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Research Article

"Phyotochemicals analysis of *Tinospora cordifolia* (Gulvel)"

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Abstract

The qualitative phytochemical studies were carried out in the solvents viz. Methanol, Chloroform and n-Butanol. The Methanol, Chloroform and n-Butanol tuber and leaves extract of *Tinospora cordifolia* shows the presence of alkaloid, glycosides, terpenoids, tannin, flavonoids, saponins, steroid and phenols but in tuber of *Tinospora cordifolia* high intensity of phytochemicals than that of leaves showed in table no.1 by twice and tannin absence in methanolic and n-butanol extract of leaves. Also, the quantitative studies were carried out in the same solvent mentioned above, Alkaloid content in tuber of *Tinospora cordifolia.*. that was 2.921, 2.546 and 3.045 µg/ml respectively, and total flavonoids in tuber, 0.845, 0.641 and 0.978 µg/ml respectively and also followed by total content of phenols that was 1.284, 0.652 and 1.361 µg/ml. Total content of Alkaloid, flavonoids and phenols in the Methanol, 1.926, 0.434 and 1.641 µg/ml. respectively, in the Chloroform 1.554**,** 0.391 and 0.856 µg/ml. and followed by in the n-Butanol leaves extract of *Tinospora cordifolia* that was 2.045, 0.423 and 0.426 µg/ml.

INTRODUCTION

Tinospora cordifolia is a popular medicinal plant which is used in several traditional medicines to cure various diseases. The common names are Amrita and Guduchi and belong to the family of *Menispermaceae.* It is considered an essential herbal plant of Indian system of medicine (ISM) and has been used in the treatment of fever, urinary problem, dysentery, skin diseases leprosy, diabetes, and many more diseases. The plant reported containing chemical compound including Alkaloids, Terpenoids, Lignans, Steroids and others that establish the phytochemistry and pharmacological activity of *Tinospora cordifolia*. The present review highlights the pharmacological importance *viz* antioxidant activity, antimicrobial activity, antibacterial activity, antifungal activity, anti-diabetic activity, antistress activity, hypolipidaemic effect, hepatic disorder, anticancer anti HIV potential, antiosteoporotic effects, antitoxic effects, wound healing, anticomplementary activity, and immunomodulating activity, systemic infection and Parkinson's disease and "Ethical Phytomedicines", which are standardized toxicologically and clinically define crude drugs, are seen as a promising low-cost alternative in primary health care. The field also has benefited in recent years from the interaction of the study of traditional ethnobotanical knowledge and the application of modern phytochemical analysis and biological

activity studies to medicinal plants. A wide range of chemical compounds including Aporphine, alkaloids, clerodane diterpenes, berberine, palmatine, tembertarine, magniflorine, choline, and tinosporin etc. have been isolated from this plant. The Alkaloids like Berberine, Palmatine, Tembetarine, Magnoflorine Choline , Tinosporin etc., Glycoside like Tinocordiside ,Tinocordifolioside etc. The trace element studies on the aqueous extract of these medicinal plants have been carried out using particle-induced X-ray emission technique for their medicinal uses. The very high concentrations of Cl, K, and Ca in all the leaf samples, appreciable levels of Mn and high Zn content in *T. cordifolia*.

So, I have selected present study on qualitative and quantitative analysis of Gulvel is a medicinal plant with various properties of therapeutic importance. It belongs to the family *Menispermaceae* and scientifically it is called *Tinospora cordifolia.* It is a deciduous shrub commonly called "Guduchi" in Sanskrit.

The plant has heart-shaped leaves with greenish-yellow flowers. It grows in tropical and sub-tropical regions of the globe, of these the African and Asian continents are abundant with *Tinospora cordifolia*. In traditional medicine, the extracts from all the parts of the plant are used including the root, stem, and leaf (aerial part) of the plant.

Material and Methods

Collection of Plant parts

Collections of *Tinospora cordifolia* plant part were collected from botanical garden Toshniwal college Sengaon, Maharashtra state, India. Plant part Stem and leaves cleaned soil dust with tap water then dried under shade and prepared fine powder and kept it in airtight bottle. The plant materials were identified by using standard floras like Cook 1907, Dhore 2005, Naik 1989, Yadav and Sardesai 2002.

Preparation of Plant Part Extract

Methanol, Chloroform and n-Butanol, extract was prepared by using Soxhlet extractor. 30 gm of each plant part powder was placed in a thimble, which was placed in chamber of the Soxhlet apparatus. 300 ml solvent in the flask and the temperature was maintained at 55 ^{0}c. for 72 hours. Then the extracts were filtered through Whatman filters paper No 1. Solvent was evaporated at 40-50 ^{0}C by using Rotary evaporator.

The collected powder was weight and dissolved in Dimethyl sulfoxide (DMSO) with 10% concentration. The extracts were used for Qualitative and quantitative evaluation of phytochemical. (Handa *et al*., 2008. Subramanian *et al*., 2011).

Qualitative analysis of *Tinospora cordifolia* plant parts

The qualitative screening test were performed for the presence of following secondary metabolites such as alkaloid, glycosides, terpenoids, tannin, flavonoids, saponins, steroid and phenols (Harborne, 1973) and Sofowara (2005).

Alkaloids test

The plant extract was evaporated to dryness and the residue was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Mayer's reagent. Formation of turbidity or yellow precipitation showed the presence of alkaloid.

Glycosides

Glycosides are compounds which upon hydrolysis give rise to one or more sugars (glycones) and a compound which is not a sugar (Glycone or Genine). To the solution of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid are added, and observed for a reddish-brown coloration at the junction of two layers and the bluish green colour in the upper layer.

Terpenoids and steroids

Four milligrams of extract were treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet colour was observed for terpenoid and green bluish colour for steroids.

Flavonoids

4 ml of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed

and metal magnesium was added. To this solution, 5 – 6 drops of concentrated hydrochloric acid was added and red colour was observed for flavonoids and orange colour for flavones.

Saponins

0.5 g of extracts was added to 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Phenols

The extract (50 mg) is dissolved in 5 ml of distilled water. To this few drops of neutral 5% ferric chloride solution are added. A dark green colour indicates the presence of phenolic compounds.

Tannins

To 0.5 ml of extract solution 1 ml of water and 1-2 drops of ferric chloride solution was added. Blue colour was observed for gallic tannins and green black for catecholic tannins.

Quantitative analysis of *Tinospora cordifolia*

Preparation of plant extracts for quantitative determination of alkaloids 5 gm of powdered plant material was taken into 20 ml of n-butanol and vigorously stirred. The content was transferred into a reagent bottle. The slurry was kept overnight at room temperature. Then it was centrifuged at 6000 rpm for 10 min and the supernatant was made up to 50 ml with n-butanol.

Estimation of total alkaloids by titrimetric methods used by Plummer, 2013 and Debnath*et al.* 2015.

Obtained supernatant of the plant sample was used for the estimation of total alkaloids by titrimetric methods. 10 ml of the supernatant was taken into a100 ml separating funnel. 10 ml of 0.1 (N) HCl was added and shaken thoroughly for 2-3 min. This results in the solubility of alkaloids. The lower layer contains alkaloids neutralized with 0.1 (N) HCl and the upper layer contains n-butanol. 10 ml HCL portion was collected in a beaker and 2-3 drops methyl red was added to it, that turns the solution into slightly reddish colour. The contents of beaker were titrated against 0.1 (N) NaOH, till colour change changed from red to pale yellow. The neutralization point was determined. Same procedure was repeated triplicate. The total amount of alkaloids was calculated by considering the following equivalent:

1 ml 0.1N HCl ≡ 0.0162 g alkaloid

Estimation of Total Phenolic Content

Total phenol content of *Tinospora cordifolia.*was assayed by modified Dewanto *et al.,* 2002 and Jothi *et al.,* 2019 procedure. The different concentrations of 10µg, 20µg, 40µg, 60µg, 80µg, and 100µg were using an aliquot of diluted extract and added to 0.25 ml of Folin Ciocalteu reagent. The elucidation was adjusted with distilled water to a final volume of 3ml and shaken thoroughly. The solution was incubated and kept in the dark placed and read at 760 nm was read against prepared blank. The total phenol content of plant parts was expressed as milligrams of gallic acid equivalents per gram of dry weight. The total sample was analyzed in three replicates.

Estimation Total Flavonoid Content

Total Flavonoid content in *Tinospora cordifolia* whole plant extract was analyzed by the aluminium chloride colorimetric system M.M Mervat, *et al* 2009 and Jothi *et al*., 2019. 0.5ml of plant part extract of at different concentrations like 10µg, 20µg, 40 µg, 60 µg, 80 µg, and 100 µg were taken and the final volume was made up to 3ml with methanol. After that, 0.1ml $AlCl_3$ (10%), 0.1ml of potassium acetate and 2.8ml of distilled water were added continuously and test solution was vigorously shaken. After 30 minutes for the incubation periods, absorbance was recorded at 415 nm. The concentration of flavonoids in test samples was calculated and expressed as the equivalent of quercetin (QE) / g of sample. The entire sample was analyzed in three replicates.

Results and Discussion

Results taken average of triplicates for different concentration of plant extract

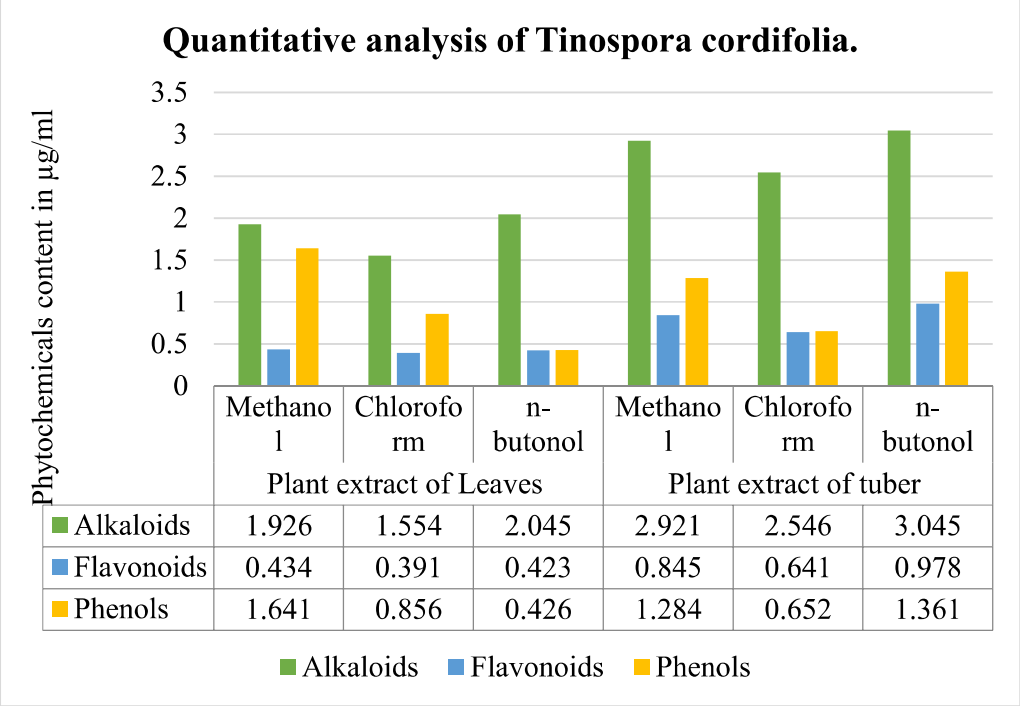
The qualitative phytochemical studies were carried out in the solvents viz. Methanol, Chloroform and n-Butanol. The Methanol, Chloroform and n-Butanol tuber and leaves extract of *Tinospora cordifolia.* shows the presence of alkaloid, glycosides, terpenoids, tannin, flavonoids, saponins, steroid and phenols but in tuber of *Tinospora cordifolia.* high intensity of phytochemicals than that of leaves showed in table no.1 by twice and tannin absence in methanolic and n-butanol extract of leaves. Also, the quantitative

Fig. Morphology of Tinospora cordifolia A) steam B) root C) leaves D) flower E) fruit F) seed.

Table No. 1. Qualitative analysis of *Tinospora cordifolia* stem and leaves

Sr. No.	Phytochemical	Plant extract of stem			Plant extract of Leaves		
		Methanol	Chloroform	n-Butanol	Methanol	Chloroform	n-Butanol
1	Alkaloids	++	++	++	++	+	+
2	Glycosides	++	+	++	+	+	+
3	Terpenoids	+	++	+	+	+	+
4	Steroids	++	+	++	++	+	+
5	Flavonoids	++	++	++	++	+	+
6	Saponins	++	++	++	+	++	++
7	Phenols	++	+	++	++	+	-
8	Tannins	++	++	++	-	+	-

Table. No. 2. Quantitative analysis of *Tinospora cordifolia*. plant parts, (leaves and Stem µg/ml).



	Plant extract of Leaves			Plant extract of tuber		
	Methanol	Chloroform	n-butonol	Methanol	Chloroform	n-butonol
Alkaloids	1.926	1.554	2.045	2.921	2.546	3.045
Flavonoids	0.434	0.391	0.423	0.845	0.641	0.978
Phenols	1.641	0.856	0.426	1.284	0.652	1.361

Studies were carried out in the same solvent mentioned above, Alkaloid content in tuber of *Tinospora cordifolia*.. that was 2.921, 2.546 and 3.045 µg/ml respectively, and total flavonoids in tuber, 0.845, 0.641 and 0.978 µg/ml respectively and also followed by total content of phenols that was 1.284, 0.652 and 1.361 µg/ml.

Total content of Alkaloid, flavonoids and phenols in the Methanol, 1.926, 0.434 and 1.641 µg/ml. respectivel, in the Chloroform 1.554**,** 0.391 and 0.856 µg/ml. and followed by in the n-Butanol leaves extract of *Tinospora cordifolia* that was 2.045, 0.423 and 0.426 µg/ml.

CONCLUSION

Tinospora cordifolia is the rich source of phytochemicals, alkaloid, glycosides, terpenoids, tannin, flavonoids, saponins, steroid and phenols. Its extraction in n-butanol solvent shows highest intensity and content of phytochemicals followed by methanolic extract of stems of *Tinospora cordifolia*.and it shows antimicrobial activities. Sagbo *et al*, 2005 and Jothi.2019, reported that Polyphenols and phenols found in plants are two secondary metabolites considered as natural antioxidants. Jothi, 2019, and Trease *et al*,1983 showed that *Tinospora cordifolia*.is an alkaloid plant, which contains alkaloid components such as colchicine and gloriosine which are mostly used in pharmaceutical formulation for drug. Noroozi *et al*, 1998 and Al-Humaid *et al*, 2010 reported Flavonoids are ketonic compounds that can induce anti-inflammatory activity and inhibits the oxygen compounds, enzyme cyclo-oxygenase dependent pro inflammation activity. Furthermore, flavonoids have a powerful anti-inflammation activity as they inhibit prostaglandin synthesis. Flavonoids in higher plants are inseparable with cardiovascular diseases and antioxidant potentials that can treat cancer disease. Pietta, 2000 and T. Sivakumar, 2017 reported that Flavonoids and antioxidants origin of vitamins A, C, E and plant source diets. So, *Tinospora cordifolia* of plant presence different phytochemical compounds useful for Further purification, identification and characterization of the active compounds of would be our priority in future studies.

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