Phytochemical screening of secondary metabolites of roots of *Tinospora cordifolia* (Willd.) Miers ex Hook. f. & Thoms

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**Abstract**

*Tinospora cordifolia* (Willd.) Miers ex Hook.f. & Thoms (Menispermaceae) contains many chemical substances in the different parts mainly the root. The present study represents phytochemical screening of root extracts of *Tinospora cordifolia* revealed presence of five different types of phytochemicals: alkaloids, steroids, flavonoids, glycosides and carbohydrates. The phytochemical analysis revealed the by using various test in *Tinospora*. Separation of secondary metabolites was observed by using thin layer chromatography with solvent system of CHCl₃ & ethanol and observing Rf value of alkaloids. Our findings provide evidence that crude aqueous and organic solvent extracts of the tested plant contain medicinally significant bioactive compounds and justify its use in the medicine for the treatment of different diseases.

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1. Introduction

*Tinospora cordifolia* (Willd.) Miers ex Hook. f. & Thoms known as “Guduchi, or Giloe or Amrita” is a common climber and medicinal plant of Chhattisgarh belongs to family Menispermaceae indigenous to the tropical areas of India, Myanmar and Sri Lanka. Due to over exploitation, its natural population is decreasing at alarming rates in the state. Phyto-nutrient or plant organic components are used to promote human health (Lenta et al., 2007). Extraction and characterization of several active phyto-compounds from these green factories have given birth to some high activity profile drugs (Mandal et al., 2007). Plants contain some organic compounds which provide definite physiological action on the human body and these phytochemical screening include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids, these compounds are synthesized by primary or rather secondary metabolism of living organisms (Mann 1978; Vasu et al., 2009). Secondary metabolites of plants serve as defense mechanisms against predation by many microorganisms, insects and herbivores (Cowan, 1999). Plant products have been part of phyto-medicines since time immemorial and medicinal plants constitute the main source of new pharmaceuticals and healthcare products (Ivanova et al., 2005). The phytochemical research based on ethno-pharmacological information is generally considered an effective approach in the discovery of new anti-infective agents from higher plants (Duraipandiyan et al., 2006). *T. cordifolia* is used in veterinary folk, ayurvedic medicine for its antipyretic, anti-spasmodic, anti-inflammatory, anti-allergic, antioxidant, anti-stress, anti-leprotic, anti-malarial, anti-diabetic activities and also used as tonic, diuretic, its constituent found in several compound preparations and root and stem are prescribed in combination with other drugs as an anti-dote to snake bite and scorpion sting. (Nadkarni et al., 1976; Kirtikar and Basu, 1975; Chopra et al., 1956; Chopra et al., 1982; Stanley et al., 1999; Zhao et al., 1991). Plant is used in fever, urinary disorders, dyspepsia, general debility,
urinary diseases, rheumatism treatment, jaundice and hypoglycaemic activity in rabbit (Zhao et al., 1991; Nayampalli et al., 1982; Stanely et al., 2000). *Tinospora cordifolia* plant has a preventive role its spray prevents insect attack (Warrier et al., 1996). The fresh plant of *Tinospora cordifolia* is said to be more effective than the dry one. However, it is traditionally dried and made into a starchy extract called Guduchi Sattva. Kiem et al. (2010) isolated and elucidated the structure of two new aporphine alkaloids in *Tinospora cordifolia*. Tembetarine, Choline, Tinosporin, Isocolumbin, Palmatine, Tetrahydropalmatine and Magnoflorine were also isolated from root extract of *T. cordifolia*.

2. Objective of Research

The main aims of our research to provide evidence that crude aqueous and organic solvent extracts of the tested plant contain medicinally significant bioactive compounds and for the treatment of different diseases.

3. Materials and Methods

3.1 Collection and identification of plant

Root of *Tinospora cordifolia* (Willd.) Miers ex Hook. f. & Thoms was collected in year 2011-12 from CCM Agriculture College Sarkanda, Bilaspur (C.G.) and was identified by one of author with the help of Flora of Madhya Pradesh in the Laboratory of Department of Botany, Dr. C.V. Raman University, Kota, Bilaspur. A herbaceous vine or twining, succulent-stemmed, fast growing with tuberous roots; Branches grey-green, up to 40 mm diameter becoming brown; Stem fleshy, roots long thread like, aerial, arise from branches. Bark thin, grayish or creamy white in color, spotted with large rosette like lenticels, when peeled fleshy stem exposed. Leaves heart-shaped; Flowers tiny creamy-greenish, male and female flowers formed on different branches; flowering in March-June, fruiting in July and mature in cold season.

3.2 Justification of Research

The phytochemical analysis revealed the presence of alkaloids, flavonoid, phenols, glycosides, carbohydrates and steroids by using various test in *Tinospora*. After phytochemical screening we can find out the antimicrobial activities in different pathogens because Giloe is most important medicinal plant. Present work may be the milestone study in the field of pharmacology and toxicology for treatment to cure diseases.

4. Observation

4.1 Preliminary screening of secondary metabolites

The shade dried plant material is powdered using mixer grinder, and subjected to soxlet extraction with petroleum ether, chloroform, 95% ethanol, and distilled water for 18h qualitative chemical tests for various phyto-constituents were carried out for all the root extracts of *Tinospora cordifolia* as explained below:

4.2 Test for Alkaloids

*Mayer’s test*: Alkaloids give cream color precipitate with Mayer’s reagent (Potassium mercuric iodide solution).

*Dragendorf’s test*: Alkaloids give reddish brown precipitate with Dragendorf’s reagent (Potassium bismuth iodide solution).

4.3 Test for Glycosides

*Raymond’s test*: Test solution when treated with dinitro-benzene in hot methanolic alkali, gives violet color.

*Legal’s test*: Treat the extract with pyridine and add alkaline sodium nitroprusside solution, blood red color appears.

4.4 Test for Sterols

*Salkowski test*: Treat extract in chloroform with few drops of conc. sulfuric acid, shake well and allow standing for some time, red color appears at the lower layer indicates the presence of steroids.

4.5 Test for Flavonoids

*Shinoda test* (Magnesium Hydrochloride reduction test): To the test solution, add few fragments of Magnesium ribbon and add concentrated Hydrochloric acid drop wise, pink scarlet, crimson red or occasionally green to blue color appears after few minutes.

*Alkaline reagent test*: To the test solution add few drops of sodium hydroxide solution; formation of an intense yellow color, which turns to Colorless on addition of few drops of dil. acid, indicates presence of flavonoids.

4.6 Test for Carbohydrates

*Benedict’s test*: Treat the test solution with few drops of Benedict’s reagent (alkaline solution containing cupric citrate complex) and upon boiling on water bath, reddish brown precipitate forms if reducing sugars are present.

5. Results & Discussion

5.1 Separation of secondary metabolites by TLC

For the thin layer chromatographic studies of secondary metabolites, pre-coated Alu gram Sil G/UV254 nm (Machery- Nagel GmbH, Germany) aluminum plates (20 × 20 cm) were used. One
gram powdered root of *Tinospora cordifolia* was extracted with 10ml ethanol on soxhlet apparatus. The filtrate was condensed by evaporation, added a mixture of water and ethyl alcohol (10:1 ml), and mixed thoroughly. The Flavonoid spots were separated using CHCl$_3$ and ethanol (9:1) solvent mixture. The colour and Rf values of these spots were recorded under ultraviolet (UV254nm) light 15. Thin Layer Chromatography was done by using solvent system of CHCl$_3$ & ethanol (9:1) and observing Rf value of alkaloids is 0.74 with dark green spot.

**Table 1:** Qualitative analysis of plant extract of *Tinospora cordifolia* (Willd.) Miers ex Hook. f. & Thoms

<table>
<thead>
<tr>
<th>Plant Constituents</th>
<th>Root</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Sterols</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>-</td>
</tr>
</tbody>
</table>

+Presence, – Absence, Pet-Petroleum, Et-Ether, Ext-Extract, Eth-Ethanol, Chl-Cholorom, Aq.-Aqueous

The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites (Singh et al., 2007). Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds (Brown and Rice-Evans 1998; Krings and Berger, 2001). Pradhan et al. (2013) reported extracts prepared in different solvents were screened for the presence or absence of phytochemicals. Tannins was absent, while, alkaloids, flavonoids, cardiac glycosides, carbohydrates, proteins and steroids were present in all the extracts. Saponins were present only in aqueous and methanolic extracts. Our results corroborates with the results of Tanwar et al. (2012) and Nasreen et al. (2010), they also reported the presence of above phytochemicals in *Tinospora cordifolia*.

Sivakumar and Dhana Rajan, (2011) also reported the presence of wide range of phytochemicals in different solvent extracts of *Tinospora cordifolia* stem. The different pharmacological actions of *T. cordifolia* like other medicinal plants can be attributed to the presence of array of secondary metabolites in it (alkaloids, flavonoids, phenols, steroids, saponins, glycosides etc. (Singh et al., 2003). The Phytochemical analysis of ethanolic, chloroform, aqueous and petroleum ether extract of Tinospora cordifolia reveals the presence of Flavonoids, Alkaloids, Sterols, Glycosides and Carbohydrate (Table 1).

Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity (Nobori et al., 1994). These simple but reliable standards will be useful for society in using the drug as a home remedy. Also the manufacturers can utilize them for identification and selection of the raw material for drug production. The information obtained from preliminary phytochemical screening will be useful in finding out the genuineness of the drug. Standardization plays a significant role in the production of phyto-pharmaceuticals of standard quality as the quality standards are based on proper selection of raw materials. Natural antioxidant mainly comes from plants in the form of phenolic compounds such as flavonoid, phenolic acids, tocopherols etc. (Ali et al., 2008).

As very little specific standards are mentioned in the official monographs evaluation of the crude drugs is of great importance for the pharmaceutical industries. This involves the determination of identity, purity & quality. Many organic & inorganic contaminations which are virtually impossible to avoid while collecting crude drugs affect the purity of any crude drug which needs proper assessment & detection based on different pharmacognostic & phytochemical parameters.

The chemical constituents reported from this shrub belong to different classes, such as alkaloids, diterpenoid lactones, glycosides, steroids, sequin terpenoid, phenolics, aliphatic compounds and polysaccharides.

**Research Highlights**

Phytochemical screening of medicinal plant and extraction of chemical constituents has been done during research work.

**Recommendations**

Present work may be recommended that after phytochemical screening of secondary metabolites, further study on the antimicrobial activities may be done.

**Funding and Policy aspects**

Present work was not done by any funding agency and source.

**Conclusion**

The wide spread use of *Tinospora cordifolia* as
traditional medicine for treatment of varied ailments is supported by various scientific studies involving its pharmacological evaluations.

Author’s Contribution and Competing Interests

Yes authors has been contributed and representing the competing the interests.

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