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INTRODUCTORY PHYTOCHEMICAL SCREENING AND FLUORESCENCE ANALYSIS IN THE LEAVES OF *TINOSPORA CORDIFOLIA* (THUNB.) MIERS

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ABSTRACT

Tinospora cordifolia (Thunb.) Miers is a herbaceous, deciduous and dioecious plant, evergreen perennial climber of the family Menispermaceae. The plant is indigenous to the tropical areas and distributed throughout India. It is a plant of significant medicinal importance in the Indian system and designated as Rasayana. Its efficacy has been also recognized by the modern system of medicine. This plant has been known to possess immunomodulatory, hypoglycaemic, antioxidant, anti-hyperglycaemic, antiallergic, anti-inflammatory The aim of this study is to determine the phytochemical constituents of leaf extracts of *Tinospora cordifolia*. Dried leaf material was grounded to coarse powder and stored in airtight container followed by the extraction with methanol, ethanol, chloroform, petroleum ether and acetone. The dry powder of leaf sample was observed under U.V. light to evaluate the fluorescence. Phytochemical screening of leaf crude extracts of *Tinospora cordifolia* in different solvents like methanol, ethanol, petroleum ether, chloroform and acetone. The quantitative studies revealed that leaves of *Tinospora cordifolia* possessed alkaloids, carbohydrates, glycosides, saponins, phytosterols, proteins, flavonoids and lignin. The preliminary phytochemical screening tests in individual plant or plant part may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and drug development.

KEYWORDS: Qualitative and quantitative analysis of phytochemicals, Fluorescence analysis, *Tinospora cordifolia*.

INTRODUCTION

India is bestowed with enormous biodiversity of medicinal plants. Among them *Tinospora cordifolia* (Thunb.) Miers has a wide array of bioactive principles as well as it has been proven medicinally important plant. A large number of plants are being used in medicine for therapeutic or prophylactic purposes. The therapeutic properties of medicinal plants are attributed owing to the presence of active substances such as alkaloids, flavonoids, glycosides, vitamins, tannins, and coumarins[1] *Tinospora cordifolia* is a large, glabrous, deciduous climbing shrub belonging to the family Menispermaceae.[2] It is distributed throughout tropical Indian subcontinent and China, ascending to an altitude of 300 m. The chemical constituents reported from this shrub belong to different classes such as alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoid, phenolics, aliphatic compounds and polysaccharides. The literature survey records a variety of medicinal properties such as anti-diabetic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arthritic, anti-oxidant, anti-allergic, anti-stress, anti-leprotic, anti-malarial, heap to protective, immune modulator and anti-neoplastic.[3] Herbal medicines are in great demand in the developed as well as developing countries for primary healthcare because of their wide biological activities, higher safety margins and lesser costs. Regarding its distribution, *Tinospora cordifolia* is indigenous to areas of India, Myanmar, Sri Lanka, China, Thailand, Philippines, Indonesia, Malaysia, Borneo, Vietnam, Bangladesh, North Africa, West Africa, and South Africa 7-10. It typically grows in deciduous and dry forests at elevations up to 1000ft.In the present study an attempt has been made the laboratory evaluations to assess the analytical and phytochemical screening of *Tinospora cordifolia* and possibly relate the constituents to their medicinal/pharmacological uses. Phytochemical studies of the plant are necessary for standardization, which helps in understanding the significance of phytoconstituents in terms of observed activities. Phytochemical screening also helps in standardizing the herbal preparations.

BOTANICAL DESCRIPTION AND MEDICINAL PROPERTIES

Tinospora cordifolia, which is known by the common names Heart-leaved Moonseed, Guduchi and Giloy, is an herbaceous vine of the family Menispermaceae indigenous to the tropical areas of India, Myanmar and Sri Lanka. It is a large, deciduous extensively spreading climbing shrub with several elongated twining branches. Leaves simple, alternate, exstipulate, long petioles up to 15 cm long, roundish, pulvinate, both at the base and apex with the basal one longer and twisted partially and half way around. Lamina broadly ovate or

ovate cordate, 10–20 cm long or 8– 15 cm broad, 7 nerved and deeply cordate at base, membranous, pubescent above, whitish tomentose with a prominent reticulum beneath. Flowers unisexual, small on separate plants and appearing when plant is leafless, greenish yellow on axillary and terminal racemes. Male flowers clustered, female usually solitary. Sepals 6, free in two series of three each, the outer ones are smaller than the inner. Petals 6 free smaller than sepals, obovate and membranous. Fruits aggregate of 1-3, ovoid smooth drupelets on thick stalk with sub terminal style scars, scarlet or orange coloured.

Tinospora cordifolia is known for its immense application in the treatment of various diseases in the traditional ayurvedic literature. It is considered as one of the most divine herbs. Recently the discovery of active components from the plant and their biological function in disease control has led to active interest in the plant across the globe. Research has shown that this plant has the following properties anti-periodic, anti-spasmodic, anti-microbial[4], anticancer[5,6], anti-osteoporotic[7], anti-inflammatory, anti-arthritic[8], anti-allergic, anti-diabetic[9] Anti-toxic[10], Anti-HIV. [11]

Scientific classification of *Tinospora cordifolia*

Kingdom	**Plantae**
Unranked	Angisoperms
Unranked	Eudicots
Order	Ranunculales
Family	Menispermaceae
Genus	*Tinospora*
Species	*Cordifolia* (Thunb) Miers

MATERIAL AND METHODS

Collection and Identification of plant materials

Fresh and healthy leaves of *Tinospora cordifolia* for the present study were collected during the summer and monsoon months of 2014 from Tiruchirappalli district, Tamil Nadu. The identity of the plant specimens was confirmed by the use of local Floras .The botanical identify was authenticated in the Department of Botany, Jamal Mohamed College, Tiruchirappalli. The collected leaf materials were washed thoroughly with tap water and dried under shade for ten days. Dried leaf material was grounded to coarse powder and stored in airtight container. It was then extracted with ethanol. The dry powder of sample was observed under U.V. light to evaluate the fluorescence. Chemical tests were performed on ethanol extract.

Qualitative analysis

Preparation of ethanol extract: About 600 gm of powered material was taken in a clean, flat-bottomed glass container and soaked in 800 ml of 95% ethanol. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through Whatman filter paper. The filtrate thus obtained was concentrated using a rotary evaporator to get the extract.

Phytochemical Screening

The plant extract was subjected to qualitative tests for the identification of the phytoconstituents present in it viz, alkaloids, carbohydrates, glycosides, phytosterols, fixed oils and fats, phenolic compounds & tannins, proteins and free amino acids, flavonoids, lignins and saponins. [12-15]

Test for Phenolic compounds

Two to three drops of 1% ferric chloride (FeCl3) solution was added into 2ml of extract. Phenolic compounds produce a deep violet colour with ferric ions.

Test for Flavonoids - Shinoda Test

To the alcoholic¬ solution of the extract a few fragments of magnesium ribbon were added. To this concentrated hydrochloric acid (HCl) was added drop wise. Magenta colour was produced after few minutes which are the characteristic reaction of flavonoids.

Test for Anthrquinines

5ml extract was boiled with 10ml of sulphuric acid and filtered while hot. The filtrate was shaken with 5ml of chloroform the chloroform layer was pipette out into another test tube then 1ml of dilute ammonia is added. The resulting solution was observed for colour changes. The change in colour indicates the presence of anthraquinones.

Test for Tannins -Ferric Chloride test

Water extract¬ was treated with 15 % ferric chloride test solution. The resultant colour was noted. A blue colour indicates condensed tannins; a green colour indicated hydrolysable tannins.

Test for Saponins

The extract was diluted with 20ml¬ of distilled water and it was agitated on a graduated cylinder for 15 min. the presence of saponins was indicated by the formation of 1cm layer of foam.

Test for Alkaloids - Mayer's test:

The Extract was¬ dissolved in chloroform. The chloroform was evaporated and the residue was acidified and added few drops of Mayer's reagent (Potassium Mercuric Iodide). Alkaloids are precipitated by Mayer's reagent to give a cream coloured precipitate.

Wagner's Test

The extract was dissolved in¬ chloroform. The chloroform layer was evaporated, to the residue was acidified and added few drops of Wagner's reagent (Iodine in Potassium Iodide). Orange precipitate indicates alkaloids.

Test for the carbohydrate:

The aqueous extract 5 ml was treated with the reagent of the starch (iodine). Any shift to blue violet indicates the presence of starch.

Test for Glycosides - Fehling's Test for reducing¬ sugars (In Glycosides)

The extract was re-dissolved in water on the water bath. To 2 ml of the solution, in the test tube was added, 1ml each of Fehling's solutions A and B. The mixture was shaken and heated in a water bath for 10min. The colour obtained was recorded. A brick-red precipitate indicates reducing sugar.

Test for Proteins - Xanthoproteic Test:

Extract was treated with few drops of Concentrated HNO3. Formation of yellow colour indicates the presence of proteins.

Test for Steroids

For testing the presence of steroids 1ml extract was dissolved in 10ml of chloroform and equal volume of concentrated sulphuric acid was added from the walls of the test tube. Appearance of red colour in the upper layer and yellow with green fluorescence indicates the presence of steroids.

Test for Phytosterols - Liebermann-Burchard's Test:

One gram of the extract was treated with chloroform and filtered. The filtrate was treated with few drops of acetic anhydride boiled and cooled, concentrated sulphuric acid was added through the sides of the test tube. The formation of brown coloured ring at the junction of two liquids confirmed the presence of steroids.

Test for Terpenoids

1ml of the extract was dissolved in 1ml of chloroform; 1ml of acetic anhydride was added following the addition of 2ml of concentrated sulphuric acid. Formation of reddish colour indicates the presence of terpenoids.

Test for fixed oil and fats

Press the extract in between¬ the two filter papers, a permanent stain indicates the presence of fixed oil. Extract was treated with few drops of 0.5N potassium hydroxide and few drops of phenolphthalein and heat, formation of soap indicates the presence of fixed oil and fats.

Fluorescence Analysis

Fluorescence analysis of the plant powder was observed in daylight and UV light (254nm) in a UV chamber (Kokoshi et.al. 1958: Harborne ,1973) after treating with different chemical reagents is reported (Table.1). It can be as a diagnostic tool for testing the adulterations.

Quantitative study of Phytochemicals

Determination of Alkaloid[16]

5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Determination of Flavonoid.[17]

10 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper. The filtrate

was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

Determination of Saponin.[18]

20 g of each grounded sample was put into a conical flask and 100cm3 of 20% aqueous ethanol was added. Then the flask was heated on a hot water bath for 4 h. with constant stirring at about 55°C. The mixture was then filtered and the residue was again extracted with another 200 ml 20% ethanol. The combined extract was reduced to 40 ml on a hot water bath at about 90 °C. The concentrate was transferred into a 250 ml separating funnel, added20 ml diethyl ether in it followed by vigorous shaking. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in oven, weighed and saponin content was calculated as percentage.

Determination of Glycosides

Test 1: Extract of 200mg of the sample was taken warmed in a test tube with 5ml of diluted 10% sulphuric acid on the water bath at 1000 c for 2 minutes. Centrifuged; pipette off the supernatant. The acid extract was neutralized with 5% solution of NaOH. Added 0.1 ml of Fehling's solution A and then Fehling's solution B until alkaline (tested with pH paper) and heated on the water bath for 2 minutes. Noted the quality of red precipitate formed and compared with that formed in Test 2.

Test 2: Extract of 200mg of the sample was taken an added 5ml of water instead of Sulphuric acid. After boiling added a volume of water equivalent to the volume of NaOH used in the Test 1, step 2 and above. Added 0.1 of Fehling's solution B until alkaline and heated on the water bath for 2 minutes. Note the quantity of red precipitate formed (Test 2). Compared the quality of the precipitate formed in test 2 that formed in Test 1. The precipitate in Test 1 was greater than that in Test 2. That indicated the presence of glycoside in the crude drug.

Determination of Lignins

2gms of the oven-dry material passed through an 80-mesh sieve were extracted in a Soxhlet apparatus with 200ml, 95% alcohol for 4 hours. The extracted residue was transferred to a round-bottomed flask of 300ml capacity and extracted with 150ml boiling water under a reflux for 1 hour. The contents of the flask are then filtered and residue was transferred back

to the flask and hydrolyzed with 150ml. 5% H one hour. The hydrolyzed product was then collected in a weighed sintered glass crucible after being washed free of acid. The residue is weighed and well powdered. 0-2g lots were weighed out into 1000 ml beakers and treated with 20ml 72% H2SO4. The powder was well mixed with the acid and allowed to stand overnight, at a temperature varying between 20 and 220 (this was the range between 4p.m and 8a.m the following morning). The following morning the contents of the beakers were made up to 800ml. with water and boiled for 2 hours, the volume being maintained by occasional addition of water. Leave the beakers overnight to settle down the Lignin's, thereby facilitating filtration. The filtration was done under suction in crucibles with Whatmann filter paper. After drying, the precipitates were weighed and ignited and the lignin's calculated on an ash-free basis.

Determination of Phytostroids

1g of powdered dried sample was extracted 3 times using a vortex mixture (15min) with 7.5 ml chloroform. All the extracts were combined and evaporated to dryness. This chloroform extract contains free sterols and terpernoids. The residue was hydrolyzed with 2N HCl in methanol (2hr, 75-800 c), neutralized with 10N NaOH, then diluted with 25ml water, and then the steroidal alkaloids and saponins were extracted 3 times with 10ml chloroform. The chloroform phase was collected and evaporated to dryness. The amounts of phytosteroids were weighed.

Determination of Fixed Oil

Transfer a 50gm of the air dried, crushed drug to an extraction thimble, extract with Solvent ether in a continuous Soxhlet extraction apparatus for 6 hours. Filter the extract quantitatively into a tarred evaporating dish and evaporate off the solvent on a water bath. Dry the residue at 105º to constant weight. Calculate the percentage of ether-soluble extractive with reference to the air-dried drug.

RESULTS AND DISCUSSION

The result of fluorescence analysis, qualitative and quantitative analysis of *Tinospora cordifolia* have been presented and discussed herewith. Fluorescence analysis of drug showed in Table 1 is an important parameter in detecting adulteration or improper handling of drugs. It can serve as a valuable source of information and provide appropriate standards to establish the quality of this plant material in future study or application.

Table.1: Fluorescence analysis of *Tinospora cordifolia* in day light and in UV light.

S.No.	Chemical test	Day light	UV light
1	Sample	Pale yellow	Dark yellow
2	Powder + 1N aq. NaOH	yellow	Green
3	Powder+ 1N alc. NaOH	Yellowish green	Green
4	Powder + 1N HCl	Greenish yellow	Dark green
5	Powder + 50% HNO3	Dark green	Light Green
6	Powder + 50% H2SO4	Yellow	Green
7	Powder + Methanol	Green	Light green
8	Powder + NH3	Pale White	Green
9	Powder + I 2	Reddish orange	Yellowish brown
10	Powder + FeCl3	Brownish orange	Light brown

The phytochemical analysis of leaf extracts of *Tinospora cordifolia* were tested by different specific tests. Methanol, ethanol, petroleum ether, chloroform and acetone leaf extracts of *Tinospora cordifolia* were analyzed for their compounds such as tannins, saponins, flavonoids, steroid, cardiac glycosides, alkaloids and presented in Table 2. Phytochemical analysis table explained that methanol and ethanol extracts showed more phytochemicals than acetone, petroleum ether and chloroform. Phytochemicals like tannins, phenols, flavonoids, cardiac glycosides, steroids were found to present in all the tested extracts. On the other hand saponins are found in methanol, ethanol and acetone they were not found in petroleum ether and chloroform.

Terpenoids and anthraquinones were not found in all the tested extracts. Whereas alkaloids are present in all the extracts but absent in chloroform. Tannins are present in all the extracts but volatile oils are absent. A variety of constituents have been isolated from different parts of *Tinospora cordifolia*. They belong to different classes such as alkaloids, diterpenoid lactones, steroids, glycosides aliphatic compounds, polysaccharides.

Some constituents have been isolated from plant mainly they are tinosporone, tinosporic acid, cordifolisides A to E, syringen, berberine, giloin, gilenin, crude giloininand, arabinogalactan polysaccharide, picrotene, bergenin, gilosterol, tinosporol, tinosporidine, sitosterol, cordifol, heptacosanol, octacosonal, tinosporide, columbin, chasmanthin, palmarin, palmatosides C and F.[19]

Table. 2: Phytochemical screening of the leaf extracts of *Tinospora cordifolia* in various solvents.

S.No.	Phytochemical constituents	Methanol	Ethanol	Chloroform	Petroleum ether	Acetone
1	Alkaloids	+	+	-	+	+
2	Carbohydrates	+	+	-	+	-
3	Glycosides	+	+	+	+	+
4	Saponins	+	+	-	-	+
5	Tannins	+	+	+	+	+
6	Phytosterols	+	+	+	-	+
7	Proteins	+	+	+	-	-
8	Flavonoids	+	+	+	+	+
9	Lignin	+	+	+	-	-
10	Volatile oils	-	-	-	-	-
11	Fixed Oils and Fats	+	+	-	-	-
12	Phenols	+	+	+	+	+
13	Anthraquinines	-	-	-	-	-
14	Terpenoids	-	-	-	-	-
15	Steroids	+	+	+	+	+

(+)- Presence (-) - Absence

The phenolic compounds are considered as being a major group to the number of the secondary metabolites that contributes to the antioxidant activity of the plant. The presence of phenolic compounds in the plant indicates that this plant may have the ability as an anti-microbial agent.[20] It has been reported that most active principles in plants are frequently flavonoids, steroids, glycosides and alkaloids. These phytoconstituents may be responsible for the many pharmacological actions of the plant like wound healing[21], cholesterol lowering and antidiabetic activity.[22] Phytosterols are an important breakthrough in the human fight against high cholesterol. It has been known that plant steroids, flavonoids are antioxidants. These antioxidants are compounds that reduce the formation of free radicals or react with and neutralize them thus potentially protecting the cell from oxidative damage. The results of quantitative phytochemicals like total alkaloids, glycosides, saponins, phytosterols, total flavonoids, lignin and fixed oil are given in Table 3.Except for total alkaloids and total flavonoids, all other constituents were found to be lesser in amount.

Table 3: Quantitative phytochemical evaluation of *Tinospora cordifolia.*

S.No.	Phytochemical constituent	Content
1	Total alkaloids (mg/Kg)	1.84
2	Glycosides(mg/Kg)	0.25
3	Saponins(mg/Kg)	0.17
4	Phytosterols(mg/Kg)	0.05

5	Total Flavonoids(mg/Kg)	0.96
6	Lignin(mg/Kg)	0.03
7	Fixed Oils(µg/lit)	0.07

The results of the micronutrient analysis of ethanol extract of *Tinospora cordifolia* showed the presence of carbohydrates, fats, proteins, reducing sugars and fixed oil (Table 4).

Table 4: Macronutrient analysis of ethanol extract of *Tinospora cordifolia.*

S.No.	Constituents	Presence/ Absence
1	Total sugars	+
2	Reducing sugars	+
3	Total Proteins	+
4	Fixed oils & fats	+

The macronutrients; proteins, carbohydrate and reducing sugar may be involved in the energy providing and body building function of this plant. *Tinospora cordifolia* is widely used in veterinary folk medicine and in ayurvedic system of medicine for its general tonic, antiperiodic, antispasmodic, anti- inflammatory,antiarthritic, anti-allergic and anti-diabetic properties.[23,24] The root of this plant is known for its antistress, anti-leprotic and anti malarial activities.[24,25] The stem is bitter, stomachic, diuretic[26], stimulates bile secretion, causes constipation, allays thirst, burning sensation, vomiting, enriches the blood and cures jaundice. Dry barks of *T. cordifolia* have anti-spasmodic, antipyretic(27), anti-allergic(28) and anti-inflammatory[29] properties. *T. cordifolia* is widely used in Indian ayurvedic medicine for treating diabetes mellitus.[30]

CONCLUSION

The plant phytochemical studies could be provide an answer to the society seeking for better therapeutic medicine from natural sources which is supposed to be more efficient with less or no side effects when compared to the commonly used synthetic chemotherapeutic agents. The results obtained from the present phytochemical analysis of the leaves of *Tinospora cordifolia* showed the presence of alkaloids, tannins, flavonoids, cardiac glycosides, steroids, saponins, phenols, flavonoids, phytosterols, lignins, and macronutrient analysis revealed the presence of proteins, carbohydrates, reducing sugar, fats and oil. Alkaloids and flavonoids have been used as antiviral, antibacterial, antiamoebial and anticancer agents. Phenols and flavonoids are the groups of secondary metabolites are of great importance as cellular support material because they form the integral part of cell wall structure by polymeric phenolics and they can protect the human body from the oxidative stress which may cause many disease,

including cancer, cardiovascular problems and ageing .This study justifies the use of *Tinospora cordifolia* in the treatment of many ailments like diabetic mellitus, carcinoma, heart disease, asthma and rheumatism. In this study it is suggested that the identified phytochemical compounds may be the bioactive constituents which are medicinally valuable. Therefore, extracts from these could be seen as a good source for useful drugs. This present study also concludes that the leaves of *Tinospora cordifolia* can be utilized as an alternative source of useful drugs. In addition to this, the phytochemicals found in the leaves of *Tinospora cordifolia* may be tested for their antimicrobial activity.

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