloroform	100	10.3±0.68*	10±0.32*
	150	13.6±0.32**	11.6±0.38*
	200	14.6±0.46**	13.3±0.39**
Ethyl acetate	100	13.6±0.57**	10.6±0.56*
	150	17.3±0.33**	13.6±0.46**
	200	19.6±0.45***	15.3±0.20***
Methanol	100	17.6±0.56**	13.3±0.23**
	150	19.3±0.36**	15±0.52**
	200	22.6±0.57***	18.3±0.33***
Ampicillin	25(µg/ml)	25.8±0.20***	22.6±0.95***

**p<0.01 each value represent Mean±SEM for n=3. Oneway ANOVA followed by Dunnet test using Instat software, compare all extract vs. standard applied. Statistically significant at

Table 5: TPC.

S. No.	Extract	Absorbance (756 nm)	Total phenolic content (µg/g equivalent of tannic acid)
1.	Hexane	0.19 ±0.035	4.8
2.	Chloroform	0.25 ± 0.025	6
3.	Ethyl acetate	0.32 ± 0.020	9.8
4.	Methanol	0.43 ± 0.030	7.6

reaction tubes and volume was made up to 5ml with distilled water. After incubation at room temperature for 30 min., The absorbance was measured at 415 NM. Total flavonoid content was calculated by extrapolating the observance of reaction mixture on the standard curve of quercetin. The experiment was repeated and the total flavonoid content was expressed as equivalent to quercetin in mg/g of the extract[17,21,26].

Determination of Antioxidant Activity

DPPH radical scavenging activity

The free radical scavenging activity of *Tinospora cordifolia* was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH). Briefly, 0.1mM solution of DPPH in methanol was prepared. Then, 1ml of this solution was added to 3ml of each extract of *Tinospora cordifolia* i.e hexane, chloroform, ethyl acetate and methanol solution at different doses (20-100µg). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm in a spectrophotometer. The lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The DPPH radical concentration was calculated using the following equation:

DPPH scavenging effect *(%)* = $A_c - A_t/A_c \times 100$

Where A_c was the absorbance of the control reaction and A_t was the absorbance in the presence of sample of *Tinospora cordifoliaI*[17,21,26].

Determination of Reducing Power

Reducing power of different extracts of *Tinospora cordifolia* was determined by the following procedure. Various concentrations of extracts were prepared in 1ml of methyl alcohol and was further mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) followed by 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50ºC for 20 mins, and then 2.5 ml 0f trichloroacetic acid (10%) was added to the mixture, which was then centrifuged for 10 mins. at 3000 rpm. The upper layer of the solution was mixed with 3 ml of distilled water and $FeCl_3$ (0.5 ml, 0.1%). The absorbance was measured at 700 nm. The higher absorbance indicates a higher reduction capability. Ascorbic acid was used as a standard[17,21,26].

Hydrogen Peroxide Scavenging Assay

The ability of the extracts to scavenge hydrogen peroxide was determined by little modification here the solution of hydrogen peroxide (100Mm) was prepared instead of 40 Mm in phosphate buffer saline of (PH 7.4), at various concentrations of hexane, chloroform, ethyl acetate and methanol extract (50-500 µg/ml) added to a hydrogen peroxide solution (2ml). Absorbance of hydrogen peroxide at 230 nm was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide. For each concentration, a separate blank sample was used for background subtraction. In case of control takes absorbance of hydrogen peroxide at 230 nm without sample extracts. The percentage inhibition activity was calculated using the following equation:

H_2O_2 scavenging effect (%) = $[(A_0-A_1)/A_0] \times 100$

Where A_0 is the absorbance of the control and A_1 is the absorbance of extract/standard taken as tannic acid[17,21,26].

RESULTS AND DISCUSSION

Extraction

The plant material (1000g) was exhaustively extracted with 2.5 L each of Hexane, chloroform, ethyl acetate and methanol respectively using a Soxhlet continuous extraction apparatus for 1 week. The final extracts were

Table 6: Flavonoid content.

S. No.	Extract	Absorbance (415 nm)	Total flavonoid content (µg/g) equivalent of Quercetin)
1.	Hexane	0.18 ± 0.04	3.6
2.	Chloroform	0.26 ± 0.02	5.6
3.	Ethyl acetate	0.30 ± 0.02	6.7
4.	Methanol	0.45 ± 0.05	10.8

concentrated and dried whose colour, nature and %age yield has been shown in table 1.

Phytochemical investigation

Phytochemical investigation covers the identification and characterization of crude drugs with respect to phytochemical constituents. The plant was evaluated for their chemical constituents. The results for the different types of phytochemicals presence are shown below in table 2.

TLC profile of Tinospora cordifolia

TLC profile of Tinospora cordifolia was done inthesolvent system of (Ethyl acetate: acetic acid: formic acid: water 10 : 1.1 : 1.1 : 2.3). Detection of spots was done in visible light as well as by derivatising agent i.e NP reagent at 366 nm. In the solvent system, three spots were detected in developed TLC of hexane extract in the range of 0.14-0.51, seven spots of chloroform extract in the range of 0.19-0.78, seven spots of ethyl acetate extract in the range of 0.06-0.81 and eight spots of methanol extract in the range of 0.05-0.87 (table 3, figure 2).

Antibacterial activity

The antibacterial activity of different extracts of Tinospora *cordifolia* was studied by cup-plate method. Antibacterial activity of Tinospora *cordifolia* was performed against one gram positive (*Staphylococcus aureus*) and one gram negative (*Escherichia coli*) bacteria.

Determination of TPC

The total phenolic content in the hexane, chloroform, ethyl acetate and methanol extracts of *Tinospora cordifolia* was calculated using standard plot of tannic acid. Phenols are very important plant constituent. There is highly positive relationship between total phenols and antioxidant activity of many plant species, because of the scavenging ability of their hydroxyl groups. It was also reported that phenolic compounds are effective hydrogen donors, making them very good antioxidant.

Determination of flavonoid content

The total flavonoid content in hexane, chloroform, ethyl acetate and methanol extracts of *Tinospora cordifolia* was calculated using quercetin as a standard. Flavonoids act as scavengers of various oxidizing species i.e super oxide anions, hydroxyl radical or peroxy radicals. They also act as quenchers of singlet oxygen.

Antioxidant activity

DPPH Radical scavenging activity

DPPH has been widely used to evaluate the free radical scavenging effectiveness of various antioxidant substances. In the DPPH assay, the antioxidants were able to reduce the stable radical DPPH to the yellow colored diphenyl-picrylhydrazine. The method is based on the reduction of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH–H by the reaction. DPPH is usually used as a reagent to evaluate free radical scavenging activity of antioxidants. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. With this method it was possible to determine the antiradical power of an antioxidant by measuring of a decrease in the absorbance of DPPH· at 517 nm. Resulting a colour change from purple to yellow, the absorbance decreased when the DPPH· was scavenged by an antioxidant through donation of hydrogen to form a stable DPPH (figure 4a)· molecule. In the radical form, this molecule had an absorbance at 517 nm which disappeared after acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule.

Reducing power

In this assay, the yellow colour of the test solution changes to various shades of green and blue depending on the reducing power of each compound. Presence of reducers causes the conversion of the Fe^{3+}/ferricyanide complex used in this method to the ferrous form. By measuring the formation of Pearl's Prussian blue at 700nm, it is possible to determine the concentration of Fe^{3+} ion. Figure. 4b. showing the reducing power of hexane, methanol, ethanol, chloroform and standard ascorbic. The reducing power of all the extracts increased with increase in concentration.

Hydrogen peroxide radical scavenging power

Hydrogen peroxide is generated in vivo by several oxidase enzymes and by activated phagocytes and it is known to play an important role in the killing of several bacterial and fungal strains. There is increasing evidence that, hydrogen peroxide, either directly or indirectly via its reduction product, OH can act as a messenger molecule in the synthesis and activation of several inflammatory mediators. When a scavenger is incubated with H_2O_2 using a peroxidase assay system, the loss of H_2O_2 can be measured (figure 4c).

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 plant based formulations, used in folk medicine 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 Pharmacological and Phytochemical investigation of *Tinospora cordifolia* was performed. The plant species were standardized and compared as per WHO guidelines. Successive solvent extraction was done using soxhlet. The deterioration time of plant material depends upon the amount of water present in the plant material. If the water content is high, the plant can be easily deteriorated due to fungus. The loss on drying at105ºC in the stem was found

to be 48.5mg/g. Preliminary phytochemical screening of *T. cordifolia* showed the presence of carbohydrates, glycosides, flavonoids, phenols, tannins and amino acids in the crude drug. *T. cordifolia* stem extracts exhibited marked dose dependent antimicrobial activity *in vitro* against both gram positive and gram negative bacteria and 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. The *T. cordifolia* stem has shown different types of phytochemicals. Methanolic extract of *T. cordifolia* stem exhibited better antioxidant potential also. Further studies on the isolation of Phyto-constituents and both *in vitro* and *in vivo* evaluation of pharmacological activities of isolated bioactive constituents of the crude drug are recommended as future works.

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