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Phytochemical analysis of stem and leaves extracts of *Tinospora cordifolia* (Thunb.) Miers

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Abstract

Tinospora cordifolia (Thunb.) Miers is a large deciduous, creeping shrub belonging to the family Menispermaceae. The phytochemical analysis of the plants is very important commercially and has great interest in pharmaceutical companies for the production of the new drugs for curing of various diseases. The present study involves the qualitative phytochemical analysis of the selected medicinal plants. The aim of this study is carried out to analyze the phytochemical compounds in stem and leaves extracts of selected plant by using phytochemical screening tests. The leaf and stem extracts of the plant is expressed the presence of several phytochemicals viz., flavonoids, amino acids, diterpines, protein, saponins and carbohydrates. The result of phytochemical screening tests revealed that diterpines and carbohydrates are positive in all extracts. But flavonoids and saponins only present in methanol and ethanol extracts. The studies justify that *T. cordifolia* use in traditional medicines. The investigation further proposed that the phytochemicals present in stems and leaves of the plant, which can be used as natural antioxidants in medicinal drugs.

Keywords: *Tinospora cordifolia*; Phytochemicals; Flavonoids; Menispermaceae; Saponins.

1. Introduction

Tinosporacordifolia (Thunb.) Miersis a climbing plant in family *Menispermaceae* and grows throughout the Indian subcontinent and in some African countries. *T. cordifolia* is important because of its medicinal properties. In Hindi, it is called giloy, which in Hindu mythology is a potion that helped the Gods stay permanently young [1,2]. It's indigenous to the tropical areas of India, Myanmar and Sri lanka. More than 30-40 species of Giloy are present all over the world. It is commonly known as Guduchi, Giloy, Amrita and Gurcha [3,4]. According to Drugs and Cosmetic Act of India (1940), giloy is considered as an ayurvedic drug. All parts of this plant like fruits, leaves, stem and seeds are useful. Most commonly stem is used and have been recognized to be of great use. Guduchi has many medicinal properties like antibiotic, immunosuppressant, anticancer, anti-spasmodic, anti-microbial, anti-osteoporotic, anti-inflammatory, anti-arthritic, anti-allergic, anti-diabetic, Anti-toxic, AntiHIV, antineoplastic, anti-oxidant, hypolipidemic, immunologic, anti-periodic, anti-stress, immunomodulatoryetc [5,6].

The medicinal plants and traditional medicines are used to maintenance of human body and to cure several type of infection in most of the developing countries. 80% of the world population uses medicinal plants as medicines. The Indian public itself uses more than 8000 species of medicinal plants [7]. Traditional medicine system, nutraceuticals, modern medicines, food supplements, pharmaceutical intermediates, folk medicines, and chemical entities used medicinal plants as rich sources for synthetic drugs [8]. The medicinal plants and their product use as medicines could be traced as far back as the beginning of human civilization. The medicinal value of plants has been mentioned earlier in Hindu culture. It is found in Rigveda, which have been written during 4500-1600 B.C. and it is earliest library of

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human awareness. It is ancient collection of Hindu culture in medicinal science. It is available in eight groups, which deals with several features of science and art of healing [9]. Guduchi is a particularly accomplished creeper. It is used in Ayurved medicine system since ancient times. It is known as Rasayana drugs in ayurved, which enhance resistance of body and develop vitality and as relaxing and adaptogen [10,11]. Various reports have been published on its chemical composition, medicinal values, and their therapeutic application [12]. Medicinal plants extensively occur in worldwide and now it's getting more consideration because they have several beneficial features to all human beings especially in pharmacological and medicine field. These plants have medicinal valuable phytochemical components that produce physiological action on the human body. These components are phenolics, tannins, flavonoids, alkaloids, steroids and terpenoids [13]. Hence, the objective of this study was proceeded to separate various phytochemical components present in leaves and stem extracts of *T. cordifolia*.

2. Materials and methods

2.1. Collection of Plant Material:

T. cordifolia (leaves and stem) were collected from Vidyabharti College Seloo Dist. Wardha in the month of March- 2024 and authenticated by the authority of Department of Botany.

2.2. Extraction of Plant

2.2.1. Grinding of selected plant materials

The plant material (leaves and stem) were taken and washed with normal water gently to separate soil particles and other contaminant and again washed with distilled water. Leaf and stem were collected and shade dried to avoid the evaporation of active phyto-constituents. Therefore, we avoided sunlight exposure during this process. The dried plant sample was cut off into small pieces and grounded to powdered form. This powdered sample of plant was used for extraction process.

2.2.2. Sample extract Preparation

Powdered and dried plant sample was extracted separately with different solvent such as chloroform, methanol and ethanol using maceration process [14]. Then, the extract was filtered and allowed to evaporate the solvent and each of the extracts was resuspended in the respective solvents for further study. Finally the percentage yield were calculated of the dried extracts by following formula-

$$\% \text{ Yield} = \frac{\text{Weight of extract}}{\text{Weight of powdered drug taken}} \times 100$$

2.2.3. Phytochemical qualitative analysis

Plant extracts, which has freshly prepared were subjected to standard phytochemical analysis to detect the phytochemical constituent's viz. flavonoids, glycosides, tannins, alkaloids, saponins, terpenoids, sugar and proteins [15,16].

2.2.4. Preparation of test solution

The test solution was prepared by taking 1gm of the extract in 25 ml of methanol.

Test for carbohydrates

There are some tests performed for carbohydrates.

- **Molisch's test:** Sample of plant extract was taken in a test tube. Then 20% alcoholic solution and concentrated sulphuric acid, which is freshly prepared is added in to test tube along the sides. This test developed reddish violet and purple colour at junction between two liquids if carbohydrates present in the sample extracts.
- **Benedict's test:** Taken a test tube, which contain small amount of plant extracts sample. In a test tube added small quantity of benedict's solution and mix properly. Then boiled this sample mixture for two minutes and cool it. If carbohydrates present in the sample, it formed red precipitate.

- **Barfoed's test:** The barfoed's solution added to 0.5 ml of solution under examination, heated to boil. If carbohydrates present in the sample extracts, it formed red precipitate of copper oxide.

Test for alkaloids

- **Dragendorff's test:** Taken a few mg of extracts sample and dissolved in 5ml water. Then 2M hydrochloric acid added until an acid reaction developed. In this mixture, 1ml of dragendorff's reagent (potassium bismuth iodine solutions) was added. If alkaloids present in sample extracts, it formed orange red precipitate.
- **Wagner's test:** Acidify the plant extract sample with hydrochloric acid (1.5% v/v) and added a few drop of Wagner's reagent (iodine potassium iodide solution) in the test tube. It formed reddish brown precipitates which indicate the presence of alkaloids.
- **Mayer's test:** 2ml of plant extracts sample was taken and 2 - 3 drops of Mayer's reagent was added (potassium mercuric iodine solution) in the test tube. If alkaloids present in the sample, it formed dull white precipitate.

Test for glycosides

- **Legal's test:** Taken a extracts sample and dissolved in pyridine then added sodium nitroprusside solution. Make this solution completely alkaline. Presence of glycosides produced pink red colour.
- **Baljet's test:** Taken a plant extracts sample in the test tube and added sodium picrate solution. Presence of glycosides produced yellow to orange colour.
- **Borntrager's test:** The test solution of plant extract was added in few ml of dilute sulphuric acid solution. This solution was filtered. Then Chloroform and ether was added in to filtrate and shaken well. In this solution ammonia was added and separated the organic layer. Organic layer showed pink, red or violet colour due to the presence of glycosides.

Test of saponins

a) 1ml of alcoholic sample extract was taken and diluted with 20ml of distilled water. This solution was shaked for 15 min in graduated cylinder. If saponins present in the extracts, it generate foam layer of 1cm.

Test for flavonoids

- **a)Shinoda test:** Taken the alcoholic sample extract in the test tube and 5-10 drops of hydrochloric acid added in the sample. Then small pieces of magnesium added in tubes. Reddish pink or brown colour was indicated the presence of flavonoids.
- **b) Alkaline reagent test:** Plant extracts sample was mixed with 2ml of 2% NaOH solution. It produced yellow colour. In this solution, 2 drops of diluted acids was added. If flavonoids present in the extracts, yellow colour changed into colourless.

Test for tannins

- Taken the sample of plant extracts in the test tube and added ferric chloride solution. If tannin present in the sample, dark blue or greenish black colour appeared.
- Taken the sample extracts and added potassium cyanide. It produced deep red colour, which indicate the presence of tannins.
- Potassium dichromate was added in to sample extracts. Yellow precipitate was formed indicate the presence of tannins.

Test for protein and amino acid

- **Biuret's test:** Taken 2-3 ml of sample extract and added 1 ml sodium hydroxide solutions (40%) and 2 drops of copper sulphate solution (1%) and mixed properly. Presence of proteins showed a pinkish - violet and purple - violet colour.
- **b)Ninhydrin's test:** Plant extracts sample mixed with freshly prepared 2 drops of 0.2% ninhydrin solution and heated to boiling for 1-2 min and allowed cooling. Blue colour appearance indicates the presence of amino acids, proteins, peptides.
- **Xanthoprotein test:** Extracts sample was taken in test tube and added conc. nitric acid. A white precipitate was obtained and upon heating turns to yellow and cool the solution carefully. 20% sodium hydroxide solution added in excess, which produce orange colour that indicate the presence of amino acids.

Test of fats or fixed oils

- **Using sodium hydroxide:** The extract was mixed in one ml 1 % of copper sulphate solution then 10% sodium hydroxide solution was added. Blue colour appeared in the solution, which showed the presence of glycerin.
- **Saponification:** plant extracts was taken and mixed with 2% sodium carbonate solution. Shaked vigorously and boiled. A clean soapy solution was formed cooled and few drops of conc. HCl was added and observed that fatty separate out and float up.

3. Results and discussion

3.1. Yield of extracts

According the result of percentage yield it is clear that ethanol was a good solvent for extraction of *T. cordifoila*. The yield of plant using ethanol and methanol solvents was higher than chloroform solvent. *T. cordifolia* exhibited higher yield in ethanol followed by methanol extracts Table 1 & Fig 1.

Table 1 Result of percentage yield of different extract

Sr. No.	Solvents	% Yeild
1	Chloroform	2.4
2	Methanol	4.7
3.	Ethanol	4.9

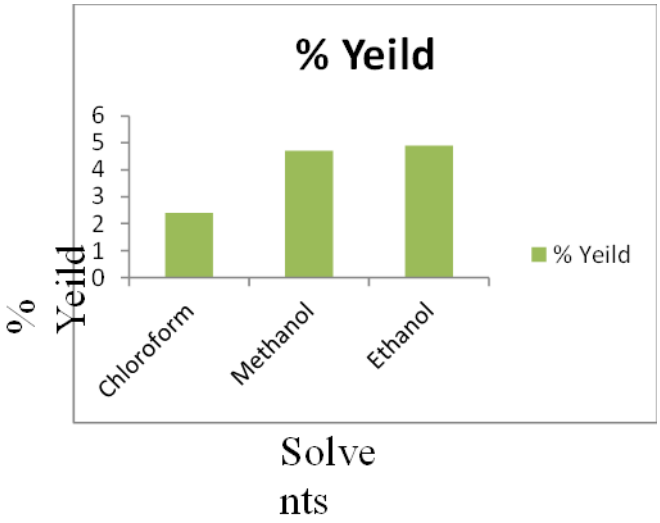

Figure 1 Yield of extract

3.2. Phytochemical screening

Phytochemical analysis of leaf and stem extract of *T. cordifolia* revealed that various phytochemicals such as flavonoids, diterpines, proteins, saponins, amino acids and sugars present in different solvent extracts (Table 2). Three different solvents (chloroform, methanol, and ethanol) were used to obtain leaf and stem extracts used for qualitative phytochemicals screening of plants using standard phytochemical tests. The methanol extract of stem showed that most of phytochemicals present in it.

Table 2 Phytochemical analysis of stem and leaves

Sr. No.	Constituents	Chloroform		Methanolic		Ethanolic	
		Stem	Leaves	Stem	Leaves	Stem	Leaves
1	Alkaloids	-	-	-	-	-	-
2	Glycosides	-	-	-	-	-	-
3	Flavonoids	+	+	+	+	+	+
4	Phenolics	-	-	+	-	-	-
5	Amino acids	-	-	+	-	+	+
6	Carbohydrates	-	+	-	-	-	-
7	Proteins	-	-	-	+	+	+
8	Saponins	-	-	-	+	+	+
9	Diterpines	+	+	+	-	+	+

{(-) Negative test; (+) Positive test}

Phytochemicals analysis is a very important steps in the medicinal plant and subsequently, it may be used for drug discovery and development. Earlier studied revealed that successfully extractions of phytochemical components depend on the solvent types used in extraction process. In this study, different solvents such as methanol, Ethanol and chloroform were used. This study concluded that solvent variation affected the phytochemical components present on the extracts [17]. Most of the phytochemical components present on methanol solvent extracts. It has more solubility for bioactive component of *T. cordifolia* than other solvent extracts. Earlier study revealed that phytochemical analysis of *T. cordifolia* showed that leaf extracts of plant has flavonoids, alkaloids, phenols, tannins, steroids and terpenoids. This plant is potential medicinal plant due to variation and availability of phytochemicals [18]. In this study, qualitative analysis of *T. cordifolia* showed that methanolic stem extracts has more phytochemical component such as flavonoid, phenols, amino acids, diterpines, saponins, proteins. Flavonoids and diterpines present in all three solvent extract. Saponins, amino acids and proteins are present in ethanol (leaves and stem) extract and methanol leaves extract only. According to present study, *T. cordifolia* is highly antioxidant plant because the presence of flavonoid component. It is similar to previous study. Thus, antioxidant rich leaf and stem extracts of *T. cordifolia* serve as a source of nutraceuticals that alleviate the oxidative stress and helps in prevention and reduction of the degenerative disease with consequent health benefits [19, 20]. Therefore, these different solvent extracts could be seen as a better source of rich phytochemical compounds. Thus, there is necessary to use these solvents for qualitative analysis of plants [21].

4. Conclusions

It can be concluded from the present study that phytochemical analysis of *Tinospora cordifolia* stem and leaves extracts indicates the presence of various phytochemicals such as flavonoids, diterpines, saponins, amino acids and proteins. These results revealed that flavonoids component were present in all solvent extracts of *T. cordifolia*. Furthermore, these results of plant sources were found to be highly significant. Hence, there are more requirements to explore the applicability of these plant resources which are rich in phytochemicals/flavonoids and may have beneficial effect on health. Our study raveled that important medicinal components present in the studied species. Many evidence proved in earlier studies also conform that recognized phytochemicals is to be bioactive. Hence, this plant can be used as a good source for beneficial drugs.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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