International Journal of Pharmacy and Pharmaceutical Sciences
ISSN- 0975-1491 Vol 2, Issue 4, 2010

Research Article

PRELIMINARY PHYTOCHEMICAL SCREENING AND EVALUATION OF FREE RADICAL SCAVENGING ACTIVITY OF *TINOSPORA CORDIFOLIA*

V. SIVAKUMAR[1]*, M.S. DHANA RAJAN[2] AND M.S. RIYAZULLAH[1]

[1]Department of Pharmacy, Sri Lakshmi Narasimha College of Pharmacy, Palluru-517 132.Andhra Pradesh, India, [2]P.G and Research Department of Biochemistry, Jaya College of Arts and Science, Thiruninravur - 602 024, Tamilnadu, India
Email: sivakumarv2k1@gmail.com, siva_bio2009@rediffmail.com

Received: 25 May 2010, Revised and Accepted: 26 Jun 2010

ABSTRACT

The present study was aimed to screen and quantify the phytoconstituents of *Tinospora cordifolia* stem methanolic extract and evaluated the *in vitro* antioxidant activity in three models *viz.* DPPH (1,1- diphenyl - 2 - picrylhydrazyl) free radical screening activity, superoxide radical scavenging activity and reducing power assay. The total phenolic and total tannins were quantitatively estimated in stem parts of *Tinospora cordifolia* in 7.2 %w/w and 8.7 % w/w present respectively. The greater amount of phenolic and tannins compounds leads to more powerful free radical scavenging effect as shown by metanolic extract of *Tinospora cordifolia* stem.

Keywords: Antioxidant, DPPH, Superoxide radicals, Reducing power assay, *Tinospora cordifolia.*

INTRODUCTION

Tinospora cordifolia is one such plant which is widely used in indigenous system of medicine[1]. It is a large, glabrous, succulent, deciduous climbing shrub belonging to the family menispermaceae[2]. It is distributed throughout tropical India subcontinent, Sri Lanka and china, ascending to an altitude of 1200m. The stem of *Tinospora cordifolia* is rather succulent with long filiform fleshy aerial roots from the branches. The bark is creamy white to grey, deeply left rosette like lenticels. The leaves are membranous and cordate. The flowers are small and yellow or greenish yellow [3].

Tinospora cordifolia known as Amrita (Guduchi) in Sanskrit, Shindilkodi in Tamil, it is a widely used in folk and ayurvedic systems of medicine. The term Amrita is attributed to its ability to impart youthfulness, vitality and longevity to the consumer[4]. The large numbers of compounds have been isolated from the aerial parts and roots of *Tinospora cordifolia.* Guduchi is widely used in Ayurvedic system of medicine "Rasayanas" to the immune system and the body resistance against infections. In modern medicine *Tinospora cordifolia* used for the treatment of general weakness, fever, dyspepsia, dysentery, gonorrhoea, urinary diseases, viral hepatitis and anaemia more recently, the immunomodulatory properties, antineoplastic activities have been reported [5]. The aim of this research work was to attempt preliminary phytochemical screening and evaluate free radical scavenging activity of methanolic extract of *Tinospora cordifolia* stem.

MATERIALS AND METHODS

Plant materials

The stem part of *Tinospora cordifolia* was collected fresh from Vellore District area in Tamilnadu. The plant stem was authentified by the Herbarium of Botany Directorate in National Institute of Herbal Science, Plant Anatomy Research Center, Chennai. A voucher specimen (No: TC08) was deposited in the center.

Preparation of plant extract

The dried powdered stem of *Tinospora cordifolia* was allowed to pass through ss sieve (20 mesh). It was defatted by treating with petroleum ether (60-80^{o}C) and then extracted to exhaustion (soxhlet) with methanol. The solvent was removed under vacuum to get the some solid mass.

Preliminary phytochemical testing

Five hundred milligrams of the dried methanolic extract was reconstituted in 10ml of methanol and it was subjected to preliminary phytochemical testing for the presence of different chemicals groups of compounds by standard methods [6].

Estimation of total phenolic content

The total phenolic content of the extract was estimated according to the method described by Singleton and Rossi[7]. Briefly, ten milligram of standard gallic acid was dissolved in 100ml distilled water in a volumetric flask (100µg/ml of stock solution concentration). From the above stock solution 0.5 to 2.5 ml of aliquots were pipetted out into 25 ml volumetric flasks. Ten ml of distilled water and 1.5 ml of Folins Ciocalteu's reagents were added to each of the above volumetric flasks. After 5min, 4ml of 20% sodium carbonate solution was added and the volume was made up to 25 ml with distilled water and incubated at room temperature for 30 min and a standard curve of absorption of the solution recorded at 765 nm and standard curve of absorbance verses concentrations of gallic acid (50 – 250 µg) was plotted.

One gram of the powdered drug was extracted with 70 % methanol, filtered, pooled and the volume was adjusted to 50ml with 70% methanol in a volumetric flask. From the stock solution, suitable quantity of the extract was taken into a 25 ml volumetric flask and 10 ml of water and 1.5 ml Folins Ciocalteu's reagents were added the above volumetric flasks. After 5min, 4ml of 20% sodium carbonate solution was added and the volume was made up to 25 ml with distilled water and incubated at room temperature for 30 min and the absorption was recorded at 765 nm in a spectrophotometer. Percentage of total phenolic was calculated from calibration curve of gallic acid (50 – 250 µg) plotted using the above procedure and phenolics were expressed as % gallic acid.

Estimation of total tannins[8]

Two grams of the powdered drug was extracted for 20 h with petroleum ether. The residue was boiled for 2 h with 300 ml of doubled distilled water. It was cooled, filtered with Whatman No.1 filter paper and diluted to 500ml with double distilled water. 25 ml of this infused was pipetted out in 2 litre porcelain dish to which 20 ml indigo solution and 750ml doubled distilled water was added. This was titrated with standard $KMnO_4$ (0.1 N) solution by adding 1 ml at a time, until blue solution changed to green, after which a few drops were added at a time until solution turned golden yellow in colour (A) . Similarly, a mixture of 20 ml indigo solution and 750 ml of doubled distilled water was titrated (B). The percentage of total tannins = [(A-B) X 1000]/ weight of drug sample taken X 0.1. Each ml of 0.1 N $KMnO_4$ = 0.004157 g of total tannins.

Free radical scavenging activity

Hundred milligrams of dried methanolic extract was dissolved in 100ml of methanol to make a stock solution of 1 mg/ml, aliquots from this stock solution were further diluted with methanol as per the concentrations required. Free radical scavenging activity of the

methanolic extract was tested in three *in vitro* models, viz., antiradical activity using DPPH[9], superoxide radical scavenging activity in riboflavin-light-NBT system[10] and reducing power assay by the transformation of Fe^{3+} to Fe^{2+} in the presence of extracts[11]. The reaction mixture for the assays is given follows;

Antiradical activity was measured by a decrease in absorbance at 517 nm of a solution of coloured DPPH in methanol brought by the samples. A stock solution of DPPH (1.3mg/ml in methanol) was prepared such that 75 µl of it in 3 ml methanol gave an initial absorbance of 0.8. Decrease in the absorbance in the presence of sample extract at different concentrations was noted after 15 min. EC_{50} was calculated from % inhibition. A blank reading was obtained using as positive control.

Assay for superoxide radical scavenging activity was based on the capacity of the sample to inhibit blue formazan formation by scavenging the superoxide radicals generated in riboflavin-light-NBT system. The reaction mixture contained 50 mM phosphate buffer (pH 7.6) 20 µg riboflavin, 12 mM EDTA, NBT 0.1mg/3ml, added in that sequence. The reaction was started by illuminating the reaction mixture with different concentration of sample extract for 150s. Immediately after illumination, the absorbance was measured at 590 nm and EC_{50} was calculated. Methanol was used for blank reading. Ascorbic acid was used as positive control.

The reducing capability of the sample extracts was measured by the transformation of Fe^{3+} to Fe^{2+} in the presence of the extract. Increased absorbance of the reaction mixture indicates increased reducing power. Different concentrations of extract in 1 ml of water were mixed with 2.5 ml of phosphate buffer and 2.5 ml of potassium ferricyanide (1%). The mixture was incubated at 50^0 C for 20min, 2.5 ml of trichloroacetic acid (10%) was added to the mixture, centrifuge at 3000rpm for 10min, 2.5 ml of upper layer of the mixture was mixed with 2.5 ml distilled water and 0.5 ml of $FeCl_3$ solution (0.1%) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicates increased reducing power. Gallic acid and tannic acid were used as positive control.

RESULTS AND DISCUSSION

In the present study, preliminary phytochemical testing shows (Table-1) the presence of high amount of glycosides, alkaloids, tannins, phenolics and other all the principal secondary metabolites were detected in methanolic extract of *Tinospora cordifolia*. The results as shown in Table-2 reveals the presence of tannins and phenolic in higher concentration of about total phenolic content 7.2%w/w and total tannic acid content 8.8%w/w subsequently quantification and this reasons prompted us to study the free radical scavenging activity of *Tinospora cordifolia*

Table 1: Preliminary Phytochemical screening of *Tinospora cordifolia* stem methanolic extract

Phytoconstituents	Inference
Alkaloids	++
Carbohydrates	+
Glycosides	++
Protein & Amino acids	+
Fixed oils & fats	+
Tannins	++
Saponins	+
Steroids (Phytosterols)	++
Flavonoids	+
Phenols	++

++ means abundant; + denotes average.

Free radicals are well known reactive molecules mainly derived from univalent reduction of oxygen. Free radicals are highly reactive and present challenges to the cellular morphology and functional integrity a decreased in membrane fluidity, loss of enzymes, receptor activity and damaged to membrane proteins leading to cell inactivation and disease conditions[12]. The living system is protected from this by enzymes such as superoxide dismutase, glutathione peroxidase and catalase and certain endogenous antioxidant such as α – tocopherol, ascorbic acid, β–carotene and uric acid, since the endogenous antioxidants acting as intracellular defense systems protecting cells from free radicals damage and extensive lyses[13]. Scavenging and diminishing the formation of oxygen-derived species are not 100% efficient, micro nutrients or antioxidants taken as supplements are particularly important in diminishing the cumulative oxidative damages[12].

Table 2: Total phenolic and total tannin content of *Tinospora cordifolia* stem methanolic extract

Total phenolic (% w/w)*	Total tannins (% w/w)*
7.2130± 046	8.7506 ± 0.03

* mean % SD (n =3)

Table 3: Free radical scavenging activity of methanolic extract of *Tinospora cordifolia* stem

Methanolic extract of *Tinospora cordifolia* sample (µg)	Antiradical activity with DPPH EC_{50} (µg)	Superoxide radical scavenging activity EC_{50} (µg)
50	07.84	24.34
100	13.94	46.46
150	21.34	62.12
200	28.64	84.51
250	34.87	98.63

Table 4: Reducing power assay of methanolic extract of *Tinospora cordifolia* stem measured by the transformation of Fe^{3+} to Fe^{2+}

Samples	Concentration in µg	Absorption at 700 nm*
Methanolic extract of *Tinospora cordifolia*	50	0.289 ± 0.002
	100	0.521± 0.004
	150	0.724 ± 0.006
	200	0.945 ± 0.013
	250	1.067 ± 0.084
Gallic acid	5	0.076 ± 0.032
	10	0.192 ± 0.042
	20	0.543 ± 0.021
	50	1.364 ± 0.013
Tannic acid	5	0.124 ± 0.014
	10	0.314 ± 0.214
	20	0.742 ± 0.313
	50	1.394 ± 0.216

*****Mean % SD (n =3)

DPPH radical scavenging activity

To elevate the antiradical activity of methanolic *Tinospora cordifolia* stem exhibited DPPH radical scavenging activity in concentration dependant manner. This method is based on the reduction of methanolic DPPH solution in the presence of hydrogen donating antioxidant (AH) due to the formation of non- radical form DPPH – H by the reaction DPPH+ AH → DPPH – H + A. The remaining DPPH measured after a certain time, corresponds inversely to the radical scavenging activity of the antioxidant[14].The sensitivity of the method is determined by the strong absorption of DPPH.

In the present study provides information on the reactivity of test compounds with a stable free radical since add electron of DPPH gives strong absorption band at 517 nm and when it is quenched by the extract there is a decreased in absorbance. Methanolic extract of *Tinospora cordifolia* stem showed (Table-3) a very greater antiradical activity in scavenging DPPH radical with maximum inhibition of above 85% (EC_{50} ranged from 07.84 µg to 34.87 µg for the sample).

Superoxide scavenging activity

Superoxide radical scavenging activity exhibited by the Methanolic *Tinospora cordifolia* stem extract at different concentration in presented in Table 4. This shows the proportionality between concentration of extract and radical scavenging activity of the stem extract the probable mechanism of scavenging the superoxide anions may be due to the inhibitory effect of methanolic extract of the stem towards generation of superoxides in the reaction mixture

Reducing power assay is a convenient and rapid screening method for measuring the antioxidant potential. The reducing ability (Fe^{3+} to Fe^{2+} transformation in terms of increasing absorbance) was found to increase with rising concentration in sample about 250µg of methanolic extract of *Tinospora cordifolia* stem were shows to have maximum reducing power, which was comparable to that of gallic acid and tannic acid which gave maximum absorption at a concentration of 50µg (Table 5).

CONCLUSION

To conclude, the above experiments clearly indicate that methanolic *Tinospora cordifolia* stem extract showed effective freeradical scaving activity which can be attributed to the presence of tannins and phenolics along with other compounds. Further investigations on the methanolic *Tinospora cordifolia* stem extract, purification of compounds and molecular mechanisms of its protective actions will be performed.

REFERENCES

1. Kiritikar KR, Basu BD. Indian medicinal plants. In: Blatterss, Canis JR, editors. *Tinospora cordifolia.* Volume I. lalit mohan publishers; India; 1933, 77- 78.
2. Anonymous. Wealth of India; Raw materials, Volume X . New Delhi : CSIR; 1976.
3. Nadkarni KM, Nadkarni Ak, editers. Indian materia medica, Vol – I 3rd ed. Mumbai : M/S. popular prakasan Pvt. Ltd; 1976.
4. Sivarajan VV, Balachandean I . Ayurvedic Drugs and their plants sources. New Delhi: Oxford and IBH publishing Co. Pvt. Ltd; 1999.
5. Singh SS,Pandey SC, Srivastava S, Gupta KS, Patro B, Ghosh AC. Chemistry and medicinal properties of *Tinospora cordifolia* (Guduchi). Indian J Pharmacol. 2003; 35: 83-91.
6. Khandelwal KR. Practical Pharmacognasy techniques and experiments. 2nd ed. Pune, Nirali prakashan, 2000.
7. Singleton VL, Rossi JA Jr. Colorimetry of total phenolics with phosphomolybdic acid. Phosphotungstic acid reagents. Am.J Enol Viticult 1965; 16: 144- 58.
8. William H,editor. Official methods of Analysis. Washington Dc: Association of Official Agriculture chemists; 1960,185.
9. Ravishankara MN, Shrivastova N, Padh H, Rajani M, Evaluation of antioxidant properties of root bark of *Hemidesmus indicus* phytomedicine 2009; 9: 153-60.
10. Bagul MS, Ravishankara MN, Padh H, Rajani M . Phytochemical evaluation and free radical scavenging properties of *rhizome* of *Bergenia ciliata* (Haw) Sternb: Forma *ligulata* Yeo. J Nat Rem 2003; 3:83 - 89.
11. Navarro CM, Montilla MP, Martin A, Jimenez J, Utrilla MP. Free radical scavenging and antihepatototoxic activity of *Rosamarinus tomentosus* planta Med 1993;59:312 – 314.
12. Vani T, Rajani M, Sankar S, shishoo CJ. Antioxidants properties of the ayurvedic formulation triphala and its constituents. Int J Pharmacog 1997; 35; 313 - 317.
13. Halliwell B, Gutteridge JM. Free radicals in biology and medicine, Oxford: Oxford University press; 1998.
14. Koleva II, van Beer TA, Linssen JP, de Groot A, Evstatieva LN. Screening of plants extracts for antioxidant activity: Acomparative study on three testing methods. Phytochem Anal 2002; 13: 8 -17.