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PHYTOCHEMICAL ANALYSIS OF *TINOSPORA CORDIFOLIA* (WILLD.) MIERS EX HOOK. F. & THOMS STEM OF VARIED THICKNESS

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ABSTRACT: *Tinospora cordifolia* (Giloe/Guduchi) belonging to the family menispermaceae, is a large extensively spreading glabrous, perennial deciduous twiner with succulent stems and papery bark. It is widely used in Ayurvedic system of medicine "Rasayanas" to the immune system and the body resistance against infections. In modern medicine, *T. cordifolia* is used for the treatment of general weakness, fever, dyspepsia, dysentery, gonorrhoea, urinary diseases, viral hepatitis and anaemia. The stems of different thickness (diameter) are being used by the pharmaceutical industries for preparation of various herbal formulations without having information about concentration of phytoconstituents. The present study was carried out with the objective to study the variation in phytoconstituents among *T. cordifoila* stems of varied thickness. *T. cordifolia* stem of varied diameter, collected from Seoni, Jabalpur and Chhindwara of Madhya Pradesh were analyzed for their phytoconstituents. Phytochemical screening revealed the presence of phenols, flavanoids, alkaloids, saponins, cardiac glycosides, steroids, carbohydrate and proteins. Quantitative analysis revealed that all the phytoconstituents increased with the increase in diameter of the stem except alkaloid content. Variation in phytoconstitutents was also found among samples collected from different locations. It can be concluded that stem of larger diameter contains more phytoconstituents and can be used by pharmaceutical industries for making quality products.

INTRODUCTION: *Tinospora cordifolia* (Willd.) Miers ex Hook.F. & Thoms is a widely used shrub in folk and Ayurvedic systems of medicine. It is distributed throughout the tropical Indian subcontinent and China, ascending to an altitude of 300 m. It is glabrous, deciduous climbing shrub belonging to the family Menispermaceae [1, 2, 3].

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T. cordifolia grows as climber; leaves are simple, alternate and long petiole, lamina broadly ovate, 7 nerved and deeply cordate at base. Flowers grow during summer and fruits during winter [4, 5].

Stem of *T. cordifolia* appears in varying thicknesses, young stems are green with smooth surfaces and swelling at nodes, while the older ones are light brown in colour with warty protuberances at the surface due to circular lenticels; transversely smoothened surface shows a radial structure with conspicuous medullary rays traversing porous tissues and is bitter in taste [6].

In Hindi, the plant is commonly known as Giloe which refers to the heavenly elixir that has saved celestial beings from old age and kept them eternally young [7]. Guduchi, its Sanskrit name, means one which protects the entire body. The term *amrita* is attributed to its ability to impart youthfulness, vitality and longevity [6]. It is an annual or perennial Ayurvedic plant which is used in several traditional medicines to cure various diseases. The plant is sometimes cultivated for ornamental value and is propagated by cuttings. The leaves afford a good fodder for cattle.

T. cordifolia finds a special mention for its use in tribal or folk medicine in different parts of the country [8]. Almost all the parts of the plant are documented to be useful in ethnobotanical surveys conducted by ethnobotanists [9]. *T. cordifolia* is reported to possess antispasmodic, anti-inflammatory and antiallergic properties. The notable medicinal properties reported are anti-diabetic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arthritic, anti-oxidant, anti-stress, anti-leprotic, anti-malarial, antipyretic, hepatoprotective, immunomodulatory and anti-neoplastic activities [10-16].

The chemical constituents reported from this shrub belong to different classes such as alkaloids, terpenoids lactones, glycosides, steroids, sesquiterpenoid, phenolics, aliphatic compounds and polysaccharides [17, 18]. The starch obtained from the stem known as Guduchi Satva is highly nutritive and digestive and used in many diseases. Starch is present throughout the parenchyma of the stem [10]. It is a tonic and useful in the treatment of chronic diarrhoea and dysentery. It is a hepato-protectant and protects the liver from damage. This is especially used when the liver has been exposed to various toxins.

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.) [8].

Geo climatic conditions are known to influence the secondary metabolites levels in medicinal plants which ultimately are reflected as a variation in the pharmacological actions.

The aim of the present research work was to evaluate phytochemical constituents in *T. cordifolia* stems of various thicknesses collected from different locations.

MATERIALS AND METHODS:

Plant material: Fresh and healthy stem of *T. cordifolia* were collected from Chhindwara, Seoni and Jabalpur districts of Madhya Pradesh. The stem was washed thoroughly in water to remove soil and other foreign particles, cut into smaller pieces of 1-2 cm length and dried under shade. The dried stem was powdered and used for extraction.

Preliminary Phytochemical Analysis: 200 mg of powdered plant material was kept overnight in 25 ml of different solvents viz. methanol, petroleum ether, water, chloroform and ethyl acetate. The solutions were filtered and extracts were then subjected to phytochemical screening as per methods prescribed in literature [19].

Quantitative Analysis:

1. **Estimation of Total Phenols:** Total phenols were determined by Folin Ciocalteau method [20]. 0.5 gm of the powdered stem was taken in a pestle and motor and grinded in 20 ml of 80% ethanol. The homogenate was then centrifuged at 10,000 rpm for 20 min. The supernatant was transferred to a beaker and evaporated to dryness. The residue was dissolved in 20 ml of distilled water. 0.2 ml of samples were then taken in test tube and volume made up to 3ml with distilled water. 0.5 ml of Folin Ciocalteau reagent was then added. After 3 min, 2 ml of 20% Na_2CO_3 solution was added to each tube, mixed thoroughly, placed in boiling water for exactly 1 min, cooled and absorbance was taken at 650 nm against blank. The standard graph was prepared by using different concentration of catechol. The concentration of phenols in samples was then calculated from the standard graph.

2. **Estimation of Total Flavonoids:** Total flavonoids were determined by aluminium chloride colorimetric technique [21]. 0.5 gm sample was weighed and kept in 95% ethanol for 24 hours. It was than filtered and volume was made up to 25 ml with 80% ethanol. 0.5 ml of filtrate was then mixed with 1.5 ml of

95% ethanol, 0.1 ml of 10% $AlCl_3$, 0.1 ml of potassium acetate and 2.8 ml water. The tubes were then incubated at room temperature for 30 min and absorbance was measured at 415 nm. The flavonoids content of the samples was calculated from the standard graph of quercetin.

3. **Estimation of Total Alkaloids:** 0.5 gm of sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 10 h. 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 alkaloid content, which was dried and weighed.

4. **Estimation of Cardiac Glycosides:** Five gram of sample was taken in 100 ml distill water. To this 10 g conc. H_2SO_4 (prediluted with 10 ml H_2O) was added. It was then reflux for 6-8 h. Cooled and extracted with chloroform (2 x 25ml). The chloroform layer was then washed with distill water till it is acid free. Transferred to a pre weighed beaker and dried in an oven to a constant weight. Percentage of cardiac glycoside was calculated from the following formula:

Percentage of Cardiac Glycoside =

$$\frac{(B - A) \times 100 \times 2}{\text{Weight of Sample}}$$

Where, B = weight of beaker with content; A = weight of empty beaker

5. **Estimation of Saponin:** 2 gm of sample was taken into a conical flask and 25 ml of 20 % ethanol was added. The samples were heated over a hot water bath for four hours at 55^{o}C. The mixture was filtered and the residue was re-extracted with another 25 ml of 20% ethanol. The combined extracts were reduced to 15 ml over water bath at about 90^{0}C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The aqueous layer was further separated by 60 ml (2X30) of n-butanol. The combined butanol extracts were washed twice with 10ml of 5% sodium chloride. The extract was then transferred to a pre-weighed beaker and dried in oven to a constant weight. Percentage of saponin was calculated from the following formula:

$$\text{Percentage of Saponin} = \frac{(B - A) \times 100}{\text{Weight of Sample}}$$

Where, B = weight of beaker with content; A = weight of empty beaker

6. **Estimation of Crude Fiber:** The crude fiber content in the samples was determined by the method given by Mayanard (1970) [22]. The ground material was extracted with petroleum ether to remove fat (initial boiling temperature 35-38^{o}C and final temperature 52°C. If fat content is below 1% extraction may be omitted. After extraction with ether, 2 gm of dried material was boiled in 200 ml of sulphuric acid for 30 min with bumping chips. The solution was then filtered through muslin and washed with boiling water until washings are no longer acidic.

   The sample was then boiled in 200 ml of sodium hydroxide solution for 30 min. Filtered through muslin cloth and washed with 25 ml of boiling 1.25% H_2SO_4, three 50 ml portion of water and 25 ml of alcohol. The residue was then transferred to pre-weighed ashing dish (w_1) and dried for 2 h at 130+2^{o}C. The dish was cooled in a desiccator and weighed again (w_2). The sample was then ignited in a muffle furnace at 600+15^{o}C till constant weight is obtained (w_3). The percentage of crude fibre in the sample was calculated from the following formula:

% Crude Fiber in Sample =

$$\frac{\text{Loss in wt. on ignition (W2 – W1) – (W3 – W1) x 100}}{\text{Weight of Sample}}$$

RESULTS AND DISCUSSION: **Table 1** represents the phytochemical screening of *T. cordifolia* stem. 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) [23] and Nasreen *et al*., (2010) [24], they also reported the presence of above phytochemicals in *T. cordifolia*. Sivakumar and Dhana Rajan, 2011 [24] also reported the presence of wide range of phytochemicals in different solvent extracts of *T. cordifolia* stem.

TABLE 1: PHYTOCHEMICAL SCREENING OF *TINOSPORA CORDIFOLIA* STEM

Phytochemical constituents	Aqueous extract	Methanol extract	Chloroform extract	Petroleum Ether extract	Ethyl acetate extract
Tannins	-	-	-	-	-
Alkaloids	+	+	+	+	+
Saponins	+	+	-	-	-
Phenols	+	+	+	+	-
Flavonoids	+	+	+	+	+
Cardiac Glycosides	+	+	+	+	+
Carbohydrates	+	+	+	+	+
Proteins	+	+	+	+	+
Steroids	+	+	+	+	+

Note: - represents absence, + shows presence

Table 2 represents quantitative phytochemical analysis of *T. cordifolia* stem collected from different places. Total phenols ranged from 0.74 ± 0.05% - 1.86 ± 0.04% at Chhindwara, 0.54 ± 0.02%- 2.09 ± 0.06% at Jabalpur and 0.62 ± 0.02% - 2.05 ± 0.1% at Seoni. Maximum concentration (2.09 ± 0.06%) of total phenols was found in 16-18 mm thick stem collected from Jabalpur while minimum (0.54 ± 0.02%) was found in 2-4 mm thick stem collected from Jabalpur. Sivakumar *et al*., 2010 reported 7.2% total phenols in the stems [26].

Total flavonoids ranged from 0.13 ± 0.02% - 0.20 ± 0.03% at Chhindwara, 0.12 ± 0.03% - 0.20 ± 0.01% at Jabalpur and 0.11 ± 0.01% - 0.3 ± 0.02% at Seoni. Maximum concentration (0.3 ± 0.02%) was found in 16-18 mm thick stem collected from Seoni while minimum (0.11 ± 0.01%) was found in 2-4 mm thick stem collected from Seoni. Premanath and Lakshmidevi reported 0.42% phenol and 0.045% flavanoid in leaves of *T. cordifolia* [27]. Cardiac glycosides ranged from 0.09 ± 0.12%- 1.52 ± 0.03% at Chhindwara, 0.09 ± 0.02% - 1.97 ± 0.02% at Jabalpur and 0.05 ± 0.01% - 2.02 ± 0.02% at Seoni. Maximum concentration (2.02 ± 0.02%) was found in 16-18 mm thick stem collected from Seoni while minimum (0.05 ± 0.01%) was found in 2-4 mm thick stem collected from Seoni.

Saponin content ranged from 2.59 ± 0.06% - 2.89 ± 0.05% at Chhindwara, 2.15 ± 0.01% - 2.73 ± 0.02% at Jabalpur and 2.40 ± 0.03% - 2.85 ± 0.01% at Seoni. Maximum concentration (2.89 ± 0.05%) was found in 16-18 mm thick stem collected from Chhindwara while minimum (2.15 ± 0.01%) was found in 2-4 mm thick stem collected from Jabalpur.

Total alkaloids ranged from 1.9 ± 0.1% - 4.03 ± 0.06% at Chhindwara, 1.71 ± 0.02% - 4.25 ± 0.03% at Jabalpur and 0.49 ± 0.04% - 4.02 ± 0.06% at Seoni. Maximum concentration (4.25 ± 0.03%) was found in 2-4 mm thick stem collected from Jabalpur while minimum (0.49 ± 0.04%) was found in 16-18 mm thick stem collected from Seoni.

The percentage of crude fiber in also varied among different accessions and increased with the increase in thickness of the stem. Amount of crude fiber ranged from 0.035 ± 0.002% - 0.106 ± 0.004% at Chhindwara, 0.015 ± 0.002% - 0.092 ± 0.003% at Jabalpur and 0.018 ± 0.002% - 0.074 ± 0.005% at Seoni. Maximum amount of crude fiber (0.106 ± 0.04%) was found in 16-18 mm thick stem collected from Chhindwara while minimum (0.015 ± 0.002%) was found in 2-4 mm thick stem collected from Jabalpur.

TABLE 2: QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF *TINOSPORA CORDIFOLIA* STEM

Location	Stem diameter class (mm)	Total Phenols %	Total Flavonoids%	Cardiac glycoside %	Saponins %	Total Alkaloids %	Crude fiber %
Chhindwara	16 – 18	1.86 ± 0.04 [a]	0.20 ± 0.03[a]	1.52 ± 0.03[a]	2.89 ± 0.05[a]	1.90 ± 0.1[a]	0.106 ± 0.004[a]
	9 – 11	1.51 ± 0.03 [b]	0.18 ± 0.02[ab]	0.68 ± 0.03[b]	2.76 ± 0.11[a]	1.88 ± 0.03[b]	0.085 ± 0.005[b]
	5 – 8	1.43 ± 0.06 [b]	0.17 ± 0.02[ab]	0.16 ± 0.02[c]	2.59 ± 0.1[b]	2.30 ± 0.04[c]	0.050 ± 0.002[c]
	2 - 4	0.74 ± 0.05[c]	0.13 ± 0.02[b]	0.09 ± 0.02[a]	2.59 ± 0.06[b]	4.03 ± 0.06[c]	0.035 ± 0.002[d]
Jabalpur	16 - 18	2.09 ± 0.06[a]	0.20 ± 0.01[a]	1.97 ± 0.02[a]	2.73 ± 0.02[a]	1.71 ± 0.02[a]	0.092 ± 0.003[a]
	9 - 11	1.81 ± 0.02[b]	0.18 ± 0.02[a]	0.56 ± 0.02[b]	2.42 ± 0.03[b]	2.12 ± 0.04[b]	0.076 ± 0.002[b]
	5 - 8	0.91 ± 0.02[c]	0.16 ± 0.02[a]	0.20 ± 0.02[c]	2.38 ± 0.02[b]	3.54 ± 0.02[c]	0.043 ± 0.002[c]
	2 - 4	0.54 ± 0.02[d]	0.12 ± 0.03[b]	0.09 ± 0.02[d]	2.15 ± 0.01[c]	4.25 ± 0.03[d]	0.015 ± 0.002[d]
Seoni	16 - 18	2.68 ± 0.02[a]	0.3 ± 0.02[a]	2.02 ± 0.02[a]	2.85 ± 0.01[a]	0.49 ± 0.04[a]	0.074 ± 0.005[a]
	9 - 11	2.19 ± 0.03[b]	0.22 ± 0.02[b]	0.72 ± 0.02[b]	2.6 ± 0.07[b]	1.05 ± 0.03[b]	0.043 ± 0.003[b]
	5 - 8	1.99 ± 0.06a[b]	0.18 ± 0.02[c]	0.18 ± 0.02[c]	2.46 ± 0.01[c]	2.64 ± 0.021[c]	0.024 ± 0.002[c]
	2 - 4	0.62 ± 0.02[c]	0.11 ± 0.01[d]	0.05 ± 0.01[d]	2.40 ± 0.03[c]	4.02 ± 0.056[d]	0.018 ± 0.002[d]

Results revealed that all the phytoconstituents increased with the increase in diameter of the stem except alkaloids. It can be concluded that stem of thicker diameter contains more phytoconstituents than thinner. It was also observed that the samples collected from Jabalpur had higher concentration of phytochemicals than other places i.e. Chhindwara and Seoni.

The phytoconstituents quantified in the present study exhibit great deal of medicinal importance like saponins acts as anti-bacterial and antineoplastic, flavonoids show anti allergic, anti-inflammatory and anti- cancer activity and alkaloids possess a good analgesic, anti-inflammatory and anti-oxidants activity.

Several studies confirmed that these phytochemicals contribute in the treatment of different ailments.

CONCLUSION: Phytochemical screening and analysis will be useful in the presence and quantification of the bioactive principles and subsequently may lead to the drug discovery and development. Our study revealed the presence of medicinally important constituents in the studied species. Many evidences gathered in earlier studies also confirm the identified phytochemicals to be bioactive.

Therefore, extracts from these plants can be used as a good source for useful drugs and their quantified values can be used as a major tool for obtaining a quality control profile for a drug. It may be concluded that thick stem may be utilized for quality products.

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