PRELIMINARY PHYTOCHEMICAL SCREENING AND QUANTIFICATION OF BIOACTIVE COMPOUNDS IN THE LEAVES OF GUDCHI (*TINOSPORA CORDIFOLIA*)

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ABSTRACT

***Tinospora cordifolia* is a large glabrous, deciduous climbing shrub, which belongs to family menispermaceae. It is also known as guduchi or amrita is an Indian medicinal plant and has been used in ayurvedic preparations for the treatment of various disorders throughout the centuries. Ayurvedic properties are antidiabetic, antiperiodic, anti-inflammatory, antispasmodic, antiarthritic, antioxidant, antiallergic, antistress, antileprotic, antimalarial, hepatoprotective, immunomodulatory and neoplastic activities. Antioxident property of *T. cordifolia* is due to presence of bioactive compounds. The preliminary phytochemical screening of *T. cordifolia* leaves in different extracts (aqueous, methanol, ethanol, and chloroform extracts) was conducted and quantified the secondary metabolites. The study reveals the presence of carbohydrates, phenols, flavonoids, saponins, alkaloids, terpinoids, cardiacglycosides and steroids in *T. cardifolia*. The quantification of bio active compounds yields 40.12% of phenols, 32.08%of flavanoids, 39.07% of saponins and 13.29% of alkaloids.**

Keywords: Alkaloids, Antioxidant, Cardiacglycosides, Phytochemicals.

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INTRODUCTION:

Medicinal plants as a source of medicine has been an ancient practice and is an important constituent of the health care system in India. The traditional medicines are derived from medicinal plants, minerals, and organic matter. The herbal drugs are prepared from medicinal plants only. The phytochemicals and provitamins that assist in maintaining good health. The role of medicinal plants in prevention or control has been attributed to antioxidant properties of their constituents. The medicinal property of plants is due to the existence of antioxidant properties of their constituents. The medicinal plant has been used for the treatments of cancer, Cardiovasucular diseases due to their antioxidant property of their phytochemicals. A veriety of constituent have been isolated from *T. **cordifolia*** belonging to different classes such as alkaloids, cardiac glycosides, terpinoids, phenols, flavonoids, saponins and steroids, *T. cordifolia* contains about 11.2% protein and rich in calcium and phosphorous (Zhao *et al.*, 1930, (Khosa *et al.*, 1971).

Tinospora cordifolia (wild) Miers, Hook. F & Thems (Family:Menispermaceae) commonly known as gudchi. It is used in veterinary folk medicine/ayurvedic systems of medicine for its general tonic, antiperiodic, antispasmodic, antiinflamatory antiarthritic, antiallergic and antidibetic properties (Singh *et al.*, 2003). According to Srivastava (2011) gudchi is also called as a magical herb due to property of curing a lot of diseases. In the present study attempts were made to screen the phytochemicals present in different solvent extracts of *T. cordifolia* leaves and to enumerate some of these compounds.

MATERIALS AND METHODS

Collection and Processing of plant samples: The leaves of the plant was collected from APSSRDI, Hindupur, Ananthapuramu, Andhra Pradesh and washed thoroughly under tap water. They were dried under shade for two weeks. The shade dried leaves were ground into a coarse powder with the help of electric blender. The powder was stored in an airtight container and kept in a cool, dark and dry place until further analysis.

Plant extraction: The shade dried plant material (leaves) was soaked in solvents such as water, methanol, ethanol and chloroform with occasional shaking at room temperature (37^{o}C) for aqueous and at 15^{o}C temperature for methanolic, ethanolic and chloroform extracts for 24 hrs. The soluble materials of individual solvents were filtered off and the extracts of plant leaves were used for further qualitative analysis.

Phytochemical Screening: As per the standard methods the phytochemical screening of plant leaves was done with four different extracts *viz.*, water, methanol, ethanol and chloroform (Rishikesh et al., 2013).

1.Test for Carbohydrates

a. **Molisch's Test:** To 2 ml of the plant extract, 2 drops of freshly prepared 20% alcoholic solution of α-napthol was added and then 2ml of concentrated sulfuric acid was added along the sides of the test tube. Formation of the violet ring at the junction of the solutions and its disappearance on the addition of excess alkali solution indicating the presence of carbohydrates.

b. **Benedict's test:** To 0.5ml of plant extract taken in a test tube, 5ml of Benedict's solution was added and boiled for 5 minutes and allowed to cool. A red color precipitate of cuprous oxide formed indicating the presence of a reducing sugar.

c. **Tollens test:** To the test solution equal volume of hydrochloric acid containing a small amount of phloroglucinol (It is a reagent of the Tollen's test) was added and heated for 10 minutes. Red color produced due to reaction of furfurals with phloroglucinol, indicating the presence of pentoses.

2. Test for Phenols

a. **Ferric chloride test**: To 1 ml of the leaf extract, 2ml of distilled water followed by a few drops of 10% aqueous ferric chloride solution was added. Formation of blue or green or violet color indicating the presence of phenols.

b. **Libermann's test:** For a small quantity of the leaf extract, 5ml of 20% sulfuric acid followed by the addition of few drops of aqueous sodium nitrate solution was added. A red color obtained, indicating the presence of phenols.

3. Test for Tannins

a. **Lead acetate test:** To 5ml of the extract, a few drops of 1% lead acetate solution was added. A yellow precipitate was formed, indicating the presence of tannins.

b. **Chlorogenic acid test:** To the plant extract, aqueous ammonia was added and exposed to air. Gradually, green color developed indicating the presence of tannins.

c. **Catechin test:** A matchstick was dipped into the plant extract, dried it and moistened with concentrated hydrochloric acid. Then the stick was warmed near the flame. The color of the stick was changed to pink. It showed the presence of tannins.

4. Test for Flavanoids

a. **Ammonia solution reduction test:** To the 1% Ammonia solution, few drops of leaf extract was added. Yellow coloration was observed, indicating the presence of flavanoid compounds.

5. Test for Saponins

Froth test: To 5ml of extract, 2.5ml of distilled water was added and shaken vigorously to obtain a stable persistent froth. To this froth 3 drops of olive oil was added and formation of emulsion was observed, indicating the presence of saponins.

6. Test for Alkaloids: The extract was stirred with a few ml of concentrated hydrochloric acid and filtered. The filtrate was tested carefully with for the presence of alkaloids by following tests.

a. **Mayer's test:** To a few ml of filtrate 2 drops of Mayer's reagent was added along the sides of the test tube. A white creamy precipitate was observed which indicated the presence of alkaloid.
b. **Wagner's test:** To a small amount of extract few drops of Wagner's reagent was added along the sides of the test tube. A reddish brown precipitate was observed, confirmed the presence of alkaloid.
c. **Dragendorff's test**: To 0.5ml of the extract, 2ml of concentrated hydrochloric acid was added. To this acidic medium, 1 ml of Dragendorff's reagent was added. Orange coloured precipitate was observed, indicating the presence of alkaloids.

7. Test for terpenoids

Salkowski's test: To 2ml of the extract, 3ml of chloroform was added and 3ml of concentrated sulfuric acid was added carefully along the sides of the test tube to form a layer. A reddish brown coloration was formed at an interface indicating the presence of terpenoids.

8. Test for Phlobatannins: 10 ml of the plant extract was boiled with 1% hydrochloric acid in a conical flask. There was no deposition of a red precipitate indicating the absence of phlobatannins.

9. Test for Cardiac glycosides:

a. **Keller-Killiani test:** To the 5 ml of extract 1ml of concentrated sulfuric acid was added and was mixed with 2 ml of glacial acetic acid containing one drop of ferric chloride. To the above mixture carefully 1ml of concentrated Sulfuric acid was added, it was underneath the mixture. Formation of brown ring at the interface, indicating the presence of cardiac glycoside constituents.
b. **Baljet's Test:** A small amount of test solution was mixed with a pinch of picric acid, formation of orange color confirmed the presence of cardiac glycosides.

10) Test for Steroids

Liberman-burchard's test: 5ml of plant extract with few drops of acetic anhydride, was boiled and cooled. Then concentrated sulphuric acid was added along the sides of the test

tube, formation of brown ring at the junction of two layers with green coloured upper layer indicating the presence of steroids.

Quantitative analysis of Phytochemicals: Quantitative analysis of phytochemicals such as flavonoids (Bohan et al., 1994)., alkaloids, saponins (Krishnaiah et al., 2009) and phenols (Iqbai et al., 2011) present in the plant powder was carried out using standard methods.

1. **Phenols:** To determine the total phenol content of the plant powder, 5gms of the plant powder was taken into a 250ml titration flask and 100ml n-hexane was added, kept for 4h and the filtrate was discarded for fat free residue. The process was repeated for residue and filtrate was collected. 50ml of diethylether was added to the residue, heated in a boiling waterbath for 15 min, cooled to room temperature and filtered into a separating funnel. About 50ml of the 10% sodium hydroxide solution was added to filtrate twice and shake well each time to separate the aqueous layer from the organic layer. The organic layer was kept aside and to the aqueous solution 75ml of de-ionized water was added. The total aqueous layer was acidified to pH 4.0 by adding 10% hydrochloric acid and to this solution 50ml dichloromethane (DCM) was added twice to the aqueous layer in the separating flask. Consequently, the combined organic layer was collected dried and then weighed.
2. **Flavanoids:** To determine flavonoid content of the plant powder, 100ml of 80% aqueous methanol was added to10gms of plant sample in a 250 ml titration flask, at room temperature and shaken for 4 hrs in an electric shaker. The entire solution was filtered through whatmann filter paper no. 42 and again, this process was repeated to residue. The residue was evaporated to dryness over a water bath and weighed.
3. **Saponins:** To 20gms of plant powder in a conical flask, 100 ml of 20% ethanol was added. The sample was heated in a hot water bath for 4 hrs with continuous stirring at about 55^0c. The solution was then filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40ml over a water bath at about 90^0C. The concentrate was then transferred into 250ml separating funnel and 20ml of diethylether was added and shaken vigorously. The aqueous layer was recovered while diethylether layer was discarded and the purification process was repeated. To this 60ml n-butanol was added and n-butanol extract was washed twice with 10ml of 5% sodium chloride. Finally the solution was heated in a water bath and evaporated. After vanishing the sample was dried in a oven to a constant weight.
4. **Alkaloids:** To 5gms of plant powder in a 250 ml beaker, 200 ml of 20% acetic acid in ethanol was added. This was covered and allowed to stand for 4h. The solution was then filtered and the extract was allowed to become concentrate in a water bath until it reached $1/4^{th}$ volume of the original volume. To this concentrate ammonium hydroxide was added until the precipitation was completed. The whole solution was left to settle down and

precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The precipitated residue was dried and weighed.

RESULTS

The different plant extracts of *T. cordifolia leaves* (aqueous, methanolic, ethanolic and chloroform) were prepared for the preliminary phytochemical analysis to identify the bioactive constituents such as carbohydrates, phenols, tannins, flavanoids, saponins, alkaloids, terpenoids, phlobatannins, cardiac glycosides and steroids. The results of various phytochemical tests performed on the extracts are presented in **Table 1**.

The aqueous extract of *T. cordifolia* showed positive result for carbohydrates, phenols, flavanoids, saponins, terpenoids, cardiac glycosides, alkaloids, steroids and showed negative result for tannins, and phlobatannins. The methanolic extract of the plant leaves revealed the presence of carbohydrates, phenols, flavanoids, saponins, alkaloids, cardiac glycosides, terpinoids, steroids and absence of phlobatannins and tannins. The ethanolic extract of the plant leaves showed the presence of carbohydrates, flavanoids, phenols, alkaloids, saponins, cardiac glycosides, terpinoids, steroids and absence phlobatannins and tannins. The chloroform extract of plant leaves showed only the presence of carbohydrates, phenols, alkaloids, flavonoids and steroids and absence of all other phytochemicals.

The quantitative analysis of phytochemicals such as flavonoids, alkaloids, saponins and phenols present in the plant powder is summarized in **Table 2**. *T. cordifolia* contains about 40.12g of total phenols, 32.29g of flavanoids, 39.07g of saponins and 13.29g of alkaloids per 100g of plant powder.

TABLE 1: Preliminary phytochemical analysis of extracts of *Tinospora cordifolia* leaves

Constituents	Test Name	Different extracts of *Tinospora cordifolia* Leaves			
		Aqueous	Methanol	Ethanol	Chloroform
Carbohydrates	Molisch's test	+	+	+	+
	Benedict's test	+	+	+	+
	Tollen's test	+	+	+	+
Phenols	Ferric chloride test	+	+	+	+
	Libermann's test	+	+	+	+
Tannins	Lead acetate test	-	-	-	-
	Chlorogenic acid test	-	-	-	-
	Catechin test	-	-	-	-
Flavanoids	Ammonia solution reduction test	+	+	+	+
Alkaloids	Mayer's test	+	+	+	+
	Wagner's test	+	+	+	+
	Dragendroff's test	+	+	+	+
Saponins	Froth test	+	+	+	-
Terpenoids	Salkowski's test	+	+	+	-
Phlobatannins	Hydrochloric acid test	-	-	-	-
Cardiacglycosides	Keller-Killiani test	+	+	+	-
	Baljet's test	+	+	+	-
Steroids	Liberman-Burchard's test	+	+	+	+

Note: (+) = Presence and (-) = Absence

TABLE 2: Percentage yield of different phytochemicals of *Tinospora cordifolia* leaves

Phytochemical Name	Weight (g/100g dry weight)
Phenols	40.12g
Flavanoids	32.08g
Saponins	39.07g
Alkaloids	13.29g

The table showed the dry weight obtained from the sequential steps of dry weight extraction methods

DISCUSSION

The secondary metabolites of aurvedic plants are nutritionally, medicinally or physiologically highly active substances. The secondary metabolites present in plants are answerable for remedial and other pharmacological properties. These secondary metabolites which are important sources of many food ingredients (Doss *etal.*, 2012). These compounds include phenolics, flavonoids, saponins, alkaloids, terpenoids, cardiac glycosides, steroids etc., to protect themselves from the incessant attack by naturally occurring pathogens, bugs and environmental stress (Kumar *etal.*, 2009, Premkumar *etal.*, 2011). For the discovery of novel drugs, the essential information concerning the chemical constituents are generally provided by the qualitative phytochemical screening of plant extracts. These components are well known for their curative activity against several human diseases (Hentschel *et al.*, 1995, Brinkhaus *et al.*, 2005).

Polyphenols are abundant micronutrients in our diet and evidence for their role in the prevention of degenerative diseases such as cancer and cardiovascular diseases is emerging. polyphenols act as antioxidants. They protect cells and body chemicals against damage caused by free radicals. According to Rice-Evans *et al.*, (1995 & 1996) the phenolic compounds have been considered as powerful antioxidants *in vitro* and proved to be more potent antioxidants than Vitamin C, E and carotenoids. The phenolics have biological and pharmacological properties such as antimicrobial activity (Prior *et al.*, 2006), antiviral (Kessler *et al.*, 2003), anti-inflammatory (Hassan *et al.*, 2004) antimutagenic (Anpin *et al.*, 2011) and anticarcinogenic activities (Mungole *et al.*, 2010).

Ghani (1998) evidenced that the flavonoids have shown a wide range of biological and pharmacological activities in *in vitro* and *in vivo* studies. Flavonoids have antioxidant property and neutralized the free radicals produced in the body. Flavonoids may also modulate cell signaling pathways and could have marked effects on cellular function by altering protein and

lipid phosphorylation and modulating gene expression. Tannins are astringent in nature and useful in treating intestinal disorders such as diarrhoea and dysentery (Akinpelu *et al.*, 2006). It also aids in wound healing (Okwu *et al.*, 2006). Flavonoids show anti allergic, antiinflammatory and anti- cancer activity.

Saponins are glycosides with a distinctive foaming characteristic and have the beneficial effects on blood cholesterol levels, cancer, bone health and stimulation of immunesystem. Saponins have been found to be potentially useful in the treatment of hypercholesterolemia which suggests that saponins might interfere with the intestinal absorption of cholesterol (Olekye 2007, Malinow *et al.*, 1977).

Alkaloids containing plants have been used by humans since ancient times for therapeutic and recreational purposes. Alkoloids have diverse and important physiological effects on humans and other animals. Alkoloids are a group of naturally occurring chemical compound contain mostly basic nitrogen atoms. Which possess good analgesic, anti inflammatory, pharmacological and anti -oxident activity. Vincamine is a vasodilating, antihypertensive alkaloid.qunine is a antipyretics, anti malerial alkaloid.

Terpenoids are naturally occurring organic compounds mainly aromatic quality. They play role in traditional herbal remidies like antibacterial, antineoplastic and other pharmaceutical functions. Terpinoids reduce complications associated with diabetes and lowers the sugar level in blood (Hawkins et al., 2006).

Cardiac glycosides (Singh *et al.*, 1970) are organic compounds containing a glycoside that act on the contractile force of the cardiac muscle. These glycosides are found as secondary metabolites in several plants. These are useful for treatment of congestive heart failure and cardiac arrhythmia.

In the current study the qualitative analysis of different extracts of *T. cordifolia* leaves showed the presence phenolic compounds, flavanoids, saponins, alkaloids, cardiac glycosides, steroids, carbohydrates and absence of phlobatannins and tannins. The quantification of phytochemicals of *T. cordifolia* leaves showed the presence of phenols, saponins, flavonoids, alkoloids justified the potent antioxidant nature of the plant, which increases its medicinal properties of the plant.

Conclusion

The phytochemical analysis of *T. cordifolia* revealed the presence of phytochemicals such as alkaloids, phenolics, flavonoids, terpenoids, cardiac glycosides, carbohydrates and steroids in different extracts. Furthermore, the quantification of phytochemicals showed the leaves of *T. cordifolia* contains 40.12g of phenolic compounds, 32.08g of flavonoids, 39.07g of saponins and 13.29g of alkaloids per 100g of plant powder. Hence the presence of these secondary metobolites imparts high medicinal value and anti-oxidant potential to *T. cordifolia.* The present

study indicates that *T. cordifolia* is an ayurvedic plant and have antioxidant property. That can be used as a therapeutic and curative medicine for many oxidative stress induced diseases.

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