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PHYTOCHEMICAL ANALYSIS AND ANTI-OXIDANT ACTIVITY OF *TINOSPORA CORDIFOLIA* (Thunb.)Meirs

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ABSTRACT

The objective of the present study was to analyse the phytochemicals and antioxidant activity of the leaf of *Tinospora cordifolia* of Menispermaceae family a promising medicinal plant with great economic potential. The *Tinospora cordifolia* leaf extracts (collected from Chennai, Tamilnadu, India) were evaluated for the presence of secondary metabolites using various solvents like aqueous, ethanol, chloroform, petroleum ether and acetone. The leaf extracts were evaluated for qualitative and quantitative antioxidant activity of aqueous, ethanol, chloroform, petroleum ether and acetone extracts by using DPPH as free radical. The ethanol leaf extracts of *Tinospora cordifolia* were rich in tannins, saponins, quinones, terpenoids, steroids, flavanoids, phenols, alkaloids, cardiac glycosides, coumarins and betacyanin followed by other extracts. The ethanolic leaf extracts of *Tinospora cordifolia* recorded higher percentage of free radical scavenging activity than aqueous followed by acetone, petroleum ether and chloroform. *Tinospora cordifolia* contains more phytochemicals and act as a good source of natural antioxidants.

KEYWORDS: *Tinospora cordifolia*, Phytochemicals, Secondary metabolites and antioxidant activity.

INTRODUCTION

Medicinal plants are the vital source of new pharmaceuticals and easily accessible remedy used in the health care products. The history of potential plants used for medicinal purpose is surely as old as history of mankind. Extraction and Characterization of several vital chemical compounds are of many kinds, but most of them are in four major biochemical classes:

alkaloids, glycosides, polypenols and terpenes. Numerous phytochemicals with potential or established biological activity have been identified and given birth to some high activity profile drugs. Secondary metabolites also called as specialized metabolites play a major role in human health.

Many plant extracts and phytochemicals show antioxidant/free radical scavenging properties. Secondary metabolites of plants serve as defense mechanisms against predation by many micro-organisms, insects and herbivores.

Antioxidants are protective molecules which will prevent and repair damage caused by free radicals. The sources of antioxidant can be natural or artificial. Certain plant based foods are rich in antioxidants. Antioxidants help to neutralize free radicals in our bodies, and this will boost our overall health. According to Garg *et al*., (2018)[1], the leaves and stem extract of *Tinospora cordifolia* have various phytochemicals, which can be used as natural antioxidants in medicinal drugs.

***Tinospora cordifolia(Thunb.)Meirs*:-** It is a woody climber or a herbaceous vine and grown in the tropical areas of India, Myanmar and Sri Lanka. It has an important place in Ayurvedic medicine and used to cure a wide ranging set of diseases. The tinospora leaves are rich in protein, calcium and phosphorus and also have tinosporin, tinosporic acid and tinosporol.

The stem is one of the constituents of several ayurvedic preparations used in general debility, dyspepsia, fever and urinary diseases(Nayampalli *et al*., (1988).[2] The stem is bitter, stomachic, diuretic, stimulates bile secretion, causes constipation, allays thirst, burning sensation, vomiting, enriches the blood and cures jaundice. The stem contain sesquiterpenes, sterols, diterpenes, tinocordifolioside, amritosides, clerodane, syringin, cordiol, tinosponone tinocordioside, tinosporoside, a novel 18-nor Clerodane diterpene Glucoside, a Candinane Sesquiterpene Glycoside, Phenylpropene disaccharides cordifoliosides A&B, Palmatosides C&F, Palmatosidesgiloin, gilenin, and gilosterol, together with columbin, chasmanthin, palmarin. The root of this plant is known for its antistress, anti-leprotic and anti-malarial activities (Nayampalli et al., (1982).[3] The root contains Isocolumbin, Palmatine, Magnoflorine, Tetrahydropalmatine and Jatrorrhizine. Regular usage of *Tinospora* can promote intelligence and mental clarity, boost memory and very effective in resolving the symptoms of diabetes, it exhibits insulin like action and reduces blood sugar. *Tinospora* is a good stimulator of bile secretion. *Tinospora* has been prescribed as an alternative medicine

for liver cancer for its immune boosting property and the chemical, dichloromethane which acts as a counter agent on the cancer cells. Use of *tinospora* leaves and stem as part of the diet to promote bowel movement and reduce the pile masses. *Tinospora* acts as a diuretic and provides a natural cure for renal obstruction like calculi and other urinary disorders. Indian scientists have found that *tinospora* stimulates other important immune cells such as neutrophils like macrophages, they also seek out and destroy infectious bugs in your body. *Tinospora's* incredible immune-boosting properties, is one of the reasons why it is so effective against cancer.

The main objective of the present study was to screen the phytochemicals and to evaluate the free radical scavenging activity of *inospora cordifolia.* This knowledge could be of great value for ascertaining the medicinal role of a plant.

MATERIALS AND METHODS

Sample Collection

The Plant specimens for the proposed study were collected from Kovilambakkam, Velachery, Padappai, Korattur, Kolathur, Chromepet and velachery. These plants were identified in the Dept of Plant Biology & Plant Biotechnologyof J.B.A.S. College for Women, Teynampet, Chennai-18 and authenticated by Dr.P.Jayaraman, Plant Anatomy and Research Centre west tambaram, Chennai-600 045.

Extract preparation

Preparation of the extracts was assessed by following method as described by Janarthanam *et al.*, (2015).[4] About 1g of dried stem powder of *Tinospora cordifolia* plant materials were extracted with 20 mL ethanol 75%, acetone, chloroform, aqueous and petroleum ether (Merck, extra pure) for 1 min using an Ultra Turax mixer (13,000 rpm) and soaked overnight at room temperature. The sample was then filtered through Whatman No. 1 paper in a Buchner funnel. The filtered solution was evaporated under vacuum in a rota-vator at 40 °C to a constant weight and then dissolved in methanol ethanol and water. The dissolving rate of the crude extracts was approximately 100 %. The solution was stored at 18 °C until use.

Phytochemical Screening of *Tinospora cordifolia*

The phytochemical screening of stem extracts of *Tinospora cordifolia* were assessed by standard method as described by Brinda *et al.*, (1981)[5]; Siddiqui and Ali, (1997)[6] and Savithramma *et al.*, (2011).[7]

Qualitative analysis of Antioxidant activity of *Hygrophila auriculata*

The antioxidant activity of leaf extracts of *Tinospora cordifolia* was determined by following the method as described by Selvaraj *et al*., (2014).[8] 50µl of leaf extracts of *Tinospora cordifolia* were taken in the microtiter plate. 100µl of 0.1% methanolic DPPH was added over the samples and incubated for 30 minutes in dark condition. The samples were then observed for discoloration; from purple to yellow and pale pink were considered as strong and weak positive respectively. The antioxidant positive samples were subjected for further quantitative analysis.

Quantitative analysis of Free radical scavenging activity of *Tinospora cordifolia*

The antioxidant activities were determined using DPPH, (Sigma-Aldrich) as a free radical. 100µl of leaf extract were mixed with 2.7ml of methanol and then 200µl of 0.1 % methanolic DPPH was added. The suspension was incubated for 30 minutes in dark condition. Initially, absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured as a control (Lee *et al.*, 2005).[9] Subsequently, at every 5 min interval, the absorption maxima of the solution were measured using a UV double beam spectra scan (Chemito, India) at 517nm. The antioxidant activity of the sample was compared with known synthetic standard of (0.16%) of Butylated Hydroxy Toluene (BHT). The experiment was carried out in triplicate. Free radical scavenging activity was calculated by the following formula:

DPPH radical –scavenging =[(Absorbance of control-Absorbance of test Sample)/ (Absorbance of control)] X 10

RESULTS AND DISCUSSION

Phytochemical screening

The Phytochemical Screening of the plant extract of *Tinospora cordifolia* was carried out by using different solvents to identify the presence of medicinally active metabolites. (Table -1). The aqueous plant extract were rich in saponins, quinones, flavonoids, Phenol, Coumarins and beta cyanin. The Ethanolic plant extract were rich in tannins, Saponins,quinones, terpenoids, steroids, flavonoids, phenol, Alkaloids, Cardiac Glycosides, coumarins and beta cyanin. The Chloroform plant extract were rich in saponins, quinones, terpenoids, steroids, Phenol and Cardiac Glycosides. The Petroleum ether plant extract were rich in terpenoids, steroids, phenol and Cardiac Glycosides. The Acetone plant extract were rich in tannins, quinines, terpenoids, steroids, flavanoids, phenol, alkaloids, Cardiac Glycosides and

Coumarins. The findings were also supported by Kaur *et al.*, (2016)[10], in the Preliminary Phytochemical screening of *Tinospora cordifolia* showed the presence of Carbohydrates, Flavonoids, Phenols, Terpenes, and Aminoacids. These findings are also in concordance with Singh *et al.*, (2017)[11] report of *Tinospora cordifolia* stem and leaf extract recorded the presence of various Phytochemicals.

Antioxidant activity

The antioxidant activity of the plant extract of *Tinospora cordifolia* was carried out using DPPH (1,1-Di-Phenyl-2-Picryl Hydrazyl) to identify free radicals. The Qualitative analysis and results are shown in Table-2. Among the five different solvent extracts of *Tinospora cordifolia*, the ethanolic leaf extract recorded the most effective DPPH radical Scavenging activity followed by aqueous and acetone.

The antioxidant positive sample were subjected for further quantitative analysis. Quantitative analysis of free radical scavenging activity was carried out on the plant extract of *Hygrophila auriculata* of aqueous, acetone, ethanol, chloroform and Petroleum ether and results are shown in Fig-1. Among the five different solvent extracts of *Tinospora cordifolia*, the ethanol leaf extract(83. 4%) recorded the most effective DPPH radical scavenging activity followed by aqueous (72.4%), acetone (59.8%), petroleum ether (51.9%) and Chloroform (51.1%). The present study showed the leaf extracts on different solvents recorded different extent of antioxidant activity. The ethanolic leaf extract of *Tinospora cordifolia* recorded the higher percentage of free radical scavenging activity than aqueous followed by ethanol, petroleum ether and chloroform. Similar results were obtained in *Tinospora cordifolia* ethanolic stem extract and showed the highest radical scavenging activity, Upadhyay *et al.*, (2013).[12] Ilaiyaraja *et al.*, (2011)[13] recommended from his findings that *tinospora cordifolia* stem extract showed more effective antioxidant activity with regard to radical scavenging activity and it can be used as a source of antioxidants for health benefits.

Statistical analysis

All experiments were repeated two or three times with two replicates for each condition tested, and similar results were obtained on all occassions. The results are expressed as the mean +SD and statistical analysis was carried out using Students t-test and one-way analysis of variance was considered to be statistically significant.

Table 1: Phytochemical screening from plant extracts of *Tinospora cordifolia.*

Phytochemicals Tested	Plant Extracts of *Tinospora cordifolia*				
	Aqueous	Ethanol	Chloroform	Petroleum ether	Acetone
Tannins	_	++	-	-	+
Saponins	+	++	+	_	_
Quinones	++	+	+	_	+
Terpenoids	_	++	+	+	+
Steroids	_	++	+	+	+
Flavonoids	++	++	-	_	+
Phenol	++	++	+	+	++
Alkaloids	_	+	-	-	+
Glycosides	_	-	-	-	_
Cardiac glycosides	_	+	+	+	+
Coumarins	+	+	-	-	+
Antho cyanin	-	_	-	-	-
Beta cyanin	+	+	-	-	+

key : + = positive, ++ = strong positive, - = negative

Table 2: Qualitative antioxidant activity of *Tinospora cordifolia.*

Extractions	*Tinospora cordifolia*
BHT(standard)	++
Aqueous	+
Ethanol	++
Acetone	+
Chloroform	--
Petroleum ether	--

Key : + = positive, ++ = strong positive, - = negative

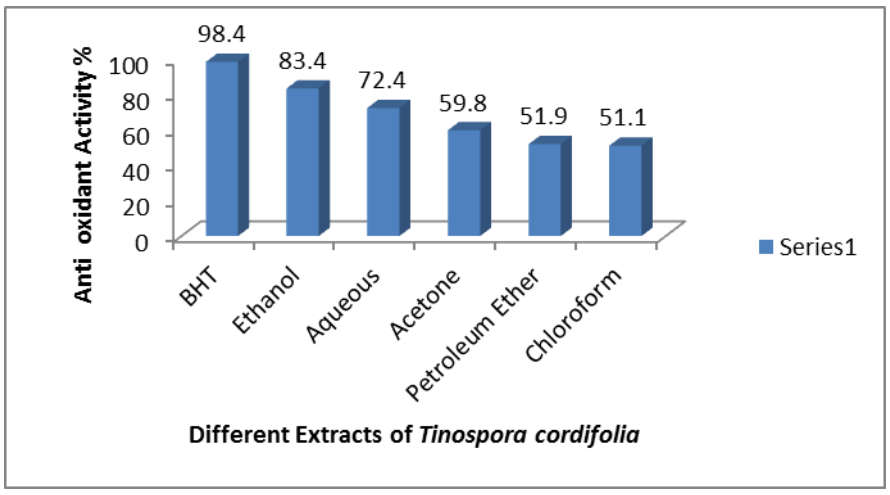

Fig. 1: Quantitative antioxidant activity of T*inosora cordifolia* leaf extract.

CONCLUSION

From this study, it was concluded that ethanolic leaf extract of *Tinospora cordifolia* has curative properties due to the presence of various Secondary metabolites such as Tannins, Saponins, Quinones, Terpenoids, Steroids, Flavanoids, Phenols, Alkaloids, Cardiac Glycosides, Coumarins and Betacyanin. The ethanolic leaf extract of *Tinospora cordifolia* showed maximum antioxidant activity both qualitatively and quantitatively. Further studies are required to elucidate whether *Tinospora cordifolia* have antidiabetic potential by *in vivo* studies for validating the traditional value.

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