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PHYTOCHEMICAL SCREENING AND QUANTIFICATION OF PLANT METABOLITES IN THE STEM EXTRACT OF *TINOSPORA CORDIFOLIA* (WILLD.) MIERS

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ABSTRACT

Tinospora cordifolia is an annual or perennial Ayurvedic plant which is still used in several traditional medicines to cure various diseases. Qualitative phytochemical screening and Quantification of metabolites were carried out in the present study. Five solvents: water, ethanol, methanol, acetone and petroleum ether were used to obtain the extracts of stem. The shade dried powdered stem material was subjected to Soxhlet extraction using different solvents and hot water extraction. The resultant extraction was subjected to screening of phytochemicals and estimation of metabolites using standard procedure. The stem extracts of varied solvents and aqueous extract were analysed and tabulated with the presence and absence of phytoconstituents. Further hot aqueous stem extract showed good amount of nutrients and medicinal properties based on the quantitative determination of primary and secondary metabolites. Thus the present findings suggest that the stem contains more active constituents and could be used in pharmaceuticals industries for the production of making quality products.

KEYWORDS: ***Tinospora cordifolia*, phytochemicals, primary metabolites and secondary metabolites**

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INTRODUCTION

Since ancient times, people have been exploring the nature particularly medicinal plants in search of new drugs. Medicinal plants are used by 80% of the world population for their basic health needs. India is the birth place of renewed system of indigenous medicines such as Siddha, Ayurveda and Unani. Traditional systems of medicines are prepared from a single plant or combinations of more than one plant. The efficacy depends upon the current knowledge about taxonomic features of plant species, plant parts and biological property of medicinal plants which in turn depends upon the occurrence of primary and secondary metabolites [1]. Phytochemicals (from the Greek word phyto, meaning plant) are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients [2].They protect plants from disease and damage. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals [3,4]. Recently, it is clearly known that they have roles in the protection of human health, when dietary intake is significant. In wide-ranging dietary phytochemicals are found in fruits, vegetables, legumes, whole grains, nuts, seeds, fungi, herbs and spices. Phytochemical screening is very important in identifying new sources therapeutically and industrially important compounds like alkaloids, flavonoids, phenolic compounds, steroids etc [5]. Plant synthesizes a wide variety of chemical compounds which can be sorted out by their chemical class, biosynthetic origin and functional group into primary and secondary metabolites. Primary metabolites directly involved in growth and development of plant. These are widely distributed in nature, occurring in one form or another in virtually all organisms. They are like chlorophylls, amino acids, carbohydrates and nucleotides have key role metabolic processes such as photosynthesis, respiration and nutrient assimilation. They are used as industrial raw material and food additives. Secondary metabolites are the basic source for the establishment of several pharmaceutical industries. The constituents present in plants play a significant role in the identification of crude drugs. *Tinospora cordifolia* commonly named as “Guduchi” in Sanskrit belonging to family Menispermaceae is a genetically diverse, large, deciduous climbing shrub with greenish yellow typical flowers, found in all plants [6-8]. In racemose panicles, the male flowers are clustered and female are solitary. The flowering season expands over summers and winters[9]. Now recently the plant is of great interest to researchers across the global because of its medicinal properties like anti - peroidic, anti-diabetic, anti – inflammatory, anti – stress, anti-arthritic, antimalarial, hepatoprotective and anti – neoplastics activities. Thus the therapeutic activites of *Tinospora cordifolia* (stem) are due to the presence of phytonutrients and secondary metabolites which aids them to exert pharmacological action against various disorders. The aim of the present study was to evaluate the phytochemical constituents and to quantify the primary and secondary metabolites.

MATERIALS AND METHODS

Collection and Identification of Plant

The fresh stem of *Tinospora cordifolia* was collected from Coimbatore district in Tamilnadu during the month of August 2015.The plant was identified and authenticated by Dr. M. Palanisamy, Botanical Survey of India, Southern Circle, Coimbatore – 641 003, (BSI/SRC/5/23/2015/Tech 1820).

Sample processing

Plant sample was washed using the distilled water and shade dried at room temperature. The sample was then made into powder, using mechanical device. The dried powder was extracted with different solvents (ethanol, methanol, acetone, petroleum ether, aqueous) in the ratio 1:10 by soxhlet apparatus. It was concentrated to dryness under reduced pressure and controlled temperature (40-50˚C) using rotary evaporator. Thus the different residual extracts were stored in refrigerator at 4˚C in small sterile glass bottles.

Qualitative phytochemical analysis

Phytochemical evaluation was carried out for all extracts as per the standard methods [10-14]. The chemical test was performed for each phytoconstituent are represented in Table – 1.

Quantitative determination of primary metabolite

Estimation of total Carbohydrate

Carbohydrate content was estimated by the method of Hedge and Hofreiter [15]. 1ml of sample was mixed with 4ml of anthrone reagent. It was then incubated in boiling water bath for 8 minutes and the absorbance was read at 630nm against a reagent blank. The estimation was done in triplicates and the results were expressed as mg/g sample.

Estimation of Proteins

Protein content was estimated by the method of Lowry *et al*[16]. 0.1ml of the sample was taken and added 5ml of alkaline copper reagent mixed well and allowed to stand for 10minutes. Then add, 0.5ml of Folin - Ciocalteau reagent. Mixed well and incubated at room temperature for 30minutes. Reagent blank was also prepared. After 30minutes, the blue colour developed was read at 660nm.

Estimation of total free Amino acids

Total free amino acid (ninhydrin method) was estimated by the method of Moore and Stein [17]. 1ml of the sample was mixed with 1ml of Ninhydrin and kept in boiling water bath for 20 minutes. Added 5 ml of diluent (equal volume of water and n propanol) and incubated at room temperature for 15 minutes. The absorbance was read at 570 nm against a reagent blank.

Quantitative determination of secondary metabolite

Estimation of Alkaloids

The estimation of alkaloids was done by method of Harborne [18]. 10 mg of plant material was homogenized in

a mortar and pestle. Add 20ml methanol: ammonia (68:2). Decanted the ammonical solution and after 24 hrs added fresh methanolic ammonia. Repeat the procedure thrice and pooled the extracts. The extracts were evaporated using a flash evaporator. Treated the residue with 1N HCl and keep it overnight. Extracted the acidic solution with 20ml of $CHCl_3$ thrice, pooled the organic layers and evaporated to dryness, basic fraction. Bascified the acidic layer with concentrated sodium hydroxide to pH-12 and extracted with $CHCl_3$ (20ml) thrice, pooled the $CHCl_3$ layers, dry over absorbent cotton and evaporated to dryness. The fraction that contains alkaloids was expressed as mg/100g.

Estimation of Flavanoids

Flavanoids was estimated by the method of Jia *et al* [19]. 1ml of the extract was mixed with 0.075ml of 5% Sodium nitrite solution and incubated at room temperature for 10 minutes.10% aluminum chloride was then added and incubated at room temperature for 6 minutes. Then 1N sodium hydroxide was added. The absorbance was read at 510 nm against a reagent blank.

Estimation of Tannins

Estimation of tannins was done by the method of Bray and Thorpe[20]. 1ml of the sample was mixed with 5 ml of vanillin hydrochloride reagent and incubated at room temperature for 20 minutes. The absorbance was read at 500 nm against a reagent blank.

Estimation of Crude fiber

The crude fiber content in the samples was determined by the method given by Mayanard [21]. The ground material was extracted with petroleum ether to remove fat (initial boiling temperature 35-38˚C and final temperature 52˚C. If fat content is below 1% extraction may be omitted. After extraction with ether, 2 gm of dried material was boiled in 200 ml of sulphuric acid for 30 min with bumping chips. The solution was then filtered through muslin and washed with boiling water until washings are no longer acidic. The sample was then boiled in 200 ml of sodium hydroxide solution for 30 min. Filtered through muslin cloth and washed with 25 ml of boiling 1.25% sulphuric acid, three 50 ml portion of water and 25ml of alcohol. The residue was then transferred to pre-weighed ashing dish (W_1) and dried for 2 h at 130+2˚C. The dish was cooled in a desiccator and weighed again (W_2). The sample was then ignited in a muffle furnace at 600+15˚C till constant weight is obtained (W_3). The percentage of crude fibre in the sample was calculated from the following formula: % Crude Fiber in Sample = Loss in wt. on ignition $(W_2 – W_1) – (W_3 – W_1)$ x 100/ Weight of Sample.

Statistical Analysis

All the estimation was performed in triplicates and the results were statistically analysed and expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

Phytochemical analysis is of paramount importance in identifying new sources of therapeutically and industrially valuable compounds having medicinal properties have been chemically investigated [22]. In the present investigation, primary and secondary metabolites of *Tinospora cordifolia* stem extract were analysed and quantified in Table 1, 2 and 3.

Table1

Preliminary phytochemical screening of Tinospora cordifolia stem extract

S.NO	Constituents	Aqueous	Ethanol	Methanol	Acetone	Petroleum ether
1	Alkaloids	+	+	+	–	+
2	Flavanoids	+	+	+	–	–
3	Tannins	+	+	–	+	–
4	Phenols	+	+	+	+	+
5	Anthraquinones	+	–	+	–	–
8	Phlotannins	+	+	–	+	+
7	Resins	+	+	+	+	–
8	Coumarins	+	–	+	+	–
9	Thiols	+	+	+	+	–
10	Terpenoids	+	+	+	–	–
11	Glycosides	+	+	+	+	+
12	Anthocyanins	–	+	–	–	–
13	Emodins	–	–	–	–	–
14	Oxalate	+	–	–	–	–
15	Gums and mucilages	+	+	+	+	–
16	Carbohydrates	+	+	+	+	+
17	Proteins	+	–	+	+	–
18	Fatty acids	+	+	+	+	+
19	Steroids	+	+	+	–	–
20	Test for oils and fats	+	–	–	+	–
21	Cardiac glycosides	+	+	+	–	–
22	Diterpenes	+	+	+	–	–

"+" Presence "-" Absent

In the present study *Tinospora cordifolia* stem extracts such as aqueous, ethanol, methanol, acetone and petroleum ether were used to reveal the presence of various phytoconstituents. Out of these five extracts ethanol and aqueous extract showed maximum number of plant constitutents whereas methanol, acetone and petroleum ether showed minimum amount of constituents which can be depicted in Table1. Alkaloids, flavonoids, tannins, steroids, phenols, crude fiber, glycosides were found universally present in *Tinospora*. *cordifolia*. The medicinal value of plants lies in some chemical substances that have definite physiological functions in the human body. Different phytochemicals have been found to possess a wide range of medicinal properties, which may help in protection against various diseases. Knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources for the synthesis of complex chemical substances. Chemical constituents may be therapeutically active or inactive. The one which are active are called active constituents and the inactive ones are called inert chemical constituents. The Preliminary phytochemical screened to analyse the essential nutrients which are actually not required by human body for sustaining life, but have important properties to prevent or fight against some common diseases.Alkaloids are heterocyclic nitrogen compounds, protects the human against chronic diseases.In plants flavonoids serve as protectors against a wide variety of environmental stress while, in humans, flavonoids appear to function as “biological response modifiers”. Flavonoids possess many pharmacological activities like anti-ulcer, anti-ageing, anti-oxidants, anti-fungal, anti-tumor, anti-diabetic, anti-hepatotoxic and anti-inflammatory. Phenols are very important plant constituents because of their scavenging ability on their hydroxyl groups possess antioxidant activity [23].Highly oxidized phenols are inhibitors[24]. Saponins possess specific physical, chemical, biological activities such as antimicrobial, anti-inflammatory, antifeedent [25] and haemolytic effects[26]. Tannins are substances capable of tanning leather or precipitating gelatin from solution. Consumption of tannins containing teas and red wines can cure or prevent a variety of ills. Glycosides are known to lower the blood pressure [27]. Coumarins have been reported to exhibit antioxidant, analgestic, anti-inflammatory and anti-mutagenic properties [28]. Biological thiols found in plants can also function as an antioxidant, an anti-mutagen and anti-carcinogen [29].

Quantitative phytochemical analysis of Tinospora cordifolia (stem extract)

Primary metabolites (Carbohydrates, Proteins, Aminoacids) have a key role in survival of the species, playing an active function in the photosynthesis and respiration. Quantitative analysis of primary metabolites in the aqueous stem extract of *Tinospora cordifolia* were depicted in Table 2 which Shows high content of carbohydrate followed by aminoacids and then proteins. Carbohydrates are sugars made up of glucose and isomers. They exist in different forms: monosaccharides, disaccharides and polysaccharides. Plant sugars are used as the sweetner and they can help the diabetic patients by supporting the body in its rebuilding [30]. A protein consists of one or more polypeptides made up of amino acids. Plants make amino acids from the process of photosynthesis through a very complex process involving the acquisition of N, usually in the form of NH_4, and involving the use of large amounts of energy, in the form of ATP and NADPH. The presence of proteins in the plants will increase the food value or protein based bioactive compounds could be isolated in the future [31].

Table 2
Determination of Primary metabolites

S.No	Primary metabolites	Quantity (mg/g)
1	Carbohydrates	3.22±0.18
2	Proteins	1.25±2.04
3	Aminoacids	1.75±0.25

Values are expressed by mean ± SD (n=3)

Secondary metabolite (Alkaloids, Flavonoids, Tannins, Crude fiber,Phenols) are necessary for extraction, purification, separation, crystallization, identification of various compounds. Quantitative analysis of secondary metabolite in the hot aqueous stem extract of *Tinospora cordifolia* were depicted in Table 3. They showed that the crude fiber content was found high followed by alkaloids, phenols, flavonoids, tannins. Phenol has an important role in defence mechanism and exhibits antioxidant properties. Flavonoids exert protection against heart disease through the inhibition of cyclooxygenase and lipoxygenase activities in platelets and macophages [32]. Tannins contributes various medicinal properties such as antimicrobial, anti-inflammatory, astringent property. They have been also reported to have anti-viral [33], antibacterial [34, 35] and anti-parasitic effects. Alkaloids are produced by large number of organisms, plants, bacteria and fungi. Alkaloids have many pharmacological activities including hypertensive effects antiarrhythmic effect [36] and antimalarial and antimicrobial activity by inhibiting DNA topoisomerase

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Table 3
Determination of Secondary metabolites

S.No	Secondary metabolites	Quantity (mg/g)
1	Alkaloids	2.02 ± 0.04
2	Flavonoids	0.85± 0.01
3	Tannins	0.35±0.10
4	Crude fiber	3.86±0.04
5	Phenols	1.03±0.09

Values are expressed by mean ± SD (n=3)

CONCLUSION

Phytochemicals found present in the stem extract of *Tinospora cordifolia* aids as principle source for synthesis of new novel medicines. Further quantification of metabolites may pave a way for drug discovery and development in pharmaceutical industries. Therefore the stem extract could be used as a good source for obtaining a quality product. Further investigation on isolation and purification of bioactive compounds may also lead to interesting research process. The Ayurvedic literature reports that it can cause constipation. If taken regularly in high doses; it has no side effects and toxicity. Yet the safety and the potential indications of human being have to be established using modern methods.

CONFLICT OF INTEREST

We declare that we do not have conflict of interest.

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