Phytochemical Screening of Tinospora cordifolia Stem Extract

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***Abstract*—Tinospora cordifolia is a member of the Menispermaceae family and has a number of medicinal applications. Giloy is a highly prized and often used plant in Ayurvedic medicine. The goal of this study was to screen for Tinospora cordifolia stems and investigate the phytochemicals that are present in the plant, both qualitatively and quantitatively. Alkaloids, glycosides, saponins, phenols, tannins, and flavonoids are among the phytochemical components found in methanolic extract of the stem of Tinospora cordifolia. Fresh stems of Tinospora cordifolia were quantitatively determined to contain 2.58 ± 0.08 % w/w of glycoside (%), 8.2501 ± 0.03 % w/w of total tannins, and 1.865 ± 0.017 % w/w of phenolics. As demonstrated by the methanolic extract of Tinospora cordifolia stem, a higher concentration of phenolic and tannin components results in a more potent free radical scavenging activity. According to the DPPH experiment, these powders' antioxidant potential was 61.18 percent.**

***Key word— Tinospora cordifolia, Stem, Screening, Tannins, Phenols, Glycosides, Free radical scavenging* activity.**

I. INTRODUCTION

Giloy (*Tinospora cordifolia*) is a huge, genetically varied climbing shrub that belongs to the Menispermaceae family and is also known as "Guduchi" in Sanskrit. It may be found at higher elevations and has distinctive greenish-yellow blossoms [26]. The heart-leaved moonseed plant, as it is generally called, is mostly found in the Konkan area of Maharashtra's natural forests. The genus Tinospora has about thirty-two species of climbing shrubs that are found in the Pacific Islands, Madagascar, Australia, and tropical Africa. It is the most significant plant from a medical and commercial standpoint. It is indigenous to several areas of China and the tropical portions of the Indian subcontinent. [35, 5]. From Kumaon eastward and southward to Sri Lanka, this year-round climber may be found in all of tropical India, reaching elevations of 900 meters. It is often collected in the summer month of May. [29]

The medicinal qualities of this plant were explored in both Ayurveda and traditional medical systems. The plant may be found at heights of up to 1,200 meters above mean sea level throughout all of tropical India, from Kumaon and Assam in the north to West Bengal, Bihar, Deccan, Konkan, Karnataka, and Kerala in the south. It grows over tiny trees and hedges and is very widespread in dry and deciduous woodlands. It thrives in a variety of soil types, from acidic to alkaline, and requires a modest level of soil moisture [4]. In addition to its anti-inflammatory properties, the stem is bitter, stomachic, cholagogue, diuretic, and tonic. It also lowers fever and thirst, purifies blood, has hepatoprotective properties, and cures urinary tract infections, upper respiratory tract infections, jaundice, and burning sensations. [39]

One of the most valued and popular herbs in Ayurvedic therapy is Giloy. On the Indian subcontinent, it has been in use for a very long period. Research on this topic has been ongoing for a long time. [8]. This plant is also used as a traditional medicine by indigenous people in tropical and subtropical parts of Africa, Australia, and Asia [28]. Over the past 10 years, the therapeutic usage of herbal medicine has significantly increased worldwide. The toxicity and side effects of allopathic medications have led to an increase in the use of herbal remedies in the field of medicine. Traditional practitioners provide the majority of medical care for around 80% of the population in our country. Therefore, it is imperative to recognize the medicinal and economic benefits of the plants and animals cultivated in the Himalayan foothills. [33]
Tinospora cordifolia is a rich source of alkaloids, furano diterpenoids, clerodane norditerpenoids,

sesquiterpenoids, phenolics, lignans, sterols, aliphatic compounds, polysaccharides, essential oil, and fatty acids [9]. It has been shown that bitter substances like tinosporin, tinosporic acid, and tinosporol, as well as alkaloids like berberine, have therapeutic advantages. The kidney, liver, and spleen are the main organs that *Tinospora cordifolia* targets when it operates pharmacologically on the body. [21]

Tinospora cordifolia has been used traditionally to treat bone fractures, fever, skin conditions, chronic diarrhea, jaundice, asthma, and overall fragility. In the form of juice, decoction, paste, powder, and tablets, it has also been used into several people's Ayurvedic therapies [36]. By invigorating the liver and spleen, *Tinospora cordifolia* serves as a blood cleanser, eliminating faulty and damaged red blood cells from peripheral blood circulation. Because of its high alkaloidal content, the stem of *Tinospora cordifolia* is permitted for use in Ayurvedic medicine and is used for therapy according to Indian Pharmacopoeia. [1]

Traditional pharmaceuticals play a major role in the global healthcare system. It is estimated that developing countries are home to 80% of the world's population. For medical treatment, they mostly rely on herbal medicines and traditional medicine, which are integral to their culture [12]. The main objective of the research to screening of phytochemical present in *Tinospora cordifolia* stem extract which was used as a herbal and tradition medicine.

II. MATERIAL AND METHODS:

Plant Material: The Giloy stems were procured from local farmers Mr. Vijay Abhyankar and Mayuresh Abhyankar, A/P.: Jambhulpada, Taluka: Sudhagad, District: Raigad (MS), Maharashtra, India. The experiment was conducted at Post Graduate Institute of Post Harvest Technology and Management Killa-Roha, under Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli. Dist-Ratnagiri (M.S).

Preparation of plant extract:
The acquired plants' stems were meticulously cleared of any extraneous material. The sample was first dried in an oven set to 60 degrees Celsius for three days. The dried material was then ground into a powder using a grinding machine. Five grams of this powder were taken in triplicate, dissolved in fifty millilitres of methanol, and shaken on a tabletop for forty-eight hours in a round joint conical flask. A vacuum rotary evaporator was used to filter and concentrate the sample after centrifugation. The concentration was increased to 10 millilitres by adding additional of the suitable solvents to make the stock solution. Samples were stored at 4°C for further examination.
Qualitative Screening: (Preliminary phytochemical evaluation)

Detection of alkaloids:
Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrates were used to test for the presence of alkaloids. The alkaloid detection was performed by different tests like Mayer's test (Filtrates were treated with Mayer's reagent (saturated solution of potassium mercuric iodide) and formation of a yellow cream precipitate indicates the presence of alkaloids), Wagner's reagent (Filtrates were treated with Wagner' reagent (saturated solution of iodine in potassium iodide) and formation of brown/reddish brown precipitate indicates the presence of alkaloids), Dragendorff's reagent (Filtrates were treated with Dragendorff's reagent (saturated solution of potassium bismuth iodide) and formation of a red precipitate indicates the presence of alkaloids) and Hager's reagent (Filtrates were treated with Hager's reagent (saturated solution of picric acid) and formation of a yellow coloure precipitate indicates the presence of alkaloids) [2].

Detection of glycosides: Extracts were hydrolyzed with dilute hydrochloric acid and then were subjected to tests for glycosides by different tests such as Modified Borntrager's test and Legal's test. [13,10]
Detection of saponins: The saponins were identified by Froth test. Extracts were diluted with distilled water to 20ml and were shaken in graduate cylinders for 15 min. Formation of foam of height of 1 cm indicates the presence of saponins [2].

Detection of phenols: (Ferric chloride test) Extracts were treated with a few drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols. [18]

Detection of tannins: (Gelatin test) Gelatin solution (1%) containing sodium chloride was added to the extracts. Formation of white precipitates indicates the presence of tannins. [18]

Detection of flavonoids:

Alkaline reagent test: Extracts were treated with a few drops of sodium hydroxide solution. Formation of intense yellow colour, which become colourless on the addition of dilute acid, indicates the presence of flavonoids.

Lead acetate test: Extracts were treated with a few drops of lead acetate solution. Formation of yellow coloure precipitates indicates the presence of flavonoids.

Zinc- hydrochloric acid reduction test: To the crude extracts, a pinch of zinc dust and small amount of concentrated hydrochloric acid were added. Appearance of magenta colour after few minutes indicates the presence of flavonoids [13].

Quantitative Screening:

Glycoside (%)

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 hrs. Cooled and extracted with chloroform (2 x 25 ml). The chloroform layer was then washed with distilled water till it is acid free. Transferred to a pre weighed beaker and dried in an oven to a constant weight. [23] Percentage of cardiac glycoside was calculated from the following formula:

$$\text{Percentage of Cardiac Glycoside} = \frac{(B-A)\times 100\times 2}{Weight\ of\ sample}$$

Where,

B = weight of beaker with content;

A = weight of empty beaker

Total tannins content (% w/w)

Two gram of the powdered drug was extracted for 20 hrs with petroleum ether. The residue was boiled for 2 hrs with 300 ml of double distilled water. It was cooled, filtered with Whatman No.1 filter paper and diluted to 500 ml with double distilled water. 25 ml of the infused was pipetted out in 2 litre porcelain dish to which 20 ml indigo solution and 750 ml double distilled water was added. This was titrated with standard KM_nO_4 (0.1N) solution by adding 1 ml at a time, until blue solution changed to green, after which a few drops were added at a time until solution turned golden yellow in colour (A). Similarly, a mixture 20 ml indigo solution and 750 ml of double distilled water was titrated (B). [32]

The percentage of total tannins = [(A-B) X 1000] / weight of drug sample taken X 0.1.

Each

ml of 0.1 N KMnO4 = 0.004157 g of total tannins.

Total phenolic content (% w/w)

Preparation of extract by soxhlet extraction method

A thimble was prepared by using 0.5 mm Whatman No. 1 filter paper. About 100 gm of powdered material was uniformly packed into a thimble and run in soxhlet extractor. It was exhaustible extracted with ethanol and methanol for the period of about 48 hours or 22 cycles or till the solvent in the siphon tube of extractor become colourless. After that extracts were filtered with the help of filter paper (Whatman No. 1) and solvent was evaporated in a rotary evaporator to get the syrupy consistency, then after the extract was kept in refrigerator at 4 ºC to determine antioxidant activity [20].

Total phenolic content (TPC) in the extracts was determined using the Folin-Ciocalteu reagent method [30]. This method depends on the reduction of FCR by phenols to a mixture of blue oxides which have a maximal absorption in the region of 765 nm using a spectrophotometer (Shimadzu Uv-160A PC, Shimadzu Corporation, Kyoto, Japan). A stock solution of stem extracts was prepared to the concentration of 10 mg/ml (50 mg of powdered extract dissolved in 5 ml of ethanol). To 1.0 ml of each extract, 5 ml of Folin-Ciocalteu reagent was added. The solution was vortexed and incubated in the dark for 3 minutes. After that 5 ml of sodium carbonate (75 g/l) solution was added to the mixture and mixed thoroughly. The mixture was incubated in the dark for 1 hour. The absorbance was read at 765 nm. Blank consisted of 5 ml Folin-Ciocalteu reagent, 1 ml ethanol and 4 ml sodium carbonate solution. Based on the measured absorbance, the concentration of phenolics was read (mg/ml) from the calibration line; then the total content of phenolic compounds were expressed as tannic acid equivalents (TAE), calculated by the following formula:

$$C = c\times V/m$$

Where,

C - total content of phenolic compounds (mg of TA/g of extract)

c - the concentration of tannic acid established from the calibration curve (mg/ml)

V- the volume of extract (ml)

m - the weight of pure plant extract (g)

Free radical scavenging activity

The scavenging activity of *Tinospora cordifolia* stem extracts was determined using DPPH assay with some minor modifications [6].

This method depends on the reduction of purple DPPH (1, 1-Diphenyl-2-Picryl Hydrazyl) to a yellow colour diphenyl picrylhydrazine. The determination of the disappearance of free radicals was done using

a spectrophotometer (Shimadzu Uv-160A PC, Shimadzu Corporation, Kyoto, Japan). The remaining DPPH which showed maximum absorption at 518nm was measured. Each plant extract sample's stock solution (10 mg/ml) was diluted to final concentrations of (9, 8, 7, 6, 5, 4, 3, 2 and 1 mg/ml) in DMSO (Dimethyl sulfoxide). One ml of a 0.3 mM DPPH DMSO solution was added to 2.5 ml of sample solution of different concentrations. These are test solutions. Ascorbic acid was used as positive control and prepared in the same manner as above. As DPPH is sensitive to light, it is exposed to the minimum possible light. These solutions were allowed to react at room temperature for 30 minutes. The absorbance values were measured at 518 nm and converted into the percentage antioxidant activity using the following equation:

Free radical scavenging activity (%) = $\frac{Abs(control)-Abs(sample)}{Abs(control)} \times 100$

Where, (Abs = Absorbance)

The test was done in triplicate.

III. RESULTS

Qualitative Screening of extract:

Table No. 1: Preliminary qualitative screening of *Tinospora cordifolia* extract

Sr. No.	Phytoconstituent	Tests	Ethanolic extract
1.	Alkaloids	Dragondroff's test	+
		Wagners test	+
		Mayers test	-
		Hagers test	-
2.	Glycosides	Legal test	-
		Modified Borntrager's	+
3.	Saponins	Foam test	-
4.	Phenols	Ferric Chloride test	+
5.	Tannins	Ferric Chloride test	+
6.	Flavonoids	Alkaline reagent	+
		Lead acetate	+
		Zinc Hcl test	-

(-) A sign indicates absence of constituent in the respective screening test; (+) sign indicates the presence of a constituent in the respective screening test.

Qualitative Screening of extract:

Glycoside (%)

The Glycoside (%) of fresh Giloy stem was found to be 2.58 ± 0.08 of the fresh weight, indicating a significant presence of glycoside.

Total tannins content (% w/w)

The total tannin content (% w/w) of fresh Giloy stem was found to be 8.2501 ± 0.03 of the fresh weight, indicating a significant presence of tannins.

Total phenolic content (% w/w)

The total phenolic content (% w/w) of fresh Giloy stem was found to be 1.865 ± 0.017 of the fresh weight, indicating a significant presence of phenolic compound.

Free radical scavenging activity

The free radical scavenging activity of Giloy stem was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. The data for free radical scavenging activity are presented in Table 1 and graphically depicted in fig.1

IV. DISCUSSION

Qualitative Screening (Phytochemical investigation):

The present study reveals that the extract from the stem of *Tinospora cordifolia* shows the presence of phytochemical constituents like alkaloids, glycosides, saponins, phenols, tannins, and flavonoids in methanolic extracts, as shown in Table 1. The described phytochemical result above in current work also marked nearly a similar presence of constituents with ethanolic extract of differently processed T. cordifolia stem powders. These results are also consistent with the previously published reports [16,11].

Quantitative Screening:

Glycoside (%)

The glycoside (%) of fresh Giloy stem was found to be 2.58 ± 0.08 of the fresh weight, indicating a significant presence of glycoside. These results are consistent with the previously correlations evidence on same spices *Tinospora cordifolia* stem. [7,22,23 and 34]

Total tannins content (% w/w)

The total tannin content (% w/w) of fresh Giloy stem was found to be 8.2501 ± 0.03 of the fresh weight, indicating a significant presence of tannins. These results are in agreement with the reports of Sivakumar *et al*. [32]; Kumar *et al*. [14]; Sardhara and Gopal [27]; Sinha *et al*. [31] and Nagalakshmi *et al*. [19] on *Tinospora cordifolia* stem.

Total phenolic content (% w/w)

The total phenolic content (% w/w) of fresh Giloy stem was found to be 1.865 ± 0.017 of the fresh weight, indicating a significant presence of phenolic compound. The same line of work was done by Premanath and Lakshmidevi [25]; Praveen *et al*. [24]; Mishra *et al*. [15]; Upadhyay *et al*. [37]; Balkrishna *et al*. [3]; Kaur *et al*. [11] and Modi *et al*. [17] on *Tinospora cordifolia* stem.

Free radical scavenging activity

The free radical scavenging activity of Giloy stem was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. The same line of work was done by Sivakumar *et al*. [32]; Balkrishna *et al*. [3] and Upadhyay *et al*. [38] on *Tinospora cordifolia* stem.

V. CONCLUSION

Based on the findings of the current studies, it can be concluded that the stem of *Tinospora cordifolia* shows the presence of phytochemical constituents like alkaloids, glycosides, saponins, phenols, tannins and flavonoids in methanolic extract and total glycoside, total tannin and total phenolic content (2.58 ± 0.08 %, 8.2501 ± 0.03%, and 1.865 ± 0.017 %, respectively) was observed in the freshly harvested stem of Giloy. The free radical scavenging activity of Giloy stem was evaluated using the DPPH assay (61.18 %) recorded in the stem of Giloy. As a source of many physiologically active compounds with protective activity, *Tinospora cordiofolia* stem is very potentially useful as an ingredient for functional food development.

VI. ACKNOWLEDGMENTS

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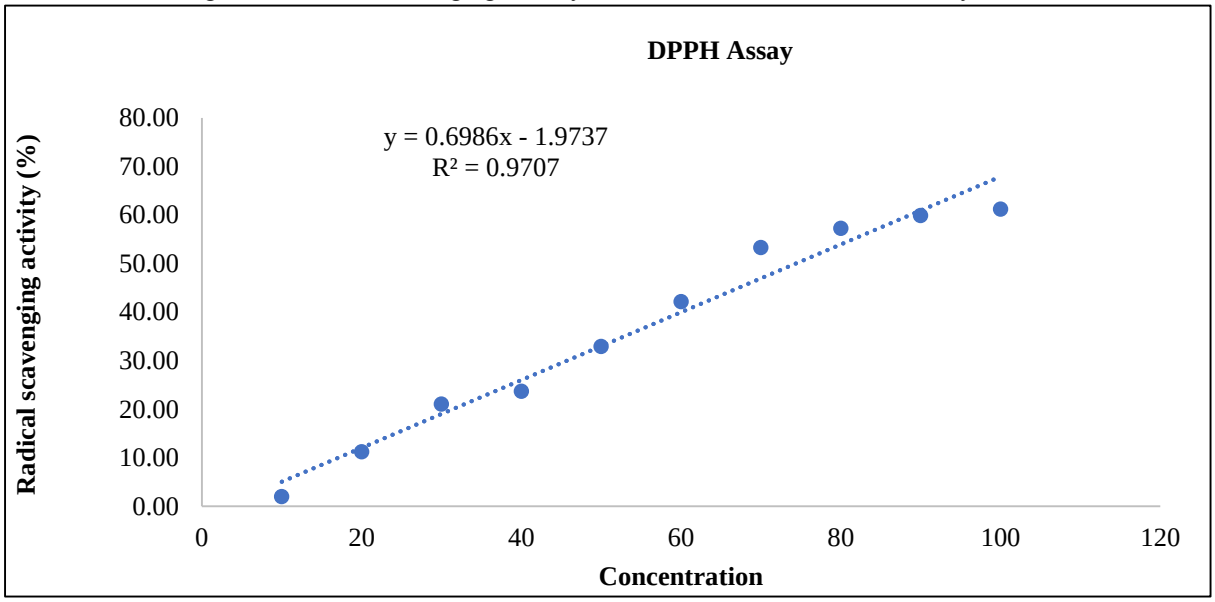
Table No.2 Free radical scavenging activity of methanolic extract of fresh Giloy stem

Methanolic extract of Giloy stem sample (µg)	(DPPH) Radical Scavenging Activity %
10	0.97
20	11.18
30	21.05
40	23.68
50	32.89
60	42.11
70	53.29
80	57.24
90	59.87
100	61.18

Fig 1 Free radical scavenging activity of methanolic extract of fresh Giloy stem



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