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RESEARCH ARTICLE

Evaluation of Antioxidant Potential of Stem and Leaf Extracts of Himalayan *Tinospora cordifolia* Hook.f. & Thomson

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Abstract:

Background:

Medicinal plants are considered a rich source of ingredients, which can be used in drug development and synthesis. *Tinospora cordifolia* (Wild.) Hook.f. & Thomson, commonly known as guduchi, heart-leaved moonseed and giloya is a herbaceous vine of the family Menispermaceae, has several beneficial properties including antioxidant activity.

Aim:

The present study was carried out to analyze the antioxidant activity of leaf and stem extracts of *Tinospora cordifolia* by using DPPH (1,1-Diphenyl-2-picrylhydrazyl) and ABTS (2,2´-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) free radical scavenging assays.

Materials and Methods:

Dried and powdered leaves and stem of *T. cordifolia* were extracted with methanol. Ascorbic acid was taken as standard. Total phenolic content was estimated by using Folin-ciocalteu's reagent while total flavonoid content by aluminium chloride reagent to find the correlation of polyphenols with antioxidant activity. ABTS assay of methanolic leaf and stem extracts showed the highest scavenging activity as compared to the DPPH assay.

Results:

Methanolic stem extract showed higher phenolic and flavonoid content along with antioxidant activity as compared to the methanolic leaf extract.

Conclusion:

The stem extract exhibited more antioxidant activity than the leaf extract with regards to the all parameters analyzed.

Keywords: Tinospora cordifolia , Antioxidant activity, Phenolic content, Flavonoid content, Leaf extract, Stem extract.

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1. INTRODUCTION

Plants are one of the most important sources of medicines. They can synthesize different bioactive molecules such as phenols, flavonoids, vitamins, alkaloids, terpenoids, tannins, glycosides, quinones and many others. Most of the plants used for medicinal purposes have been identified and their uses are well documented and described by various authors [1 - 3]. Medicinal plants have been used as natural medicines and they came into existence since prehistoric times. There are different ways in which plants have been found useful in medicines. The crude extract of plants has been used directly because of the presence of natural chemical constituents such as berberine, morphine, psilocin and vincristine [4]. Medicinal plant research has increased all over the world and collected the immense potential of medicinal plants used in various traditional

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systems. The extraction and characterization of bioactive compounds from medicinal plants have resulted in the introduction of new drugs with high medicinal value [5]. The parts of medicinal plants that may be used are different types of seeds, root, leaf, fruit, flowers or even the whole plant [6].

Tinospora cordifolia (Wild.) Hook.f. & Thomson commonly known as guduchi, heart-leaved moonseed and giloya, indigenous to the tropical areas of India, Myanmar and Sri Lanka, is a herbaceous vine of the family Menispermaceae. Guduchi is widely used in veterinary folk medicine/ ayurvedic system of medicine for its genera tonic, antiperiodic, antispasmodic, anti-inflammatory, anti-arthritic, anti-allergic and anti-diabetic properties [7, 8]. *Tinospora cordifolia* is a rich source of alkaloids, furan diterpenoids, clerodane, norditerpenoids, sequiterpenoids, phenolics, lignans, sterols, aliphatic compounds, polysaccharides, essential oil and fatty acids. In its pharmacological actions, *Tinospora cordifolia* targets body organs, mainly kidney, liver, and spleen [9]. The genus *Tinospora cordifolia* has been widely investigated by a number of workers and reported to contain a number of phytochemicals with marked therapeutic activity [10 - 26]. Free radicals form in our body as a result of biological oxidation. Oxidation is a natural process in organisms for the production of energy to fuel biological cycles. Oxidation by-products of normal metabolism cause extensive damage to DNA, protein and lipids, constituting a major contribution to ageing and also to degenerative disease. Oxidative damage is associated with chronic degenerative diseases, including cancer, coronary artery disease, hypertension and diabetes [14]. An antioxidant is a chemical that prevents the oxidation of other chemicals. They protect the key cell components by neutralizing the damaging effects of free radicals, which are natural by-products of cell metabolism [27]. Antioxidants occur naturally in many fruits and are able to neutralize free radicals by donating an electron and convert them into harmless molecules [28].

During the last few decades, there has been an increasing interest of scientists in the study of medicinal plants and surveys on their traditional use all over the world. Although some eminent work on *Tinospora cordifolia* has been reported from some areas of the world like Angat, Bulacan, Philippines [16] and some from different regions of India like Mysore [10, 11], Ranchi [13], Khandari Campus Agra [14, 15], Bengaluru [18], Gwalior (MP) [19], Udupi (Karnataka) [20], Chitrakoot region (MP) [22], West Singhkhum (Jharkhand) [23], Hazaribagh, and Bokaro (Jharkhand) [24]. However, comparative reports on Himalayan *Tinospora cordifolia* stem and leaf extracts so far have not been investigated. Therefore, keeping these points in mind, the present study was undertaken in order to identify the antioxidant potential and quantitative analysis of stem and leaf extracts of *Tinospora cordifolia* (Wild.) Hook.f. & Thomson, which were procured from the Haldwani regions of Kumaun Himalaya.

2. MATERIALS AND METHODS

2.1. Collection and Processing of Plant Materials

The plant material (*Tinospora cordifolia* (Wild.) Hook.f. & Thomson) of the present study was collected from Haldwani, District Nainital, Uttarakhand, India (Latitude: 29.17558; Longitude: 79.51718; Altitude: 424m). The identity of the collected specimen was authenticated and submitted at the Taxonomy lab of D. S. B. Campus, Nainital. The plant material used for antioxidant activity was thoroughly washed off under running tap water to remove all the debris and soil and shade dried at room temperature for two to three weeks. The air-dried plant material was finely ground into powdered and packed in self-seal airtight polythene bags for further analysis [14].

2.2. Extraction

Fivegram powder of each stem and leaf of *Tinospora cordifolia* (Wild.) Hook.f. & Thomson was soaked in 50 mL of methanol solvent in an orbital shaker at 37°C 120 rpm for 3 days. After that, the extracts were filtered with the help of Waltman No. 1 and solvent was evaporated in an incubator at 37°C to get the syrupy consistency, then 25% DMSO (Dimethyl sulphoxide) was added and after that the extracts were kept in a refrigerator at 0°C to determine the antioxidant activity [14].

2.3. Antioxidant Activity Measurement

The scavenging activity of *Tinospora cordifolia* (Wild.) Hook.f. & Thomson stem and leaf extracts was determined using DPPH and ABTS assay.

2.3.1. DPPH (1,1-Diphenyl -2-picrylhydrazyl) free radical scavenging assay

The method adopted for this study was developed by Blois (1958), which depends on the reduction of purple DPPH to yellow coloured diphenyl picrylhydrazine radical scavenger [29]. A solution of the radical was prepared by dissolving 1mg/m L DPPH in methanol. The determination of the disappearance of the free radical was done using a UV-visible spectrophotometer. Each plant extract sample stock solution (1mg/mL) was diluted in final concentration of 10, 20, 40, 80, 160, 320, 640µg/mL). Ascorbic acid was used as positive control and prepared in the same manner as an extract. As DPPH is sensitive to light, it is exposed to minimum sensitive light. These solutions were allowed to react at room temperature for 30 minutes in the dark [14].

The absorbance values were measured at 517nm and converted into percentage antioxidant activity using the following equation:

(where Abs =Absorbance)

All the determinations were performed in triplicate.

$$\text{Free radical scavenging activity (\%)} = \frac{\text{Abs (Control)} - \text{Abs (Sample)}}{\text{Abs (Control)}} \times 100$$

2.3.2. ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) Free Radical Scavenging Assay

This method depends on the reduction of sea green coloured ABTS to transparent ABTS [30]. ABTS + cation radical was produced by a reaction between 7mM ABTS and 2.45mM potassium persulfate stored in the dark at room temperature for overnight before use. The solution was diluted with 50% methanol. Each plant extract sample stock solution (1mg/mL) was diluted in a final concentration of 10, 20, 40, 80, 160, 320, 640µg/mL). Ascorbic acid was used as positive control and prepared in the same manner as above. Then 2mM ABTS added to different concentrations of extract and incubate in the dark for 30 min at room temperature. As ABTS is sensitive to light, it is exposed to minimum sensitive light at room temperature. The determination of the disappearance of the free radical was done using a UV-visible spectrophotometer. All the measurements were carried out at least three times [20]. Percent inhibition of absorbance at 745nm was calculated using the formula:

$$\text{Free radical scavenging activity (\%)} = \frac{\text{Abs (Control)} - \text{Abs (Sample)}}{\text{Abs (Control)}} \times 100$$

(where Abs =Absorbance)

All the determinations were performed in triplicate.

The concentration of any antioxidant, which shows 50% inhibition, is called as IC_{50} value. In the experiment, IC_{50} values for extracts and standards were calculated from the regression plot between serial dilutions and the % inhibition.

2.4. Quantitative Analysis

2.4.1. Total Phenolic Content

Total phenolics were estimated by following Folin-Ciocalteu's phenol reagent (FCR) method of Upadhyay *et al*. with little modification [14]. Different concentrations (0.5,1, 2, 4, 8mg/mL) of standard and test samples were prepared in methanol from the stock solution. 0.5mL of each concentration was mixed with 0.2 mL of FCR and 0.5 mL Na_2CO_3 (20%). After 30min of incubation at room temperature, absorbance was measured at 750nm against blank (methanol). The concentration of total phenolic content was calculated as mg gallic acid equivalent/g extract from the calibration curve of the standard solution of gallic acid.

2.4.2. Total Flavonoid Content

Total flavonoid content was estimated by aluminium chloride ($AlCl_3$) method. Different concentrations (0.5, 1, 2, 4, 8mg/mL) of standard and test samples were prepared in methanol from the stock solution. 0.5ml of each concentration was mixed with 0.2 ml of $AlCl_3$, 0.5mL $NaNo_3$ (20%). The concentration of total flavonoid content was calculated as mg quercetin acid equivalent/g extract, from the calibration curve of the standard solution of quercetin acid. Then allow the solution at room temperature for 30 min. The absorbance was measured at 506nm by spectrophotometer [22].

3. RESULTS AND DISCUSSION

3.1. Antioxidant Activity

3.1.1. DPPH (1,1-Diphenyl-2-picrylhydrazyl) Free Radical Scavenging Activity of Stem and Leaf Extracts

Both the stem and leaf extracts of *Tinospora cordifolia* (Wild.) Hook.f. & Thomson showed effective scavenging activity against the free radicals. The DPPH standard (ascorbic acid) had a maximum (96.58%) free radical scavenging activity at 640µg/mL and with the lowest IC_{50} value of 22.38 µg/mL (Tables **1**-**3**). The methanolic stem extract showed the highest scavenging activity (55.94%) at 640µg/mL and lowest (25.32%) at 10µg/mL (Table **1**). It was found that when the concentration of the extract increased, the absorbance value gets decreased regularly. The methanolic leaf extract of *T. cordifolia* showed comparatively lesser activity with the highest scavenging activity (48.03%) at 640 µg/mL and the lowest (25.01%) at 10µg/mL (Table **1**) as compared to the methanolic stem extract. Similar results were also observed by Upadhyay *et al*. [14] in the methanolic stem extract of *T. cordifolia* and found that at a concentration of 10 mg/mL, DPPH free radical scavenging activity was low (44%). In contrast, Praveen *et al*. [31] reported that methanolic leaf extract showed the highest scavenging activity. Scavenging effect on DPPH radical was observed to be higher in methanolic leaf extract as compared to the methanolic extract of stem of *T. cordifolia* procured from the local market of Mysore [11]. The leaf and stem extracts of the methanolic solvent of *T. cordifolia* showed greater percentage inhibition with IC_{50} values of 0.54 mg/mL and IC_{50} 0.74 mg/mL, respectively, which was more than the present study [11]. In a previous study, the methanolic stem extract of *T. cordifolia* from the Philippines exhibited good free radical scavenging activity potential with an IC_{50} value of 0.861 µg/µL [16].

3.1.2. ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) Free Radical Scavenging Activity of Stem and Leaf Extracts

All the concentration of the test solution more or less scavenged the free radicals. Both the stem and leaf extracts of *Tinospora cordifolia* showed effective scavenging activity against ABTS free radical. The ABTS standard (Ascorbic acid) had maximum free radical scavenging activity (91.55%) at 80µg/mL concentration with the least IC_{50} value of 9.36 µg/mL as represented in Table **2**. The methanolic stem extract showed the highest scavenging activity (74.21%) at 640µg/mL concentration and the lowest (4.51%) at 20µg/mL (Table **2**). The methanolic leaf extract of *T. cordifolia* showed comparatively lesser activity with the highest scavenging activity (73.86%) at 640 µg/mL concentration and the lowest (6.33%) at 10µg/mL concentration as compared to the methanolic stem extract (Table **2**). The stem extract of *T. cordifolia* showed better IC_{50} value in ABTS (126.93 µg/mL) and DPPH (500.40 µg/mL) assays as compared to the leaf extract of the plant with IC_{50} values if 379.31 µg/mL and 677.78µg/mL in ABTS and DPPH assays respectively (Table **3**). With all the extracts (leaf and stem), dose-dependent radical scavenging was observed, *i.e*., when the concentration of the extract was increased, the free radical scavenging activity also increased. These results are in agreement with reports of Upadhyay *et al*. [14]. ABTS scavenging assay of leaf and stem extracts of the methanolic solvent of *T. cordifolia* from Mysore showed greater inhibition as compared to the present study with the least IC_{50} values of 95µg/mL and 107 µg/mL [11]. The ABTS scavenging assay of both leaf and stem extracts was the most potent method as compared to the DPPH scavenging assay with the highest scavenging activity and least IC_{50} values.

3.2. Quantitative Analysis

3.2.1. Total Phenolic Content (TPC)

TPC was measured by folin-ciocalteu's phenol reagent method and gallic acid was taken as standard in different concentrations (mg/mL). TPC of methanol extract of *Tinospora cordifolia* leaves and stem was calculated as gallic acid equivalent (GAE) (mg GAE/g), using the calibration curve, which was based on the following equation: Y= 1.5118x-0.1533 R^2=0.9664 shown in Fig. (**1**). It was clear that the phenolic content in methanolic leaf extract (8.51 mg GAE /g) was lower as compared to the methanolic stem extract (17.44 mg GAE /g) of *Tinospora cordifolia* as shown in Table **4**. The assay supported that there was a positive relationship between total phenolic content and antioxidant activity. The TPC of methanolic stem extract was less than the TPC in the

Table 1. DPPH radical scavenging activity of methanolic stem and leaf extracts of *Tinospora cordifolia*.

Concentration (µg/mL)	Ascorbic Acid (% Scavenging Activity)	Stem Extract (% Scavenging Activity)	Leaf Extract (% Scavenging Activity)
10	21.42	25.32	25.01
20	53.57	25.54	26.64
40	80.43	26.69	27.92
80	84.00	27.48	28.94
160	89.05	33.76	30.66
320	93.78	44.10	39.28
640	96.58	55.94	48.03

Table 2. ABTS scavenging activity of methanolic stem and leaf extracts of *Tinospora cordifolia*

Concentration (µg/mL)	Ascorbic Acid (% Scavenging Activity)	Stem Extract (% Scavenging Activity)	Leaf Extract (% Scavenging Activity)
5	30.46	-	-
10	58.49	-	6.33
20	85.41	4.51	12.79
40	90.55	11.89	18.81
80	91.55	26.89	30.05
160	-	56.50	49.77
320	-	73.43	73.43
640	-	74.21	73.86

Table 3. IC_{50} values of leaf and stem extracts against DPPH and ABTS activities in, µg/mL.

Antioxidant Activity	Ascorbic Acid (Standard)	Stem Extract	Leaf Extract
DPPH	22.38	500.40	677.78
ABTS	9.36	126.93	379.31

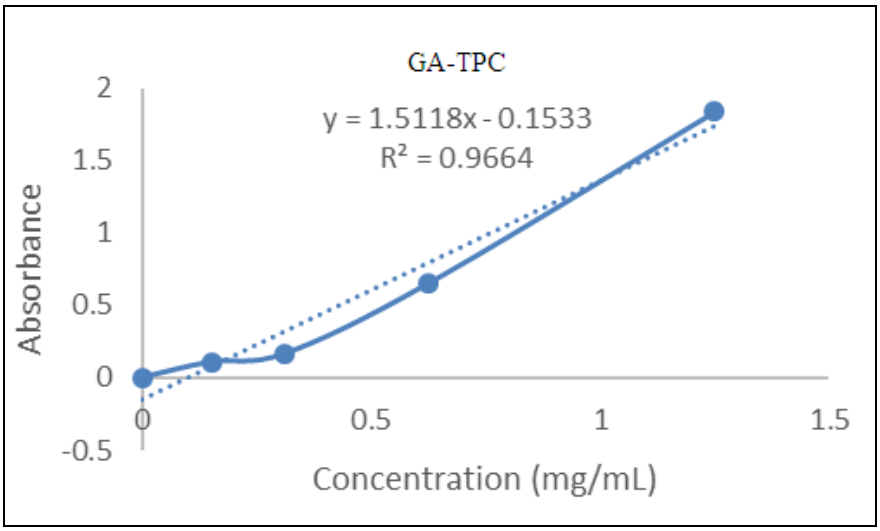

Fig. (1). Standard curve of gallic acid for total phenolic content.

Table 4. Quantitative analysis of stem and leaf extracts of *Tinospora cordifolia*.

Samples	TPC (mg GAE /g of extract)	TFC (mg QE /g of extract)
Methanolic stem extract	17.48±0.01	23.83±0.00
Methanolic leaf extract	8.51±0.012	20.94±0.02

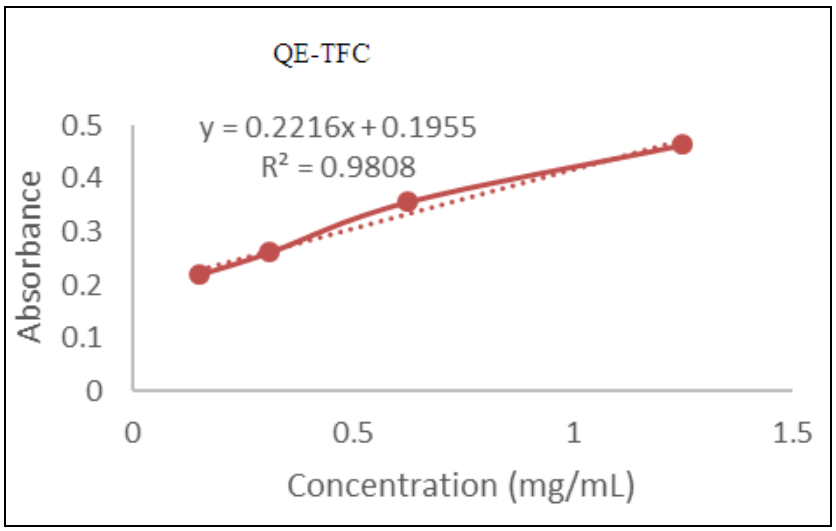

Fig. (2). Standard curve of quercetin for total flavonoid content.

methanolic stem extract (51.86 mg GAE /g), as reported by Upadhyay *et al*. [14]. Similar results were reported by IIaiyaraja and Khanum [11] with total phenolic content of 33.93 mg GAE /g and 52.17 mg GAE /g in the methanolic stem and methanolic leaf extracts of *T, cordifolia,* respectively. The total phenolic content of methanolic leaf extract of *T. crodifolia* was reported to be 2.00 mg GAE /g by Shwetha *et al*. [18], which was less than the present findings.

3.2.2. Total Flavonoid Content (TFC)

The TFC was measured by aluminium chloride reagent method and quercetin was taken as standard. TFC of methanol extract of *Tinospora cordifolia* leaves and stem extracts was calculated as Quercetin Equivalent (QE) (mg/g), using a calibration curve, which was based on the following equation: Y = 0.2216x+ 0.1955 R^2=0.9808 as shown in Fig. (**2**). The flavonoid content of the methanolic leaf extract (20.94 mg QE/g) was lower as compared to the methanolic stem extract

(23.82 mg QE/g) of *T. cordifolia* as shown in Table **4**. The high flavonoid content was found to be positively correlated with the antioxidant activity. The TFC in the methanolic leaf extract of *T. cordifolia* was 0.7 mg QE/g reported by Shwetha *et al*. [18], which was less than the present findings. The methanolic stem extract showed higher antioxidant activity and higher phenol and flavonoid content as compared to the Methanolic leaf extract. The similar results were observed by Garg and Garg [22] showing lesser flavonoid content in methanolic stem extract (2.14 mg QE/g) as compared to the methanolic leaf extract (4.53 mg QE/g).

CONCLUSION

The plant kingdom is a treasure of potential drugs and the drugs from the plants are easily available, less expensive, safe, efficient and rarely have side effects. In the present study, *Tinospora cordifolia* (Wild.) Hook.f. & Thomson, which was collected from Haldwani (Nainital), was assessed for its antioxidant activity and quantitative analysis. The stem extract exhibited more antioxidant activity than the leaf extract with regards to the all parameters analyzed.

ETHICAL STATEMENT

The plant material (Tinospora cordifolia (Wild.) Hook.f. & Thomson) of the present study was collected from Haldwani, District Nainital, Uttarakhand, India (Latitude: 29.17558; Longitude: 79.51718; Altitude: 424m). The identity of the collected specimen was authenticated and submitted at the Taxonomy lab of D. S. B. Campus, Nainital.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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