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ORIGINAL ARTICLE

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Qualitative and Quantitative Phytochemical Screening of *Tinospora cordifolia* (Willd.)

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Background: *T. cordifolia* contains a lot of secondary metabolites which are produced from various parts of the plant. They have their specificity in many diseases to cure. By considering the importance of *T. cordifolia* as medicinal plant to investigate the phytochemical properties.

Results: The preliminary phytochemical study was carried out to identify the presence of different phytochemicals and the results revealed the presence of Carbohydrates, Glycosides, Proteins, amino acids, Alkaloids, Flavonoids, Phenolic compounds, Tannins, Saponins, steroids and triterpenoids. When we compare the presence of phytocompounds in different solvents, methanol and aqueous extracts revealed the presence of more phytoconstituents followed by petroleum ether and chloroform solvents. The quantitative analysis of phytochemicals such as Flavonoids, phenols, alkaloids, tannins and saponins were also estimated from methanol extract by various methods. Total phenolic content of methanolic extract of the *T. cordifolia* was found to be 6.53mg/g. Total flavonoid content was found to be 4.07mg/g, alkaloid content was 5.62mg/g and the tannin content was found to be 5.83 mg/g. Saponin content was 5.02mg/g. The results revealed that the phenol content was more in stem extract of *T. cordifolia* and it was followed by tannins, alkaloids, flavonoids, and saponins. FT-IR analysis was performed to identify the various functional groups of the biomolecules found in the methanolic stem extract of the plant.

Conclusions: The results of present study revealed the presence of medicinally important constituents in *Tinospora cordifolia*. Traditional medicine practice is recommended strongly that further work should be carried out to isolate, purify and characterize the bioactive compounds responsible for the activity of these plants.

Key words: Tinospora cordifolia, Stem, Methanol extract, Phytochemical properties, FT-IR analysis

Phytochemicals, also known as secondary metabolites, are compounds that plants produce primarily to ensure their survival. Though not directly involved in plant growth, these compounds perform a variety of important functions. In human life, these compounds, particularly essential oils, are used as medicines, flavourings, or relaxing drugs (Justin *et al*., 2014). *Tinospora cordifolia* (Willd.) is a widely available and important medicinal plant that is widely used in the ayurvedic system of medicine for its adaptogenic and rejuvenating properties. It is also known as "Guduchi" (Singh, 2008) and, more commonly, "Giloya," a Hindu mythical word that means the divine concoction that prevents Godly spirits from growing old and keeps them young forever (Nadkarni *et al*., 1954). The entire plant contains a diverse range of chemical constituents, as well as a pharmaceutical approach to various ailments. The plant is used in ayurvedic medicine, "Rasayan," to boost the immune system and the body's resistance to infections (Abhimanyu *et al*., 2010). *T. cordifolia* contains numerous secondary metabolites derived from various parts of the plant. They specialise in curing a wide range of diseases.

This plant's bitter principle has antiperiodic, antispasmodic, antiinflammatory, immunomodulatory, antitumor, cognition, antineoplastic, antihyperglycemia, antihyperlipidemia, antioxidant, antituberculosis, antiosteoporotic, antiangiogenic, anti-malarial, and antipyretic properties (Nasreen *et al*., 2010; Mukheshwar *et al*., 2012). Considering the importance of *T. cordifolia* as a medicinal plant, the phytochemical screening was conducted.

MATERIALS AND METHODS

Plant material

The study was carried out on *Tinospora cordifolia* (Willd.) commonly known as Guduchi, Giloya and Amrita. It is widely distributed throughout India, especially in the tropical parts ascending to an altitude of 300 m. and also in certain parts of China. Besides India and China, it is also distributed in Burma and Sri Lanka, ascending to an altitude of 1200 m.

Collection and preparation of plant material

T. cordifolia was collected from pachaimalai hills in Tiruchirappalli District, Tamil Nadu, and India in the month of February. The collected plants were washed with water to remove dust. The plant material (stem) was dried in shade for four to five days and chopped into small pieces. Then it was pulverized into coarse powder. The powder was used for the extraction of active principles.

Extraction of plant

The maceration extraction method was used to extract plant material. The dried powders were first soaked separately in aqueous (1:4 w/v) for 24 hours at room temperature. Suction filtration was used to filter the extracts through Whatmann no.1 filter paper. This was repeated for two more days, and similar extracts were pooled together, concentrated, and vacuum evaporated using a 40°C rotary evaporator. Similarly, petroleum ether, methanol, and chloroform were used in the extraction process. The dried extracts were either dissolved in the appropriate solvents or refrigerated until use.

QUALITATIVE PHYTOCHEMICAL STUDIES

The extracts obtained from successive solvent extraction (Aqueous, Petroleum ether, methanol and chloroform) were then subjected to various qualitative chemical tests to determine the presence of various phytoconstituents like carbohydrates, glycosides, proteins, amino acids, alkaloids, phenolics compounds, tannins, saponins, steroids and triterpenoids using reported methods. (Harborne, 1984; Khandelwal *et al*., 2006; Evans, 2009)

QUANTITATIVE PHYTOCHEMICAL STUDIES

Estimation of alkaloids (Indian Pharmacopoeia, 2010)

5 grams of extract were weighed and placed in a 250ml beaker. In the same beaker, an aliquot of 8ml of 20% acetic acid and 32ml of ethanol was added, covered, and left to stand for 4 hours. The extract was filtered after 4 hours, and the filtrate was concentrated to one-quarter of its original volume using a water bath. A few ml of concentrated ammonium hydroxide was added to the concentrate until the precipitation was complete.

The entire solution was provided time to settle. Filtration was used to collect and weigh the precipitate.

Estimation of total flavonoids

Using the aluminium chloride method, the total flavonoid content of plant extracts was calculated. One ml of extract was combined with 4 ml of de-ionized water and 0.3 ml of 10% sodium nitrate. The solution is allowed to sit for 5 minutes. 0.3ml of 10% aluminium chloride solution was added to this solution. Allow the solution to stand for one minute. An aliquot of 2ml of 1M sodium hydroxide solution was added to this. The test tube was shaken vigorously to thoroughly mix the reagents before being left to stand for some time. The optical density (OD) was measured at 510nm. For the preparation of the standard curve, 80% methanol was used as a blank and Rutin as a standard. The total flavonoid content of extracts was determined using the standard curve. The total flavonoid content was expressed as mg/g of the extract (Hossain and Nagooru, 2011).

Estimation of phenol

One ml of extract was taken in a test tube to which 1ml of Folin phenol reagent was added and 2ml of 20% sodium carbonate solution was also added. One ml of 80% methanol was used as a blank and all the above reagents were added to it. One ml of Catechol solution was used as a standard for the preparation of standard curve. The test tubes are shaken well and heated in a boiling water bath for 1 minute. After cooling, the solution turns blue and it was diluted to 25ml with distilled water. The absorbency of the solution was read at 650nm against the solvent blank of 80% methanol. (Bray & Thorpe, 1954).

Estimation of Tannins

The tannins were measured using the Folin-Ciocalteu method. A volumetric flask (10 ml) was filled with 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent, 1 ml of 35% sodium carbonate solution, and diluted to 10 ml with distilled water. The mixture was thoroughly shaken and left at room temperature for 30 minutes. Tannic acid reference standard solutions (20, 40, 60, 80, and 100 g/ ml) were prepared in the same manner as previously described. An UV/ Visible spectrophotometer was used to measure the absorbance of test and standard solutions against a blank at 700 nm. The estimation of the tannin content was carried out in triplicate. The tannin content was specified in terms of mg of tannic acid equivalents/ g of dried sample (Govindappa *et al*., 2011).

Estimation of Saponins

In a 250 ml conical flask, 100 ml of 20% aqueous ethanol was added to five grams of each powder sample. At 55°C, the mixture was heated in a hot water bath for 4 hours with continuous stirring. After filtration, the mixture residue was re extracted with another 100 ml of 20% aqueous ethanol and heated for 4 hours at a constant temperature of 55°C with constant stirring. The combined extract was evaporated to 40 mL over a 90°C water bath. In a 250 ml separator funnel, 20 ml of diethyl ether was added and vigorously agitated to recover the aqueous layer while discarding the ether layer. This purification procedure was carried out twice. 60 ml of n-butanol was added and extracted twice with 10 ml of 5% sodium chloride. After discarding the sodium chloride layer the remaining solution was heated in a water bath for 30 minutes, after which the solution was transferred into a crucible and was dried in an oven to a constant weight. (Obadoni & Ochuko, 2002; Ejikeme *et al*., 2014).

EVALUATION OF IR SPECTRUM

Dried powder of extracts of the plant materials were used for FTIR analysis. 10 mg of the dried extract powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample was loaded in FTIR spectroscope (PERKIN ELMER, IR), with a Scan range from 500 to 4000 cm^{-1} with a resolution of 4 cm^{-1}.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of the *T. cordifolia* stem extract of different solvents (Aqueous, Petroleum ether, methanol and Chloroform) presented in Table 1. The phytochemical analysis of the *T. cordifolia* extract was analysed for the compounds such as Carbohydrates, Glycosides, Proteins, amino acids, Alkaloids, Flavonoids, Phenolic compounds, Tannins, Saponins, steroids and triterpenoids. The preliminary phytochemical analysis of aqueous extract revealed the

presence seven compounds i.e. Carbohydrates, Glycosides, Alkaloids, Proteins, and amino acids, flavonoids, and Phenolic compounds. The absence of tannins, saponins and steroids. Petroleum ether extract revealed the presence of 6 compounds i.e. glycosides, proteins, amino acids, flavonoids, phenolic compounds, steroids and triterpenoids. Preliminary phytochemical analysis of methanol extracts revealed the presence of all the test compounds. Chloroform extracts revealed the presence of 5 compounds respectively. As a result the phytochemical analysis of solvents methanol revealed the presence of more phytoconstituents followed by aqueous, petroleum ether and chloroform solvents. (Fig. 1). Similar results were reported by Nagaprashanthi *et al*., (2012) and Sivakumar *et al*., (2010) but Venkanna *et al*., (2012) reported that presence of all these secondary metabolites in the aqueous extract of *T. cordifolia* also. Dwivedi *et al*., (2014) reported that the methanolic extract of *T. cordifolia* showed the presence of all secondary metabolites except anthraquinones. Mishra *et al*., (2013) reported positive tests for terpenoids. Similar experiments were carried out by Grover *et al*., (2013) on the stem part of the *T. cordifolia*. The extractions of any crude with a particular solvent yield a solution contains different phytoconstituents. The composition of these phytoconstituents in the particular solvent can be the means of providing preliminary information on the quality of a particular drug sample. The water soluble extract values play an important role for the evaluation of crude drugs. It can be used to indicate poor quality, adulteration with any unwanted material or incorrect processing of the crude drug during the drying, storage etc. Phytochemical testing of plant is very useful for determination of the active constituents in different solvents and their yields. Most of the phytochemicals are found in alcoholic and aqueous extracts. Methanol is known to be one of the best solvents for extracting compounds such as phenolics and other polar materials in plants (Velioglu *et al*., 1998)

Quantitative phytochemical analysis of methanolic extract of the *T. cordifolia* represented in Table 2. Methanolic extract of the *T. cordifolia* contains all the tested phytoconstituents than the other solvents. Hence we select this extract for quantitative phytochemical studies. Total phenolic content of Methanolic extract of the *T. cordifolia* was found to be 6.53mg/g. Total flavonoid content was found to be 4.07mg/g, alkaloid content was 5.62mg/g and the tannin content was found to be 5.83 mg/g. Saponin content was 5.02mg/g. The results revealed that the phenol content was more in stem extract of *T. cordifolia* and it was followed by tannins, alkaloids, flavonoids, and saponins shown in Fig. 2. Yadav & Agarwala, (2011) reported that presence of phenols in the leaf and stem were 18 mg/gm and 6 mg /gm respectively.

Table 1 Qualitative phytochemical screening of *Tinospora cordifolia*

S.No	Constituents	ASE	PEE	MEE	CFE
1.	Carbohydrates	+	-	+	+
2.	Glycosides	+	+	+	-
3.	Proteins	+	+	+	+
4.	Amino acids	+	+	+	+
5.	Alkaloids	+	-	+	-
6.	Flavonoids	+	+	+	+
7.	Phenols	+	+	+	+
8.	Tannins	-	-	+	-
9.	Saponins	-	-	+	-
10.	Steroids and triterpenoids	-	+	+	-

ASE- Aqueous Extract, PEE- Petroleum ether Extract, MEE- Methanol Extract, CFE- Chloroform Extract.

(+) = Present and (-) = Absent

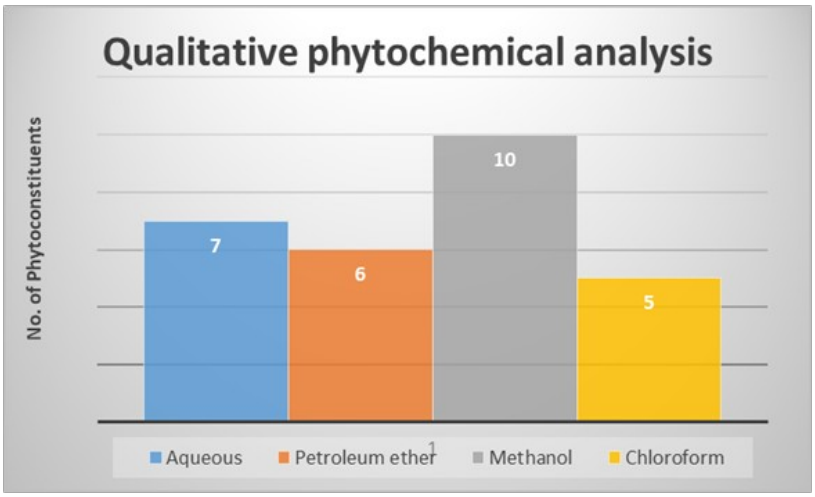

Figure 1 Qualitative screening of various solvent extracts of *T. cordifolia*

Table 2 Qualitative phytochemical screening of *Tinospora cordifolia*

Phytoconstituents	Quantity (mg/g) dry. wt.
Alkaloids	5.62
Flavonoids	4.07
Phenols	6.53
Tannins	5.83
Saponins	5.02

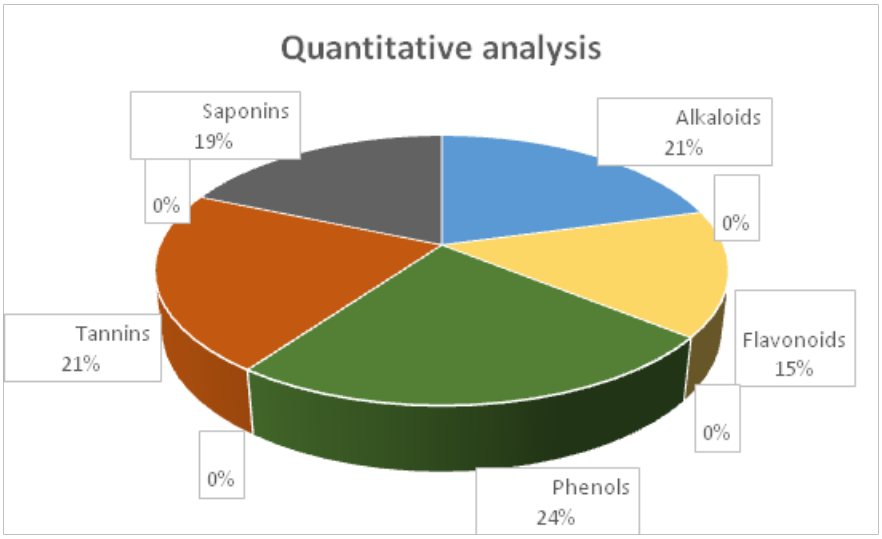

Figure 2 . Quantitative phytochemical analysis of *T. cordifolia*

Table 3 FTIR frequency range and functional group of *T. cordifolia*

Frequency range	Molecular Motion	Functional group
3906.30	O-H (Non-bonded)	Hydroxyl group
3850.45	O-H (Non-bonded)	Hydroxyl group
3768.40	O-H (Non-bonded)	Hydroxyl group
3717.11	O-H (Non-bonded	Hydroxyl group
3635.61	O-H (Non-bonded	Hydroxyl group
3520.50	O-H stretch	Alcohol
3410.30	O-H stretch	Alcohol
3338.78	O-H stretch	Alcohol
3172.22	N-H stretch	Amides
3065.34	=C-H stretch	Alkenes
2903.18	C-H stretch	Alkanes
2824.50	C-H stretch	Aldehydes
2746.37	C-H stretch	Aldehydes
2654.83	O-H stretch	Carboxylic Acids
2457.33	C≡N stretch	Nitriles
2311.25	C≡N stretch	Nitriles
2174.26	C≡C stretch	Alkynes
1728.11	C=C stretch	Aromatic Compounds
1613.36	C=C stretch	Aromatic Compounds
1516.01	C=C stretch	Aromatic Compounds
1386.36	C=C stretch	Aromatic Compounds
1023.08	C-F stretch	Alkyl & Aryl Halides
668.28	C-H bend	Aromatic Compounds
592.55	C-I stretch	Alkyl & Aryl Halides

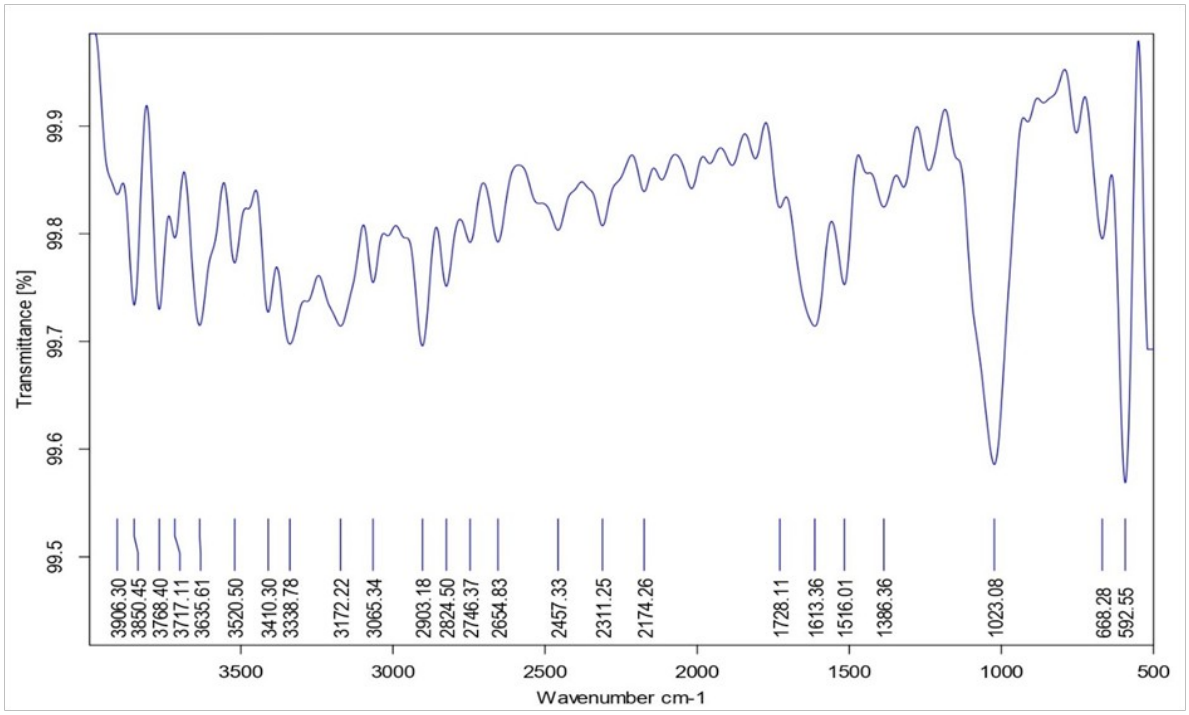

Figure 3 FTIR spectrum of stem extract of *Tinospora cordifolia*

FT-IR analysis was performed to identify the various functional groups of the biomolecules found in the methanolic stem extract of the plant (Fig. 3). Different wave numbers which correspond to different functional groups such as are present in stem of *T. cordifolia* having the presence of Hydroxyl group, Alcohols,

Amides, Alkanes Aldehydes, Alkenes, Carboxylic acids, Aromatic compounds, Nitriles, Alkyl and Aryl halides which shows O-H (Non-bonded), O-H stretch, N-H, C-H, C≡N, C≡C, C=C, C-F, C-H bend, and C-I stretches (Table 3). The results revealed that, *Tinospora cordifolia* stem contains phytocompounds like phenols, flavonoids, tannins, saponins, etc. The results obtained from FTIR analysis are significant with the result of preliminary qualitative analysis.

CONCLUSION

The results of present study revealed the presence of medicinally important constituents in *Tinospora cordifolia*. Several studies confirmed that the presence of phytochemicals contribute medicinal as well as physiological properties to these plants in the treatment of different ailments. Traditional medicine practice is recommended strongly that further work should be carried out to isolate, purify and characterize the bioactive compounds responsible for the activity of these plants.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest

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