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QUALITATIVE AND QUANTITATIVE ANALYSIS OF LEAVES AND STEM OF *TINOSPORA CORDIFOLIA* IN DIFFERENT SOLVENT EXTRACT

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ABSTRACT

Tinospora cordifolia is known as Giloe and Guduchi, with significant importance in the traditional medicinal systems. It is dioeciously plant. It is mostly used in Ayurved system. It is also known as a 'Rasayans' of medicinal system, which develops immune system of the body and protect against infection. The aim of this study is carried out to analyse the phytochemical compounds in leaves and stem extracts of *T. cordifolia* by using phytochemical screening tests and estimate total flavonoid content (TFC) by using aluminium chloride method in the sample extracts. The leaf and stem extracts of *T. cordifolia* 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 of *T. cordifolia*, but flavonoids and saponins only present in methanol and ethanol extracts. TFC of *T. cordifolia* was higher in ethanolic leaves extracts than mathanolic leaves extracts. The studies justify that *T. cordifolia* use in traditional medicines. The investigation further proposed that the phytochemicals present in stems and leaves of *T. cordifolia*, which can be use as natural antioxidants in medicinal drugs.

Keywords: *Tinospora cordifolia*, Phytochemicals, Flavonoids

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INTRODUCTION

Various plants have medicinal properties and it was a source of medicine in the past days and now these plants and their valuable products use as a source of medicines, which has recognized by general public and scientists[1]. These plants have some chemical components that produce physiological action on the human body. These chemical components possess different medicinal property[2]. 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[3]. Traditional medicine system, nutraceuticals, modern medicines, food supplements, pharmaceutical intermediates, folk medicines, and chemical entities used medicinal plants as rich sources for synthetic drugs[4]. 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 earliar in Hindu culture. It is found in Rigveda, which have been written during 4500-1600 B.C. and it is earliest library of 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[5]. *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[6, 7] . Various reports have been published on its chemical composition, medicinal values, and their therapeutic application[8, 9]. *T. cordifolia* (Willd.) is commonly known as Guduchi, Giloy or Heart- leaved

Moonseed is a genetically different, large, transitory clamber shrubs belong to menispermaceae family[10, 11]. In Ayurvedic medicinal system, it is used as most divine shrubs for its enormous properties like as anti-inflammatory, antiarthritis, antidiabetic, antioxidant, antiallergic, antileprotic, antiperiodic, hepatoprotective, antimalarial, antineoplastic and immunomodulatory activities [12, 13]. 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[14]. Hence, the objective of this study was proceeded to separate various phytochemical components present in leaves and stem extracts of *T. cordifolia* and to calculate the Total flavonoids content in the plant sample.

MATERIALS AND METHODS

Sample collection

Tinospora cordifolia (leaves and stem) were collected from Ruler area of chitrakoot (M.P.) region in the month of April, 2015 and authenticated by Botanical Department of Govt. P.G. College, Satna, (M.P.).

Plant extraction

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 dried at 37°C. Plants have active phytochemical constituents, which losses their activity due to exposure of sunlight. 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.

Preparation of sample extract

Powdered and dried plant sample was extracted separately with different solvent such as chloroform, methanol and ethanol using maceration process [15]. 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-

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

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[16,17].

Preparation of test solution

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

A. Test for carbohydrates

There are some tests performed for carbohydrates.

a) **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.

b) **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.

c) **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.

B. Test for alkaloids

a) **Dragendorff's test:** Taken a few mg of extracts sample and dissolved in 5ml water. Then 2 M 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.

b) **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.

c) **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.

C. Test for glycosides

a) **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.

b) **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.

c) **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.

D. 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.

E. 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.

F.Test for tannins

a) 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.

b) Taken the sample extracts and added potassium cyanide. It produced deep red colour, which indicate the presence of tannins.

c) Potassium dichromate was added in to sample extracts. Yellow precipitate was formed indicate the presence of tannins.

G. Test for protein and amino acid

a) 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.

c) 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.

H. Test of fats or fixed oils

a) 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.

b) 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.

Estimation of total flavonoids content (TFC)

Estimation of total flavonoids component was based on aluminum chloride ($AlCl_3$) method[18]. Taken 50 mg quercetin component and dissolved in 50 ml methanol. Then different aliquots of 5-25µg/ml were prepared in methanol. Quercetin was used as a standard. 10gm of dried extracts of plant were dissolved with 10ml methanol and filter. Three ml (1mg/ml) of this extract was used for the estimation of flavonoids. Take 3 ml of extract or standard and added 1 ml of 2% $AlCl_3$ methanolic solution, then allowed this mixture to stand at room temperature for 60 min. Then absorbance was measured at 420 nm by spectrophotometer.

RESULTS AND DISCUSSION

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 *Tinospora cordifolia* 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

S. No.	Solvents	% Yield
1.	Chloroform	2.3
2.	Methanol	4.5
3.	Ethanol	4.8

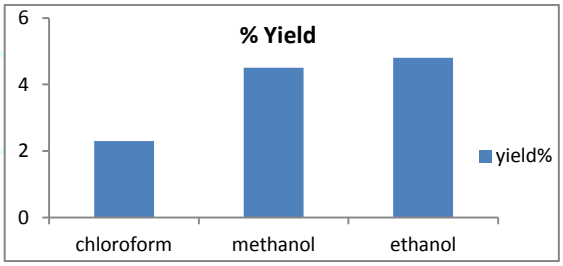

Figure 1: Yield of extract

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: Result of phytochemical analysis of *T. cordifolia*

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

+ = presence, - = absence

Phytochemicals analysis is a very useful step 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[19]. 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[20]. 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[21, 22].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[23].

Results of total flavonoids content

Total flavonoids content was determined by the preparation of Quercetin calibration curve. Quercetin was taken as standard in different concentration (µg/ml) and measured the absorbence of quercetin at 420nm. According to this calibration curve, flavonoids content of the leaf and stem was determined by using following equation: **Y=0.040 X+0.009, R^2=0.999**, where X is the Quercetin equivalent (QE) and Y is the absorbance Table 3 & Fig 2.

Table 3: Preparation of calibration curve of quercetin

S. No.	Concentration (µg/ml)	Absorbance (Mean) λmax=420 nm
0	0	0
1	5	0.216
2	10	0.425
3	15	0.625
4	20	0.815
5	25	1.021

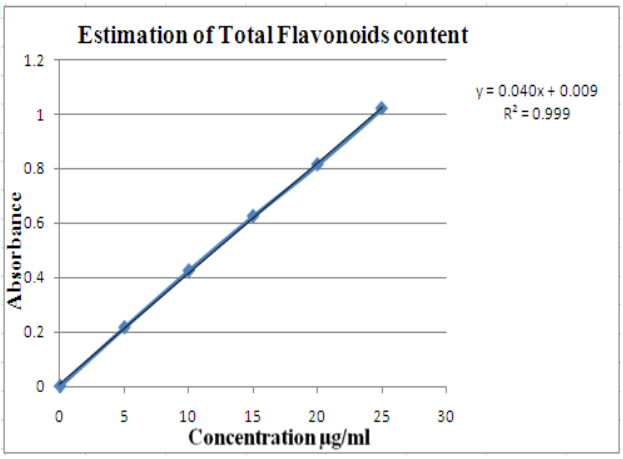

Figure 2: Calibration curve of Quercetin

Results of total flavonoids content in different extract

It has been accepted that more antioxidant activity present in flavonoids content and it is very effective on human health and their nutritions. Total flavonoids content (TFC) of methanol and ethanol extract of *T. cordifolia* leaves and stem was calculated as quercetin equivalent (mg/100mg), using calibration curve, which is based on following equation: **Y= 0.040X+0.009, R^2 = 0.999**, where X is the quercetin equivalent (QE) and Y is the absorbance which has

Table 4: Total flavonoids content of *T. cordifolia* extract

Estimation	Methanolic extract		Ethanolic extract	
	Leaves	Stem	Leaves	Stem
Absorbanc	1.813	0.857	1.820	0.700
Total Flavanoids (mg/100mg)	4.53	2.14	4.55	1.75

This table raveled that TFC was higher in methanolic and ethanolic leaves extract than stem extract of methanol and ethanol. According to this study, *T. cordifolia* show high free radical scavenging activity due to the presence of high amount of flavonoids component. Highly flavonoid content is correlated with high antioxidant activity. Results of present study are similar to the earlier findings.

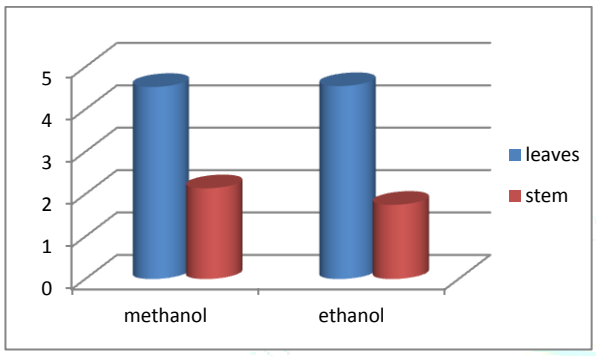

Figure 3: Total flavonoid content of different solvent extract

Previous finding had shown that the efficiency of the flavonoids extraction depend on the type of the plant and kind of solvent used [24, 25]. The earlier study concluded that ethanol was good solvent for the flavonoids extraction in medicinal plants. [26]. The current study revealed that ethanol and methanol extracts of the leaves has significantly high flavonoid content than methanol and ethanol stem extract of the plants. It suggests that these solvents are better for the flavonoids extraction in the studied plants. Study had shown that antioxidant activity of plant is depends on the types of extraction solvent [24]. The plants studied here can be seen as a potential source of useful drugs. Further studies are going on these plants in order to isolate, identify, characterize and elucidate the structure of the phytochemical compounds.

CONCLUSION

India has a large flora which require in traditional treatments of medical system. The medicinal properties of these plants could be based on the therapeutic and antioxidant effect of different phytochemicals present in them. The leaves and stem extracts of this plant have various phytochemicals such as flavonoids, diterpines, saponins, amino acids and proteins which are responsible for these activities. These results revealed that flavonoids component were present in all solvent extracts of *T. cordifolia*. TFC was high in methanolic and ethanolic leaves extract of *T. cordifolia*. Furthermore, these results of plant sources were found to be highly significant. Hence, there is more requirements to explore the applicability of these plant resources which are rich in phytochemicals/flavonoids and may have beneficial effect on health. This is observed that leaves and stem of the plant has antioxidant activity. Thus it can be use as natural antioxidants. Phytochemical screening and analysis can be beneficial for drug discovery and development. 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 use as a good source for beneficial drugs and its quantified values can be use as a tool for a drug to obtain a quality control profile.

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