GAU Research Journal **49** (2) : 208-217 (July 2024)

Phytochemical screening of dioecious Characters of *Tinospora cordifolia* (Willd.) Miers

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Received : May, 2024 Accepted : June, 2024

ABSTRACT

The genus *Tinospora* has been widely investigated by several researchers reported to contain many phytochemicals with marked therapeutic activity. *Tinospora cordifolia* (Willd.) Miers is a dioecious plant where male and female plants are differentfrom each other. In this article we performed quantitative as well as qualitative analysis andmorphological, anatomical and seasonal variationsof their phytochemical studies. Qualitative analysis studies showed their presence and absence ofglycosides, alkaloids, tannins, phenolics, saponins, flavonoids, andterpenoids. Where terpenoids absent in aqueous extract of male stem and steroid was not found in leaf aqueous extact. Similarly in stem of female plant steroids and terpenoids both were absent and in leaf aqueous extract steroids and flavonoids were not found. Quantitative analysis, studies were also performed for studying the total phenolic content and, total alkaloid content in all three seasons.

Key words : Dioecious, Phytochemical, *Tinospora cordifolia*

INTRODUCTION

Tinospora cordifolia, a dioecious plant is commonly known as "Guduchi" or "Giloy,". This botanical gem, belonging to the family Menispermaceae is native to the Indian subcontinent and some parts of Southeast Asia. It has been a subject of great interest because of its versatile therapeutic potential.It has large source ofalkaloids and terpenes(Joshi et al., 2016). *Tinospora cordifolia* is generally found as a climber onshrubs and trees,scattered in tropical India and reaching upto an altitude of 1000 feet in South Asia, Indonesia, the Philippines, Thailand, Myanmar, China, and Sri Lanka worldwide (Mishraetal., 2013). There are some researches available for the use of these plants as anti-inflammatory, antiallergic, anticancer and immunosuppressive, hypoglycaemic, immuno modulatory, anthelminthic agents etc (Mishra et al., 2013)Thechemical constituents reported from the shrub belong to different classes such as aliphatic, glycosides, alkaloids,steroids, sesquiterpenoid, diterpenoid lactones, phenolics, and polysaccharides (Mishra et al., 2013). The leaves of this plant are rich inprotein (11.2%) and are fairly rich in calcium andphosphorus (Kauret al., 2016). It has been of considerable interest in research in

recent years especially during the times of the pandemic Covid-19 and also in the post covid times. *T. cordifolia* is one of the most promising medicinal herbs with multifarious pharmacological paradigms (Gupta et al., 2024). It incorporates anextensive range of pharmacological properties (Modietal.,2021). Female giloy is having a larger concentration of secondary metabolites and superior antioxidant potential than male variety (Modiet al.,2021). Many other reports also concluded that there are some morphological, genetic, microscopical, and phytochemical differences in male and female character of giloy(Nidhiet al., 2013). Indian Tinosporaspecies provide an alternative to conventional chemotherapy for autoimmune diseases because of their good immune modulatory properties (Nidhiet al., 2013).

The plant is dioecious;although, this factor is not given proper thought while collecting the plant material for medicinal purposes. Less studies are available on male and female varieties of giloy and minimal published information on the comparative physicochemical study of *Tinospora cordifolia*is available so far. The values of water and alcohol soluble extractives were found relatively higher in the male variety, while according to studies the total alkaloid, total starch and total mucilage contents were higher in the female variety. All functional groups were found to be approximately the same in both varieties(Rohit et al., 2015).

The findings of thecurrent study support the reports of Choudhary et al., 2014) wherein, the total alkaloid contents were found more in female plant than in the male plant. Relatively higher starch and mucilage contents in the female variety suggest greater yield of “giloysatva” (the aqueous extract, starchy substance of *Tinosporacordifolia*) can be expected from the female variety.Comparing the male and female plants of dioecious species like *Tinospora cordifolia* can provide valuable understandings into potential differences in their phytochemical studies and biological activities. Morphology between male and female *Tinospora cordifolia* plants may include variations in leaf shape, size, stem characteristics, and any other visible distinctionsmale and female plants exhibit.They also differ in the concentration of phenolic compounds likegallic acid (Choudhary et al., 2014) The ecological and environmental factors including habitat, soil type and climate etc might also impact the phytochemical differences between male and female plants.

MATERIALS AND METHODS

Collection of samples-Plant samples, stem and leaf of both male and female varieties of *Tinospora cordifolia* were collected from the local region of Durg (Chhattisgarh).



Fig (1.1) Tinospora cordifolia stem



Fig (1.2) Tinospora cordifolia leaf

Washing and drying-Collected samples were washed with firstly tap water and as well as distilled water for removing dust and other

contamination or impurities. Sample was kept for shade drying at room temperature for a minimum of 15-20 days.

Preparation of extract-Standard methodology was followed for this work (*Logpriya* et. al.,2018). Forth e extraction, Soxhlet apparatus was used. Extract was prepared in three different solvents in Methanol, Acetone and distilled water. After extraction It was kept for further analysis. To determine the antioxidant potential and for quantification of total phenol and total alkaloid was performed with the prepared samples (Logpriya et al., 2018). For this method the samples were dried and grounded to fine powder. The fine powder of sample was used for extraction where sample was extracted with ethanol using soxhlet apparatus at 60°C for 4hr. The extract was filtered through Whatman No.1 filter paper and was concentrated on rotatory evaporator at 40°C to obtain the solid extracts for each sample. These extracts were used for the respective analysis at the required concentrations (Umar et al., 2015).

Qualitative phytochemical test performed by the given tests (Ansari,2006).

Test for Phenols$FeCl_3$ Solution test- Plant aqueous extract (2 ml) then added 2ml of 2% $FeCl_3$ solution. Appearance of blue/green/ black colour indicated the presence of phenol.

Test for Steroid-Liebermann Burchard test- Added5ml of aqueous plant extract to 2ml of chloroform followed by addition of equal amount of conc H_2SO_4which resulted in formation of green colour on top of the colour less chloroform layer formed at the base of test tube.

Test for Flavonoid-Alkaline reagent test- Treated 2 ml of 2% NaOH with aqueous plant extract whichgaveyellow colour appearance then added 2 drops of diluted acid to the mixture. The solution changedto colourless.

Test for Glycoside-Keller kilani test-2ml of stem and leafextract then added 2ml of glacial acetic acid followed by1-2 drops of $FeCl_3$ Solution. Then took another test tube with 2ml of conc.$H_2SO_4$and poured the solution which was prepared before into it. Abrown ring appeared at the interphase which indicates the presence of glycosides.

Test for Saponin-Shaking method- 2ml of plant aqueous extract then added 5ml of distilled water and shookvigorously, formation of a stable foam indicating the presence of saponin.

Test for Terpenoid-Salkowski's test- 2ml of stem and leaf aqueous extract then added 2ml chloroform, 2ml conc. H_2SO_4 then shookgently where reddish-brown colour appeared which indicated the presence of terpenoid ring.

Test for Alkaloid-Hager's test- 2ml of plant aqueous extract haen added 1-2 drops of Hager's reagent (saturated picric acid) where bright yellow colour appeared which confirmed the presence of alkaloids.

Quantitative phytochemical analysis

Total Phenolic Content- The extract 0.1ml (1mg/ml) was added to 2.5ml of 10% Folin-Ciocalteau's reagent (v/v), followed by 2.0ml of 7.5% sodium carbonate solution. The reaction mixture was incubated for 1.5 hour at room temperature in dark, and the absorbance was measured at 765 nm using a UV-Vis Double Beam Spectrophotometer (Systronic). The similar treatment was performed for variable concentrations of Gallic acid (10-50µg/ml). The total phenol content was subsequently calculated using Gallic acid standard curve. The results were

expressed as milligram Gallic acid equivalents (GAE)/g of sample (Hossain *et al.*, 2017; Mariani et al., 2019).

Total Alkaloid Content - For Quantitative determination of alkaloid, 100mg of extract was dissolved in 50ml of 10% ethanolic acetic acid and allowed to stand for 4 hours. The extract was concentrated on a water bath to one-quarter of the original volume followed by addition of 5 drops of concentrated ammonium hydroxide drop wise to the extract until the precipitation was seen. The precipitate was allowed to stand for 3 hours to sediment, then the supernatant was discarded, and the precipitates were washed with 10ml of 0.1M ammonium hydroxide and then filtered using filter paper. The residue on the paper was dried in an oven and its weight was measured. The percentage of alkaloid is expressed mathematically as-% alkaloid= weight of residue/ Weight of sample ×100

Morphological study-Morphological characteristics such as general appearance i.e., shape, size, the surface of leaf and stem, colour, odour, taste, etc. was examined.

Systemic Position

- Tinospora cordifolia
- Kingdom- Plantae
- Division – Magnoliophyta
- Class- Magnoliopsida
- Order- Ranunculales
- Family-Menispermaceae
- Genus- Tinospora
- Species- cordifolia

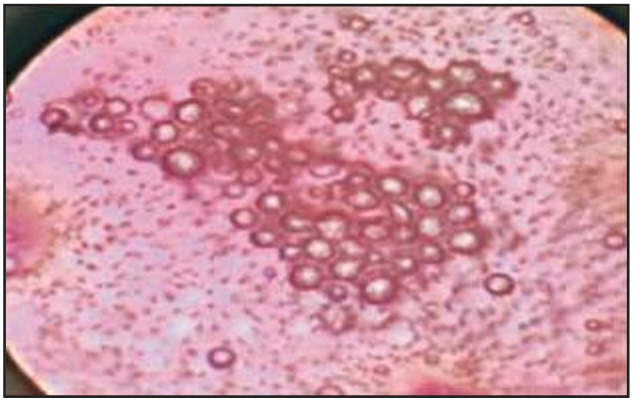


Fig (2.1) T.S. of stem Tinospora cordifolia Male



Fig (2.2) T.S. of stem Tinospora cordifoliaFemale

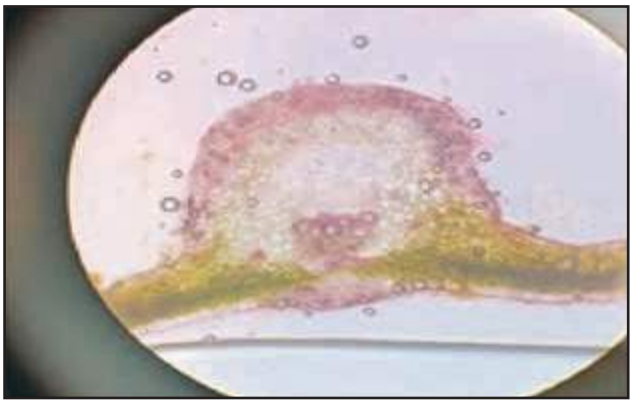


Fig (2.3) T.S. of leaf Tinospora cordifolia Male



Fig (2.4) T.S. of Leaf Tinospora cordifoliaFemale

Anatomical studies

Qualitative Phytochemical study-The extract of the plant of *Tinospora cordifolia*was subjected to different chemical tests for the detection of phytoconstituents such as glycosides, alkaloids, tannins, phenolics, saponins, flavonoids, terpenoids.

Table (i) T. S. characters found of leaf and stem of *Tinospora cordifolia* male and female plant

S. No.	Characters	Male plant	Female plant
		STEM	
1	Hypodermis	Thick Collenchymatous cells	Less thickening Collenchymatous cells
2	Cortex	Broad	Comparatively narrow
3	Mucilaginous canals	less in number	more in number
4	Vascular bundles	6-8 (more in numbers)	6-7 (comparatively less in numbers)
5	Hypodermis and ground tissue	only collenchymatous	Differentiation present
		LEAF	
6	Midrib region	Some sclerenchymatous cells just below the collenchyma cells	Similar to male leaf except the sclerenchymatous patches

Table (ii) : where A represent Stem and BrepresentLeaf of Tinospora cordifoliaof male plant whereresults showing + (presence) and - (absence) of qualitative phytochemicals in aqueous, acetone, methanol solvent

S. NO.	FUNCTIONAL GROUP	TEST/ REAGENTS	RESULTS					
			AQUEOUS		ACETONE		METHANOL	
			A	B	A	B	A	B
1	Phenols	2% $FeCl_3$ Sol.	+	+	+	+.	+	+
2	Steroid	Liebermann BurchardTest	+	-	-	+	-	-
3	Flavonoids	Alkaline Regents	+	+	+	+	+	+
4	Glycosides	Keller kilaniTest	+	+	+	+	-	+

S. NO.	FUNCTIONAL GROUP	TEST/ REAGENTS	RESULTS					
			AQUEOUS		ACETONE		METHANOL	
			A	B	A	B	A	B
5	Saponins	Shaking in Test Tube	+	+	+	-	-	-
6	Terpenoids	Salkowski's Test	-	+	-	-	+	+
7	Alkaloid	Hager's	+	+	+	+	+	+

Table (iii) : Where A represent Stem and B represent Leaf of Tinospora cordifolia female plant, results showing + (presence) and - (absence) of qualitative phytochemicals in aqueous, acetone, methanol solvent

S. NO.	FUNCTIONAL GROUP	TEST/ REAGENTS	RESULTS					
			AQUEOUS		ACETONE		METHANOL	
			A	B	A	B	A	B
1	Phenols	2% $FeCl_3$ Sol.	+	+	+	+	+	+
2	Steroid	Liebermann Burchard Test	-	-	-	+	-	-
3	Flavonoids	Alkaline Regents	+	-	+	+	+	+
4	Glycosides	Keller kilani Test	+	+	+	-	-	+
5	Saponins	Shaking in Test Tube	+	+	+	-	+	-
6	Terpenoids	Salkowski's Test	-	+	-	-	+	+
7	Alkaloid	Hager's	+	+	-	+	+	-

Table (iv) : Quantitative analysis of Alkaloid compound, stem and leaf of Tinospora cordifolia in Female plant of summer, rainy and winter season

S. No.	SEASON	PLANT PART	WEIGHT OF RESIDUE	WEIGHT OF EXTRACT	ALKALOID CONTENT %
1	SUMMER	STEM	0.107 g	0.5g	21.40%
		LEAF	0.109g	0.5g	21.80%
2	RAINY	STEM	0.126 g	0.5g	25.20%
		LEAF	0.119g	0.5g	23.80%
3	WINTER	STEM	0.128g	0.5g	25.60%
		LEAF	0.111g	0.5g	22.20%

Table (v) : Quantitative analysis of Alkaloid compound, stem and leaf of Tinospora cordifoliain male plant of summer, rainy and winter season

S. No.	SEASON	PLANT PART	WEIGHT OF RESIDUE	WEIGHT OF EXTRACT	ALKALOID CONTENT %
1	SUMMER	STEM	0.122 g	0.5g	24.40%
		LEAF	0.109g	0.5g	21.80%
2	RAINY	STEM	0.105 g	0.5g	21%
		LEAF	0.119g	0.5g	23.80%
3	WINTER	STEM	0.135g	0.5g	27%
		LEAF	0.106g	0.5g	21.20%

Table (vi) : Quantitative analysis of Total Phenolic compound in stem and leaf of *Tinospora cordifolia* Female plant of summer, rainy and winter season

S.NO.	SEASON	PLANT PART	O.D. AT 765 NM			MEAN+SD	TPC	TPC GAE (mg/g)
			R_1	R_2	R_3		y= mx + c	X*V/sample wt.(g)
1	SUMMER	STEM	0.341	0.342	0.347	0.3433+0.0032	333.3µg/mL	3.33mg GAE/g
		LEAF	0.412	0.416	0.42	0.416+0.00326	406 µg/mL	4.06 mg GAE/g
2	RAINY	STEM	0.422	0.39	0.397	0.403+0.0168	393µg/mL	3.93 mg GAE/g
		LEAF	0.412	0.427	0.42	0.4196+0.0075	409.6µg/mL	4.10 mg GAE/g
3	WINTER	STEM	0.39	0.297	0.317	0.3346+0.0489	324.6µg/mL	3.25 mg GAE/g
		LEAF	0.442	0.414	0.408	0.421+0.0181	411µg/mL	4.11 mg GAE/g

Table (vii) : Quantitative analysis of Total Phenolic compound in stem and leaf of Tinospora cordifolia male plant of summer, rainy and winter season

S.NO.	SEASON	PLANT PART	O.D. AT 765NM			MEAN+SD	TPC y= mx + c	TPC GAE(mg/g)
			R_1	R_2	R_3			
1	SUMMER	STEM	0.347	0.342	0.342	0.343+0.0028	333.3µg/mL	3.33 mg GAE/g
		LEAF	0.406	0.409	0.416	0.410+0.00513	400µg/mL	4.00 mg GAE/g
2	RAINY	STEM	0.412	0.409	0.39	0.403+0.0119	393µg/mL	3.93 mg GAE/g
		LEAF	0.405	0.402	0.41	0.405+0.0040	395µg/mL	3.95 mg GAE/g
3	WINTER	STEM	0.39	0.397	0.41	0.399+0.0101	398µg/mL	3.98 mg GAE/g
		LEAF	0.41	0.412	0.405	0.409+0.00360	399 µg/mL	3.99 mg GAE/g

RESULT AND DISCUSSION

Qualitative phytochemical study-were analyzed where different phytochemicals were found such as phenol, flavonoid, steroid, glycoside, saponin, Terpenoid and alkaloids in stem and leaf extract of three different solvent such as methanol, acetone and aqueous which can be seen in table (ii) and (iii) above.

Quantitative study-Total amount of phytochemical study was performed by UV-Spectrophotometer for the determination of total phenolic content (TPC), gallic acid was used as a reference compound for the construction of calibration curve. The average absorbance values (at 765 nm) obtained for specific gallic acid concentrations were used to construct the calibration curve for TPC. Using calibration curve and absorbance values of aqueous extract of leaves and stem of male and female plant of different seasons (summer, rainy, winter) for the summer season we obtained 3.33mg GAE/g,4.06 mg GAE/gin stem and leaf respectively, 3.93 mg GAE/g,4.10 mg GAE/gfor stem and leaf respectively in rainystem3.25 mg GAE/g and leaf 4.11 mg GAE/g was found in winter for female *Tinospora* plant. In male plant in summer season3.33 mg GAE/g, 4.00 mg GAE/g in stem and leaf respectively for rainy season3.93 mg GAE/g, 3.95 mg GAE/g stem and leaf respectively for winter 3.98 mg GAE/g, 3.99 mg GAE/gstem and leaf respectively were found. Studies were reported total phenolic content in *Tinospora* is ranging from 0.77 to 6.35mg GAE/g (Yashika et al.,2024).The comparison of the total phenolic compound in male we found better TPC in rainy season and in female plant also gave the better TPC in rainy as well as winter season.

Total alkaloid content-Total alkaloid content

was studied in stem and leaf of *Tinospora cordifolia*. In male plant, we get greater percentage of alkaloid content in summer and winter season than in rainy season, where 24.4%,21% and 27% alkaloid contents in stem and 21.8%, 23.8%, 21.2% alkaloid contents inleaf part in summer, rainy and winter season respectivelyfound Similarly for female plant part, 21.4%, 25.2% and 25.6% alkaloid contents were found in stem, 21.8%, 23.8% and 22.2% in leaf part in summer, rainy and winter season respectively.

CONCLUSION

Phytochemical composition is varies between both the plant parts which can influence their properties male plant has found more alkaloid content which is responsible for good immunity similarly female plant found great TPC content which is effective for antioxidant, anti-inflammatory treatment.

ACKNOWLEDGEMENT

The authors thank theDepartment of Botany, Govt V.Y.T. PG Autonomous college Durg, Chhattisgarh for the necessary help and support.

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