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RESEARCH PAPER

The biochemial and phyotochemical study of *Tinospora cordifolia* in water extracts

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ABSTRACT

Tinospora cordifolia (TC) is a widely used medicinal herb in Indian ayurvedic system as this plant has great antioxidant potential which is due to their contents of variable phytoconstituents. The aqueous extract showed significant antioxidant potential and also possess metal chelation and reducing power activity. *Tinospora cordifolia* aqueous extract was found to have the total phenolic content 8.0 mg/g, flavonoids 3.0 mg/g and tannins 18.0 mg/g.

Key Words : *Tinospora cordifolia*, Antioxidant, Metal chelation, Phenolic content

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In ayurveda, *Tinospora cordifolia* (TC), an indigenous plant, is used widely in ayurvedic medicine. *Tinospora cordifolia*, commonly known as Gulancha, is a glabrous, deciduous climbing shrub on large trees; belong to the family Menispermaceae. *T. cordifolia* is usually used in Indian ayurvedic medicine for treating diabetes mellitus (Modak *et al.*, 2007). The extract of *Tinospora cordifolia* stem is also useful in skin diseases, jaundice, arthritis, gout, and anemia (Stanely Mainzen and Menon, 2001). The root and stem of *T. cordifolia* are prescribed in combination with other drugs as an antidote. Bishayi *et al.* (2002) has reported hepatoprotective and immunomodulatory effects of *Tinospora cordifolia* in rats. The aqueous extract of roots of *T. cordifolia* act as anti-oxidant in alloxan diabetes rats (Singh *et al.*, 2003).The water soluble fraction of *Tinospora cordifolia* leaf has immunostimulatory and disease resistance properties and has probable to be used as an immunoprophylactic agent (Alexander *et al.*, 2010). In diabetes, protein glycation and glucose autoxidation may generate free radicals, which in turn catalyze lipid peroxidation (Mullarkey *et al.*, 1990). The free radicals are well recognized reactive oxygen molecules, mostly derived from univalent reduction of oxygen and giving rise to numerous by products through their reactions with almost all

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unsaturated normal living cells (Baynes, 1991). Antioxidant can catch the free radicals directly or scavenge them through a series of coupled reactions with antioxidant enzymes. *Tinospora cordifolia* is a valuable source in various secondary metabolites such as alkaloids, flavonoids, tannins, and terpenoids which show some antioxidant effects. The information regarding the *in vitro* antioxidative properties of *Tinospora cordifolia* is very little. Hence, the phytochemical components such as total phenols, flavonoids, tannins and metal chelating property were evaluated to assess *Tinospora cordifolia* as potential antioxidant.

RESEARCH METHODOLOGY

Plant material :

The leaves of plant *Tinspora cordifolia* were freshly collected in winter season and the taxonomic identification of the plant was confirmed. Dried 60gm fine powder from leaves of *Tinospora cordifolia* was soaked in 600ml of water. It was kept at room temperature (RT) for 48 hours with intermittent mixing. Aqueous extract of *Tinospora cordifolia* obtained, after 48 hours of soaking, was filtered using Whatman paper and stored at 4°C. Protein precipitation was done by 90 per cent ammonium sulphate solution. To remove precipitate, content was centrifuged at 10000 rpm for 30 minutes. The supernatant was decanted off and pellet was resuspended in PBS.

Metal chelating activity :

The metal chelating property was determined as described by Bhawya and Anilakumar (2010). In brief, the extracts were mixed with solution of 2 mM $FeCl_2$. The reaction was initiated by the addition of 200 µl of 5 mM ferrozine. The mixture was shaken vigorously and left at room temperature for 10 min. The absorbance of the solution was then measured spectro photometrically at 562 nm. The per cent of Inhibition of ferrozine-Fe complex was calculated by the formula given below :

$$\text{Per cent inhibition} = [(A_0 - A_1) / A_0] \times 100$$

where, A_0 is the absorbance of the control and A_1 is the absorbance in the presence of the samples of *Tinospora cordifolia* aqueous extract. All the values are mean values of triplicate. BHA was used as a standard compound.

Total phenolic contents :

The total phenolic contents were estimated spectro photometrically as per the method Folin-Ciocalteau with minor modification. In brief, different concentrations of *Tinospora cordifolia* aqueous was made up using the distilled water. After that 100 µl of *Tinospora cordifolia* aqueous extract was mixed with 500 µl folin-Ciocalteau reagent and kept the sample for 10 min at room temperature. Now to this mixture, 2ml of 7 per cent sodium carbonate solution was added and again kept for 1 min in boiling water bath. After cooling, the absorbance of samples was read at 650nm against blank. The different concentration of gallic acid was used as standard, and the results were expressed as mg gallic acid equivalents gm^{-1} extract.

Flavonoids :

The estimation of flavanoids was carried out as per the protocol described by Delcour and Varebeke (1985). The different concentration of the *Tinospora cordifolia* aqueous extract in distilled water and 5 ml chromogen reagent (1g L^{-1} 4-dimethyl amino cinnamaldehyde) was added and after 10 min absorbance was measured at 640 nm against a blank consisting of water instead of extract and the flavanoids content was calculated with (±) Catechin and the concentration was expressed as (±) catechin equivalents.

Tannins :

0.5ml *Tinospora cordifolia* aqueous extract was weighed and boiled with 75 ml of water for 30 min. This content was centrifuged at 800 × g for 20 min and the finally made upto the volume to 100 ml using distilled water. Aliquots were treated with Folin-Denis reagent and absorbance was measured at 700 nm.

RESULTS AND REMONSTRATION

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Metal ions chelating activity :

A metal ion chelating activity (ferrous ion-chelating) of *Tinospora cordifolia* aqueous extract is shown in Fig. 1. Under the oxidative stress condition the production of superoxide anion radicals, hydrogen peroxide, and hydroxyl radicals is increased. This reactive oxygen

species (ROS) degraded the biomolecules like DNA damage, protein oxidation and lipid oxidation. The transition metals are capable to react with ROS and lower the effect of ROS. Among the transition metals, ferrous state of iron accelerates lipid oxidation by breaking down hydrogen and lipid peroxides to reactive free radicals via the fenton reaction, Fe^{3+} ion also produces radicals from peroxides although the rate is 10-fold lower than that of Fe^{2+} ion (Haber and Weiss, 1934). Fe^{2+} ion is the most dominant pro-oxidant among the various species of metal ions (Halliwell and Gutteridge, 1985). Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of chelating agents, the complex formation is disrupted, resulting in a decrease in the red colour complex. Therefore, measurement of colour reduction allows estimating the metal chelating activity of the coexisting chelator.

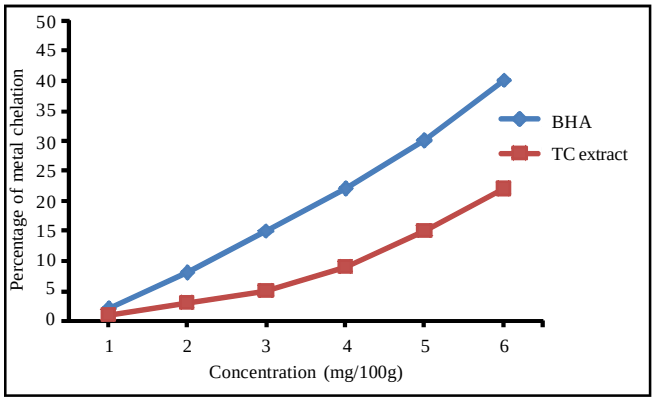

Fig. 1 : Metal ions chelating activity of *Tinospora cordifolia*

Polyphenols, tannins and flavonoids :

Antioxidant rich plant extracts serve as source of nutraceuticals that alleviate the oxidative stress and therefore, prevent or reduce the onset of degenerative diseases. It is very well-known that plant phytochemicals /phenolics, in general, are highly effective free radical scavengers and antioxidants. The total phenolic content was found to be 8.0 mg/g, flavonoids 3.0 mg/g and tannins 18.0 mg/g of *Tinospora cordifolia* aqueous extract (Fig. 2). Polyphenolic compounds have good property to donate hydrogen. These compounds are responsible for the inhibition of free radical induced lipid peroxidation (Yen *et al.*, 1993). As a result the antioxidant activities of plant/ herb extracts are often explained with respect to their total phenolics and flavonoid contents, with good association.

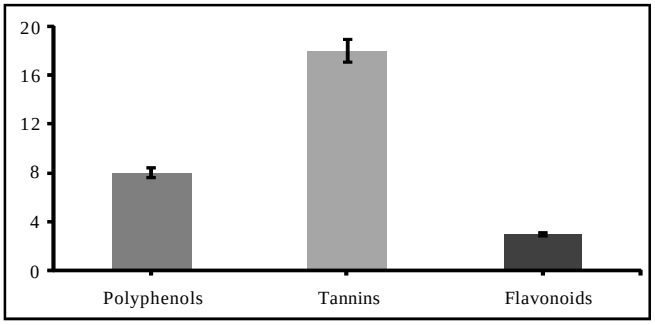

Fig. 2 : Phytochemicals analysis of *Tinospora cordifolia*

Each value is presented as mean standard error (n=3).The vertical bars indicate standard errors where they exceeded the symbol size.

It has been found that phytochemicals contribute to the etiology of a variety of ailment. Antioxidants can prevent the risk of so many diseases by interacting with free radicals. The present study was undertaken to determine the metal chelation, phenolic, flavonoid and tannin contant in this plant. The *Tinospora cordifolia* aqueous extract was found to be effective in scavenging superoxide anion radical. The extract significantly acted as strong electron-donating agents in the Fe^{3+} to Fe^{2+} assay. In addition, they possess phytochemicals which exhibit a strong free radical scavenging activity.

The antioxidant activity of *Tinospora cordifolia* aqueous extract is due to several secondary metabolites especially, *e.g.*, phenolic compounds. In addition, they possess phytochemicals such as polyphenols, flavonoids and tannins which attribute to a strong free radical scavenging activity (Kitts *et al.*, 2000). *Tinospora cordifolia* has potential application as antioxidant that is why this plant has occupied a special place in ayurvedic, “Rasayanas” to improve the anti oxidant and the body resistance against infections.

Conclusion :

The high antioxidant activity of the *Tinospora cordifolia* aqueous extracts indicate the potential of the leaves as a source of natural antioxidants or nutraceuticals to reduce oxidative stress with consequent health benefits.

REFERENCES

Alexander, C. P., Kirubakaran, C. J.W. and Michael, R.D. (2010). Water soluble fraction of *Tinospora cordifolia* leaves enhanced the non-specific immune mechanisms and disease resistance in Oreochromis mossambicus. *Fish & Shellfish Immunol.*, **29** : 765-772.

Baynes, J.W. (1991). Role of oxidative stress in development of complications in diabetes. *Diabetes*, **40** : 405-412.

Bhawya, D. and Anilakumar, K.R. (2010). *In vitro* antioxidant potency of *Tinospora cordifolia* (gulancha) in Ssquential extracts. *Internat. J. Pharmac. & Biolog. Archive*, **1**:448-456.

Bishayi, B., Roychowdhury, S., Ghosh, S. and Sengupta, M. (2002). Hepatoprotective and immunomodulatory properties of *Tinospora cordifolia* in CCl_4 intoxicated mature albino rats. *J. Toxicolog. Sci.*, **27** : 139-146.

Delcour, J.A. and Varebeke, D.J.D. (1985). A new colourimetric assay for flavanoids in pilsner beers. *J, Institute Brewing*, **91** : 37-40.

Haber, F. and Weiss, J. (1934). The catalytic decomposition of hydrogen peroxide by iron salts. In Proceedings of the Royal Society of London A: Mathematical, Physical and Engineering Sciences. *Royal Society*, **147** : 332-351.

Halliwell, B. and Gutteridge, J.M. (1985). *Free radicals in biology and medicine*. Clarendon Press, *Oxford*, 331-332.

Kitts, D.D., Wijewickreme, A.N. and Hu, C. (2000). Antioxidant properties of a North American ginseng extract. *Molecular & Cellular Biochem.*, **203** : 1-10.

Modak, M., Dixit, P., Londhe, J., Ghaskadbi, S. and Devasagayam, T.P.A. (2007). Indian herbs and herbal drugs used for the treatment of diabetes. *J. Clinical Biochem. & Nutrit.*, **40** : 163.

Mullarkey, C.J., Edelstein, D. and Brownlee, M. (1990). Free radical generation by early glycation products: a mechanism for accelerated atherogenesis in diabetes. *Biochem. & Biophysical Res. Commun.*, **173** : 932-939.

Raghunathan, K. and Mitra, R. (1982). Pharmacognosy of indigenous drugs. Central council for research in Ayurveda and Siddha, New Delhi, 433.

Singh, S.S., Pandey, S.C., Srivastava, S., Gupta, V.S., Patro, B. and Ghosh, A.C. (2003). Chemistry and medicinal properties of *Tinospora cordifolia* (guduchi). *Indian J. Pharmacol.*, **35**: 83-91.

Stanely, Mainzen Prince P. and Menon, V.P. (2001). Antioxidant action of *Tinospora cordifolia* root extract in alloxan diabetic rats. *Phytotherapy Res.*, **15** : 213-218.

Yen, G.C., Duh, P.D. and Tsai, C.L. (1993). Relationship between antioxidant activity and maturity of peanut hulls. *J. Agril. & Food Chem.*, **41** : 67-70.

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