# Studies on Anti-oxidant activity of *Tinospora cordifolia* (Miers.) Leaves using *in vitro* models

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**Abstract:** Plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. A regular and widespread use of herbs throughout the world has increased serious concern over their quality, safety and efficacy. Thus, a proper scientific evidence or assessment has become the criteria for acceptance of herbal health claims. In the present study, we examined the anti-oxidant effects of leaves of *Tinospora cordifolia*. Dried and powered leaves of *T. cordifolia* were extracted with hexane, chloroform, methanol, ethanol and water. Total phenolic and flavonoid contents of different solvent extracts were determined. Of the different solvent extracts, ethanol extract had the highest phenol and flavonoid content of 5.1±0.25 mg/g and 0.52±0.02 mg/g respectively. Antioxidant assays were carried out by using different *in vitro* models such as total reducing power, total antioxidant activity, lipid peroxidation inhibitory activity, DPPH radical scavenging activity and superoxide radical scavenging activity. Ethanol extract showed the highest total antioxidant activity of 41.4±0.45 µM Fe(II)/g. The $EC_{50}$ values of ethanol extract for lipid peroxidation inhibitory activity and DPPH radical scavenging activity was found to be 0.1 and 0.5 mg/ml respectively. The anti-oxidant activities of other solvent extracts were poor when compared to the ethanol extract. These results suggest that, the active antioxidant compounds are better extracted in ethanol and there is a direct correlation between the total polyphenols extracted and its anti-oxidant activity. The *in vitro* anti-oxidant activity of *T. cordifolia* justifies the ethno medical use of this plant. [Journal of American Science 2010;6(10):736-743]. (ISSN: 1545-1003).

**Key words:** Medicinal plant; *Tinospora cordifolia*; solvent extracts; anti-oxidant activity

## 1. Introduction

Plants have been a source of medicine in the past centuries and today scientists and the general public recognize their value as a source of new or complimentary medicinal products. Recently, wide array of research investigations highlight the potential health beneficial principles from phytal sources. Over the past twenty years, interest in medicinal plants has grown enormously from the use of herbal products as natural cosmetics and for self-medication by the general public to the scientific investigations of plants for their biological effects in human beings. Beyond this pharmaceutical approach to plants, there is a wide tendency to utilize herbal product to supplement the diet, mainly with the intention of improving the quality of life and preventing the diseases of elderly people (Maffei, 2003).

The WHO estimates that up to 80% of people still rely mainly on traditional remedies such as herbs for their medicine (Tripathi and Tripathi, 2003). India has been identified as a major resourceful area in the traditional and alternative medicines globally. Multi-factorial health beneficial activity of these plant extracts has been attributed to multi-potent anti-oxidant, anti-microbial, anti-cancer, anti-ulcerative and anti-diabetic properties. Generally, anti-oxidants have been identified as major health beneficial compounds reported from varieties of medicinal plants and are sources for alternative medicines (Daniel, 2005).

Free radicals or reactive oxygen species (ROS) are formed in our body as a result of biological oxidation. The over production of free radicals such as hydroxyl radical, super oxide anion radical, hydrogen peroxide can cause damage to the body and contribute to oxidative stress (Diplock, 1994; Thomson, 1995). Oxidative damage of proteins, DNA and lipid is associated with chronic degenerative diseases including cancer, coronary artery disease, hypertension, diabetes etc (Lee *et al*., 2000) and compounds that can scavenge free radicals have great potential in ameliorating these disease processes (Kris-Etherton *et al*., 2002; Di Malteo and Esposito, 2003; Behera *et al*., 2006). Most of the reactive oxygen species are scavenged by endogenous defense systems such as catalase, superoxide dismutase and peroxidase-glutathione system (Rice-Evans and Bourdan, 1993). But these systems may not be completely efficient requiring them to depend on exogenous anti-oxidants from natural sources.

Medicinal plants constitute one of the main sources of new pharmaceuticals and health care

products. A whole range of plant derived dietary supplements, phytochemicals and pro-vitamins that assist in maintaining good health and combating disease are now being described as functional ingredients and neutraceuticals. The role of medicinal plants in disease prevention or control has been attributed to antioxidant properties of their constituents (Ivanova *et al*., 2005). The protective effect of plant products are due to the presence of several components such as enzymes, proteins, vitamins (Halliwell, 1996), carotenoids (Edge *et al*., 1997), flavonoids (Zhang and Wang, 2002) and other phenolic compounds (Argolo *et al*., 2004).

Since synthetic anti-oxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have restricted use in foods, the search for natural anti-oxidants has greatly increased in the recent years. The researchers have focused on natural anti-oxidants and numerous crude extracts and pure natural compounds have been recognized to have beneficial effects against free radicals in biological systems as anti-oxidants (Reena *et al*., 2004; Hazra *et al*., 2008; Demiray *et al*., 2009).

*Tinospora cordifolia* Miers. is a widely used shrub in folk and Ayurvedic systems of medicine. It is a large, glabrous, deciduous climbing shrub belonging to the family menispermaceae. It is distributed throughout tropical Indian subcontinent and China. It is reported to possess anti-spasmodic, anti-inflammatory, anti-allergic, anti-diabetic, anti-oxidant properties (Singh *et al*., 2003).

The objective of the present study was to determine the anti-oxidant activity of *T. cordifolia* leaves in different solvent extracts using standard methods. The findings from this work may add to the overall value of the medicinal potential of the shrub, since most of the studies have focused on antioxidant activities of root and stem of *T. cordifolia*.

## 2. Materials and Methods

### *2.1. Plant material*

Fresh and healthy leaves of *T. cordifolia* were collected from local growers. The leaves were washed thoroughly in distilled water and the surface water was removed by air drying under shade. The leaves were subsequently dried in a hot air oven at 40 $^0$C for 48h, powdered and used for extraction.

### *2.2. Preparation of aqueous extract*

Fifty grams of powdered leaves of *T. cordifolia* was macerated with 100 ml sterile distilled water in a blender for 10 min. The macerate was first filtered through double layered muslin cloth and centrifuged at 4000 g for 30 min. The supernatant was filtered through Whatman No.1 filter paper and heat sterilized at 120 $^0$C for 30 min. The extract was preserved aseptically in a brown bottle at 4 $^0$C until further use.

### *2.3. Preparation of solvent extract*

Fifty grams of shade dried powered leaf material was extracted successively with chloroform, hexane, methanol and ethanol until the plant material became colorless. It was then filtered with sterile Whatman filter paper into a clean conical flask and the filtrate was transferred into the sample holder of the rotary flash evaporator. The extracts so obtained was weighed and preserved at 4 $^0$C in airtight bottles until further use.

### *2.4. Total phenolic content*

Total soluble phenolic content was estimated by Folin-Ciocalteau reagent method (Malick & Singh, 1980) using gallic acid as a standard phenolic compound. One ml of stock solutions of different solvent extracts was prepared (1g/ml) from which different aliquots were pipetted out into test tubes. The volume was made up to 3 ml with distilled water to which freshly prepared Folin-Ciocalteau reagent was added. After 3 min, 2 ml of 20% sodium carbonate solution was added to each tube and mixed thoroughly. The tubes were placed in boiling water for one minute, cooled and the absorbance was measured at 650 nm in a spectrophotometer against a reagent blank. The concentrations of the total phenolic compounds in the extracts were obtained by extrapolating the absorbance of gallic acid on standard gallic acid graph. The experiment was repeated thrice and concentration of total phenols was expressed as mg /g of dry extract.

### *2.5. Total flavonoid content*

The total soluble flavonoid content was estimated by aluminium chloride colorimetric method for both aqueous and solvent extracts (Woisky & Salatino, 1998). 0.5ml of stock solution (1g/ml) of the extract, 1.5 ml methanol, 0.1ml potassium acetate (1M) was added to reaction test tubes and volume was made up to 5 ml with distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm. Total flavonoid content was calculated by extrapolating the absorbance of reaction mixture on standard curve of rutin. The experiment was repeated thrice and the total flavonoid content was expressed as equivalent to rutin in mg/ g of the extracts.

### *2.6. Antioxidant activity assays*

#### *2.6.1. Total reducing power*

The determination of reducing power was performed as described by Yen and Duh (1993). Various extracts (0.1 - 0.9 mg/ml) were mixed with

phosphate buffer (500 µl, 20 mM, pH 6.6) and 1% potassium ferricyanide (500 µl), and incubated at 50 $^0$C for 20 min; 500 µl of 10% trichloro acetic acid were added, and the mixture was centrifuged at 2500 rpm for 10 min. The supernatant was mixed with distilled water (1.5 ml) and 0.1% ferric chloride (300 µl) and the absorbance was read at 700 nm. The experiment was repeated thrice. Increase in the absorbance of the reactions mixture indicated increase in the reducing power.

***2.6.2. Ferrous reducing antioxidant power assay (Total antioxidant activity assay)***

The method employed was a modification method of Benzie & Strain (1996) method. The stock solutions included 300 mM acetate buffer (pH 3.6), 10 mM 2, 4, 6-tripyridyl-s-tri-azine solution in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$ solution. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ and 2.5 ml $FeCl_3.6H_2O$. The temperature of the solution was raised to 37 $^0$C before using. Plant extracts (150 µl) were allowed to react with 2850 µl of the FRAP solution for 30 min in the dark condition. Readings of colored product (ferrous tripyridyltriazine complex) were taken at 593 nm. The experiment was repeated thrice. Results were expressed in µM Fe (II)/g dry mass and compared with that of BHT.

***2.6.3. Lipid peroxidation inhibitory activity***

The lipid peroxidation inhibitory activity of the leaf extracts was determined according to the method of Duh & Yen (1997). Egg lecithin (3 mg/ml phosphate buffer, pH 7.4) was sonicated in an ultrasonic sonicator for 10 min to ensure proper liposome formation. Test samples (100 µl) of different concentrations (0.1 - 0.9 mg/ml) were added to liposome mixture (1 ml); the control was without test sample. Lipid peroxidation was induced by adding ferric chloride (10 µl, 400 mM) and L-ascorbic acid (10 µl, 200 mM). After incubation for 1 h at 37$^0$ C the reaction was stopped by adding hydrochloric acid (2 ml, 0.25 N) containing trichloroacetic acid (150 mg/ml) and thiobarbutyric acid (3.75 mg/ml). The reaction mixture was subsequently boiled for 15 min, cooled, centrifuged at 1000 rpm for 15 min and the absorbance of the supernatant was measured at 532 nm and compared with that of BHA. Percentage radical scavenging was calculated using the following formula:

% Inhibition = $[(A_{control} – (A_{samaple} - A_{sampleblank} / A_{control}] \times 100$

***2.6.4. DPPH radical scavenging activity***

The free radical scavenging activity of the leaf extracts was assayed using a stable free radical, 1, 1-diphenyl-2-picryl hydrazyl (DPPH). The DPPH scavenging assay employed in the present study was a modification of the procedure of Moon & Terao (1998). 0.1 ml of test sample at different concentration (0.1 - 0.9 mg/ml) was mixed with 0.9 ml of Tris-HCl buffer (pH 7.4); then 1 ml of DPPH (500 µM in ethanol) was added. The mixture was shaken vigorously and left to stand for 30 min. The absorbance of the resulting solution was measured at 517 nm in a spectrophotometer and compared with that of BHA. The experiment was repeated thrice. The percentage of DPPH scavenging was calculated using the following formula:

% Scavenging = $[(A_{control} – (A_{samaple} - A_{sampleblank} / A_{control}] \times 100$

***2.6.5. Superoxide radical scavenging activity***

The measurement of superoxide anion scavenging activity was based on the method by Fontana, Mosca, & Rosei (2001). Superoxide radical is generated in phenazine methosulfate-nicotinamide adenine dinucleotide (PMS-NADH) systems by oxidation of NADH and assayed by the reduction of nitroblue tetrazolium (NBT) to a purple formazan. The 1 ml reaction mixture contained phosphate buffer (20 mM, pH 7.4), NADH (73 µM), NBT (50 µM), PMS (15 µM) and various concentrations of sample solution. After incubation for 5 min at ambient temperature, the absorbance at 562 nm was measured against an appropriate blank to determine the quantity of formazan generated. The experiment was repeated thrice. The results were compared with that of quercetin. The % inhibition of superoxide anion generation was calculated using the following formula:

% Scavenging = $[(A_{control} – (A_{samaple} - A_{sampleblank} / A_{control}] \times 100$

**3. Results**

In this study, some of the biological activities of *T. cordifolia* leaves have been investigated, whereby; hexane, chloroform, methanol, ethanol and aqueous extracts were assayed for their total phenolic and flavonoid contents and anti-oxidant activities using different *in vitro* models.

***3.1. Total phenolic and flavonoid content***

Results obtained in the present study revealed that the level pf polyphenols in the ethanol extract was 5.1±0.25 mg/g which was higher when compared to methanol, chloroform, hexane and aqueous extracts of *T. cordifolia*. Ethanol extract of the leaves had a flavonoid content of 0.52±0.02 mg/g. The flavonoid content of other extracts tested was lower than the ethanol extract. Aqueous extract had the least polyphenol and flavonoid content (**Table 1**).

**Table 1: Polyphenol and Flavonoid content of *Tinospora cordifolia* leaves in different solvent extracts**

| Solvents | Total phenols (mg/g) | Flavonoid content (mg/g) |
|---|---|---|
| Ethanol | 5.1±0.25 | 0.52±0.02 |
| Methanol | 4.2±0.30 | 0.45±0.03 |
| Chloroform | 2.1±0.25 | 0.25±0.02 |
| Hexane | 1.5±0.35 | 0.19±0.04 |
| Aqueous | 1.1±0.05 | 0.12±0.02 |

### *3.2. Antioxidant activity assays*

#### *3.2.1. Total reducing power*

The reducing power of different solvent extracts using the potassium ferricyanide method is shown in **Figure 1.** The result indicates that the reducing ability of the extracts increased with the concentration. Among all the extracts tested for their reducing abilities ethanol extract of *T. cordifolia* showed better reducing power as shown by the increasing optical density at 700 nm.

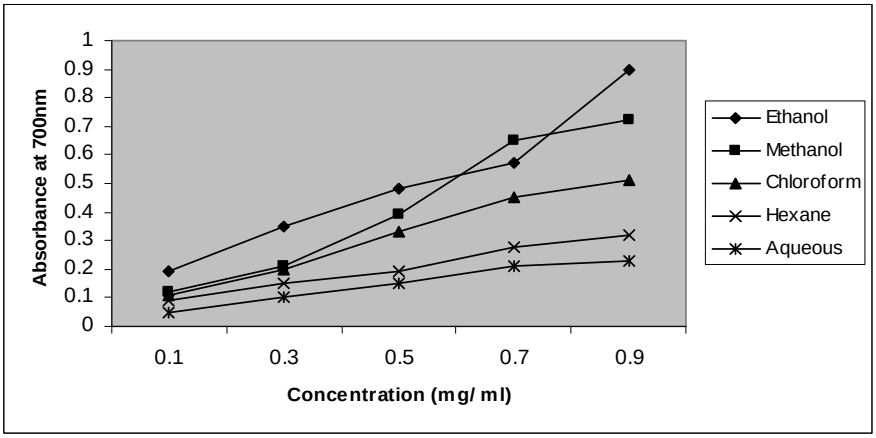


**Figure 1: Reducing power of *Tinospora cordifolia* leaf extract at different concentrations (mg/ml).**

#### *3.2.2. Total antioxidant activity*

The ability of the plant extracts to reduce ferric ions was determined by FRAP assay (**Figure 2**). An anti-oxidant capable of donating a single electron to the ferric-TPTZ (Fe (II)-TPTZ) complex would cause the reduction of the complex into the blue ferrous TPTZ (Fe (II)-TPTZ) complex which absorbs strongly at 593 nm. The FRAP values for the extracts were lower than that of BHT (63±0.35 µm/g fw). Among the extracts tested, ethanol extract had a total anti-oxidant activity of 41.4±0.45 µm/g fw followed by methanol 33.9±0.49 µm/g fw. Aqueous extract had the least reducing ability of 4.8±0.30 µm/g fw.

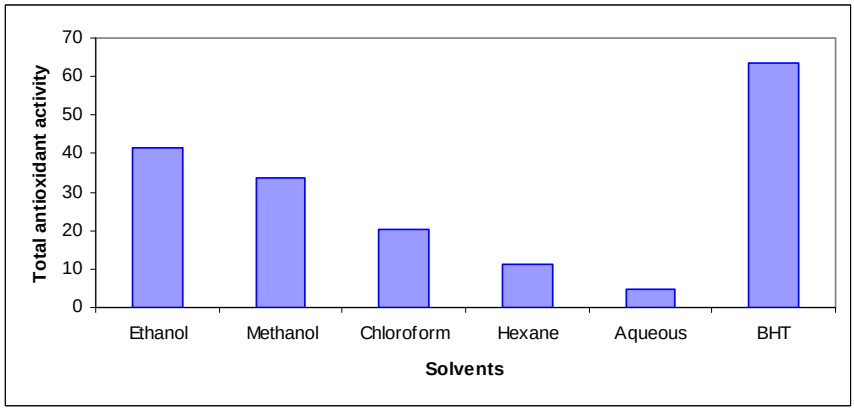


**Figure 2: Total Antioxidant activity of *Tinospora cordifolia* solvent extracts (µM Fe (II)/g).**

***3.2.3. Lipid peroxidation inhibitory activity***

The anti-oxidative action of *T. cordifolia* leaf extracts in the liposome model, induced by ferric chloride plus ascorbic acid and determined by thiobarbutyric acid method is shown in **Figure 3**. Ethanol extract had an $EC_{50}$ value of 0.1 mg/ml which showed an inhibition of 57.5%. $EC_{50}$ value could be achieved only with methanol extract at a concentration of 0.7 mg/ml. As with other extracts, 50% inhibition could not be achieved even at 0.9 mg/ml. BHA showed very strong lipid peroxidation inhibitory activity with an $EC_{50}$ value 12 µg/ml. Lipid peroxidation inhibitory activity of ethanol extract gradually decreased with an increase in concentration.

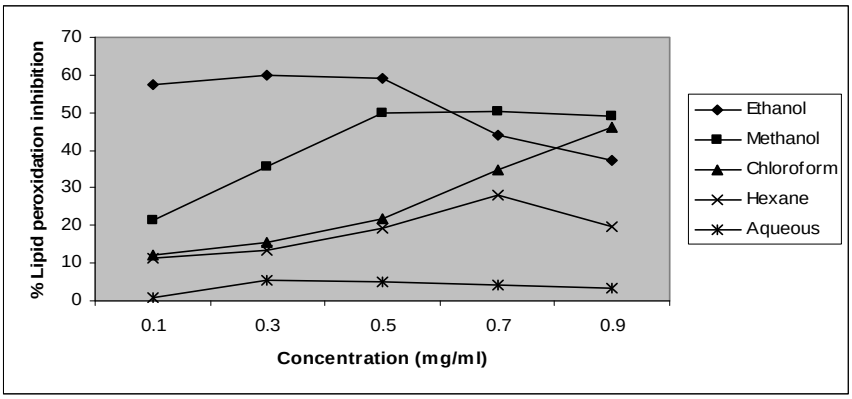


**Figure 3: Lipid peroxidation inhibitory activity of *Tinospora cordifolia* leaf extract at different concentrations (mg/ml).**

***3.2.4. DPPH radical scavenging activity***

The DPPH radical scavenging activity of *T. cordifolia* leaf extracts is shown in **Figure 4**. Among the extracts tested, ethanol extract had better scavenging activity ($EC_{50}$ value of 0.5 mg/ml) followed by methanol ($EC_{50}$ value of 0.9 mg/ml). When compared to BHA which had an $EC_{50}$ value of 5.3 µg/ml, the e $EC_{50}$ value of ethanol was quite high.

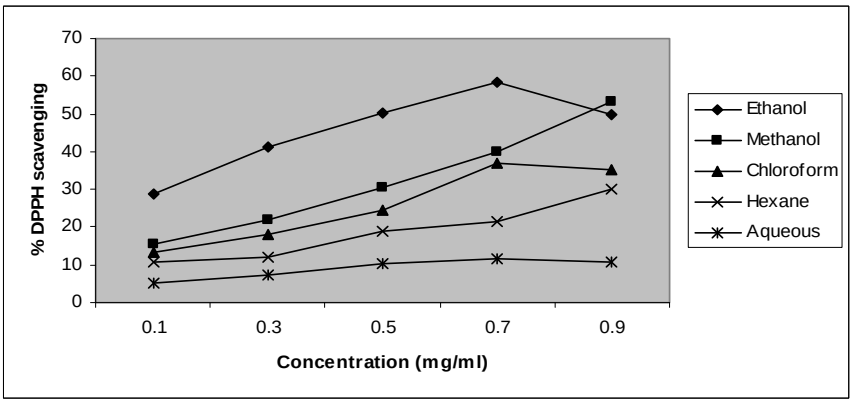


**Figure 4: DPPH radical scavenging activity of *Tinospora cordifolia* leaf extract at different concentrations (mg/ml).**

***3.2.5. Superoxide radical scavenging activity***

The superoxide radical generated from dissolved oxygen by PMS-NADH coupling can be measured by their ability to reduce NBT. The decrease in absorbance at 562 nm with the plant extracts and the reference compound quercetin indicates their abilities to quench superoxide radicals in the reaction mixture. As shown in **Figure 5** the quenching ability generally was low with all the solvent and aqueous extracts. As with ethanol extract, even at 0.7 mg/ml concentration the percentage radical scavenging was 40.1%. Percent radical scavenging abilities of other extracts were lower than ethanol extract. Quercetin was found potent with an $EC_{50}$ value of 155 µg/ml.

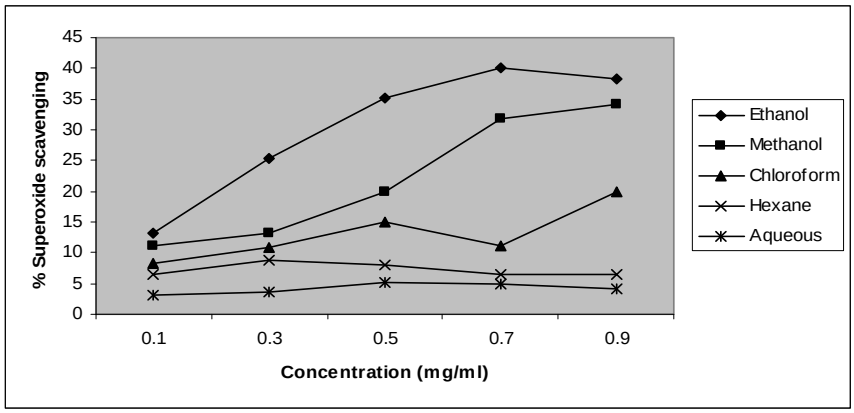


**Figure 5: Superoxide radical scavenging activity of *Tinospora cordifolia* leaf extract at different concentrations (mg/ml).**

## 4. Discussion

### *4.1. Total phenolic and flavonoid content*

Medicinal plants are an important source of antioxidants (Rice-Evans, 2004). Natural anti-oxidants increase the anti-oxidant capacity of the plasma and reduce the risk of certain diseases (Prior and Cao, 2000). Polyphenols are the major plant compounds with anti-oxidant activity. Typical phenolics that possess anti-oxidant activity are known to be mainly phenolic acids and flavonoids (Demiray *et al*., 2009). It is reported that the phenolics are responsible for the variation in the anti-oxidant activity of the plant (Luo *et al*., 2004). They exhibit anti-oxidant activity by inactivating lipid free radicals or preventing decomposition of hydro peroxides into free radicals (Pokorny 2001; Pitchaon *et al*., 2007). Flavonoids are phenolic acids which serve as an important source of anti-oxidants found in different medicinal plants and related phytomedicies (Pietta, 1998). The anti-oxidant activity of flavonoids is due to their ability to reduce free radical formation and to scavenge free radicals.

### *4.2. Antioxidant activity assays*

#### *4.2.1. Total reducing power*

Reducing power is associated with its anti-oxidant activity and may serve as a significant reflection of the anti-oxidant activity (Oktay *et al*., 2003). Compounds with reducing power indicate that they are electron donars and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary anti-oxidants (Yen and Chen, 1995).

#### *4.2.2. Total antioxidant activity*

FRAP assay is based on the ability of anti-oxidants to reduce $Fe^{3+}$ to $Fe^{2+}$ in the presence of 2, 4, 6-tri (2-pyridyl)-s-triazine (TPTZ) forming an intense blue $Fe^{2+}$ -TPTZ complex with an absorption maximum at 593 nm. This reaction is $P^H$ dependent (optimum $P^H$ 3.6). The absorbance decrease is proportional to the anti-oxidant content (Benzie and Strain, 1996). In the present study there was an increase in the anti-oxidant activity which was proportional to the polyphenol content. Ethanol extract exhibited high anti-oxidant activity due to its high polyphenol content. Similar results have been observed in *Calpurnia aurea* leaf and stem extracts, where in methanolic stem extract has shown high anti-oxidant activity than the leaf extract due to high polyphenol content (Adedapo *et al*., 2008).

#### *4.2.3. Lipid peroxidation inhibitory activity*

To evaluate the lipid peroxidation inhibitory activity of the leaf extracts of *T. cordifolia*, a liposome model was used. Anti-oxidant effect of polyphenols (flavonoids) on lipid peroxidation is the result of scavenging of hydroxyl radicals at the stage of initiation and termination of peroxyl radicals has been reported by Hussain *et al*. (1987). Earlier study by Prasad *et al*. (2005) has shown the lipid peroxidation inhibitory activity of a flavonoid isolated from *Ipomea aquatica* leaf. From the present study, it was found that the percent inhibition of lipid peroxidation by ethanol leaf extract decreased after a certain concentration which may be due to the degradation or peroxidation of the source.

#### *4.2.4. DPPH radical scavenging activity*

The stable radical DPPH has been used widely for the determination of primary anti-oxidant activity (Brand-Williams *et al*., 1995; Katalinic *et al*., 2004). The DPPH anti-oxidant assay is based on the ability of DPPH a stable free radical, to decolorize in the presence of anti-oxidants (Ara and Nur, 2009). The present study indicates that the $IC_{50}$ value of ethanol leaf extract is quite high when compared to the standard which may be attributed to its poor proton

donating ability (Voravuthikunchai *et al*., 2009). A study carried out by Hasan *et al*. (2009) has shown the DPPH radical scavenging activity of *T. cordifolia* aerial parts with an $EC_{50}$ value of 0.02 mg/ml. The difference in the $EC_{50}$ value can be attributed to the distribution of secondary metabolites that may fluctuate between different plant organs (Lissiewska *et al*., 2006).

### *4.2.5. Superoxide radical scavenging activity*

Although superoxide anion is a weak oxidant, it gives rise to generation of powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute to oxidative stress (Meyer and Isaker, 1995). Among the extracts of *T. cordifolia*, ethanol extract showed mild scavenging activity and $EC_{50}$ value could not reached even at 0.9 mg/ml concentration. The result supports the earlier study by Mathew and Kuttan (1997), which showed that the $EC_{50}$ value for superoxide scavenging could be as high as 6 mg/ml.

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## 5. References

1. Adedapo AA, Jimoh OF, Koduru S, Afolayan JA, Masika JP. Antibacterial and antioxidant properties of the methanol extracts of the leaves and stems of *Calpurnia aurea*. BMC Complementary and Alternative Medicine 2008;8:53-60.
2. Angolo AC, Sant Ana AE, Pletseh M, Coelho LC. Antioxidant activity of leaf extracts from *Bauhinia monandra.* Bioresource Technology; 95:229-233.
3. Ara N, Nur H. *In vitro* antioxidant activity of methanolic leaves and flower extracts of *Lippia alba.* Research Journal of Medicine and Medical Science 2009;4(1):107-110.
4. Behera BC, Verma N, Sonone A, Makhaija U. Determination of antioxidant potential of lichen *Usnea ghattensis in vitro*. LWT Food Science and Technology 2006;39:80-85.
5. Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Analytical Biochemistry 1996;239:70-76.
6. Brand-Williams W, Cuvelier ME, Berset C. Use of free radical method to evaluate antioxidant activity. Lebanon Wissen Technology 1995;28:25-30.
7. Daniel M. Medicinal plants: Chemistry and properties. Science Publishers, USA. 2005:1-9.
8. Demiray S, Pintado ME, Castro PML. Evaluation of phenolic profiles and antioxidant activities of Turkish medicinal plants: *Tilia argentea*, *Crataegi folium* leaves and *Polygonum bistorta* roots. World Academy of Science, Engineering and Technology 2009;54:312-317.
9. Di Malteo V, Esposito E. Biochemical and therapeutic effects of antioxidants in the treatment of Alzheimers disease, Parkinson disease and amyotrophic lateral sclerosis. Current Drug Target- CNS and Neurological Disorders 2003;2:95-107.
10. Diplock AT. Free radical damage and control. In: Rice Evans CA and Burden RH, ed. Antioxidant and free radical scavengers. Elsevier, New York, USA. 1994.
11. Duh PD, Yen GH. Antioxidative activity of three herbal water extracts. Food Chemistry 1997;60:639-645.
12. Edge R, Mc Greevy DJ, Truscott TG. The carotenoids as antioxidants: A review. Journal of Photochemistry and Photobiology B: Biology 1997;41:189-200.
13. Fontana M, Mosca L, Rosei MA. Interaction of enkephalines with oxyradicals. Biochemical Pharmacology 2001;61:1253-1257.
14. Halliwell B. Ascorbic acid in the prevention and treatment of cancer. Alternative Medicine Reviews 1996;3:174-186.
15. Hasan SMR, Hossain M, Akter R, Jamila M, Mazumder EH, Rahman S. DPPH free radical scavenging activity of some Bangladeshi medicinal plants. Journal of Medicinal Plant Research 2009;3(11):875-879.
16. Hazra B, Biswas S, Mandal N. Antioxidant and free radical scavenging activity of *Spondias pinnata*. BMC Complementary and Alternative Medicine 2008;8:63-75.
17. Hussain SR, Cillard J, Cillard P. Hydroxyl radical scavenging activity of flavonoids. Phytochemistry 1987;26:2489-2491.
18. Ivanova D, Gerova D, Chervenkov T, Yankova T. Polyphenols and antioxidant capacity of Bulgarian medicinal plants. Journal of Ethnopharmacology 2005;97(1-2):145-150.
19. Katalinic V, Milos M, Modun D, Music I, Boban M. Antioxidant effectiveness of selected wines in comparison with (+)- calectin. Food Chemistry 2004;86:593-600.
20. Kris-Etherton PM, Hecker KD, Bonanome A, Cival SM, Binkasi AE, Hilpert KF. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. American Journal of Medicine 2002;113:71s-88s.
21. Lee KG, Mitchell AE, Shibamoto T. Determination of antioxidant properties of aroma extracts from various beans. Journal of Agriculture and Food Chemistry 2000;48:4817-4820.
22. Lisiewska Z, Kmiecik W, Korus A. Content of vitamin C, carotenoids, chlorophylls and polyphenols in green parts of dill (*Anethum graveolens* L.) depending on plant height. Journal of Food Compound Analysis 2006;19:134-140.

23. Luo Q, Cai Y, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Science 2004;74:2157-2184.
24. Maffei M. Dietary supplements of plant origin-Nutrition and health approach. Taylor and Francis, e-library 2003;18.
25. Malick CP, Singh MB. In: plant Enzymology and Histo Enzymology. Kalyani Publishers. New Delhi.1980:286.
26. Mathew S, Kuttan G. Antioxidant activity of *Tinospora cordifolia* and its usefulness in the amelioration of cyclophosphamide induced toxicity. Journal of Experimental Clinical Cancer Research 2997;16(4):407-411.
27. Meyer AS, Isaksen A. Application of enzymes as food antioxidants. Trends in Food Science and Technology 1995;6:300-304.
28. Moon JH, Terao J. Antioxidant activity of caffeic acid and dihydrocaffeic acid in lard and human low density protein. Journal of Agriculture and Food Chemistry 1998;46:5062-5065.
29. Oktay M, Gulcin I, Kufre Vioglu OI. Determination of *in vitro* antioxidant activity of fennel (*Feoniculum vulgare)* seed extracts. Lebanon Wissen Technology 2003;36:263-271.
30. Pietta PG. In: Flavonoid in Health and Disease; Rice-Evans CA, Packer L ed. Marcel Dekker. New York. 1998:110.
31. Pitchaon M, Suttajit M, Pongsawatmani R. Assessment of phenolic content and free radical scavenging capacity of some Thai indigenous plants. Food Chemistry 2007;4:1409-1418.
32. Pokorny J, Yanishlieva N, Gordan M. Antioxidants in food: Practical applications. Woodhead Publishing Limited. Cambridge. 2001:3.
33. Prasad KN, Divakar S, Shivamurthy G.R. and Aradhya S.M. Isolation of free radical scavenging antioxidant from water spinach (*Ipomea aquatica* Forsk.). Journal of the Science of Food and Agriculture 2005;85:1461-1468.
34. Prior RL, Cao G. Antioxidant phytochemicals in fruits and vegetables: Diet and Health Implications. Horticulture Science 2000;35:588-592.
35. Reena R, Yuan-Tong Lin MS, Shetty K. Phenolics, their antioxidants and antimicrobial activity in dark germinated fenugreek sprouts in response to peptide and phytochemical elicitors. Asia Pacific Journal of Clinical Nutrition 2004; 13(3):295-307.
36. Rice-Evans CA, Bourdon R. Free radical lipid interaction and their pathological consequences. Progressive Lipid Research 1993;12:71-110.
37. Rice-Evans C. Flavonoids and isoflavones: absorption, metabolism and bioactivity. Free Radical Biology and Medicine 2004;36:827-828.
38. Singh SS, Pandey SC, Srivastava S, Gupta VS, Patro B. Chemistry and medicinal properties of *Tinospora cordifolia* (Guduchi). Indian Journal of Pharmacology 2003;35:83-91.
39. Thomson MJ. The role of free radicals and antioxidants; How do we know that they are working? Critical Reviews of Food Science and Nutrition 1995;35:21-29.
40. Tripathi L, Tripathi NJ. Role of biotechnology in medicinal plants. Tropical Journal of Pharmaceutical Research 2003;2(2):243-253.
41. Voravuthikunchai PS, Kanchanapoom T, Sawangjaroen N, Hutadilok-Towatona N. Antioxidant, antbacterial and antigiardial activities of *Walsura robusta* Roxb. Natural Product Research 2009;24(9):813-824.
42. Woisky R, Salatino A. Analysis of Propils: Some parameters and procedure for chemical quality control. Journal of Agricultural Research 1998;37:99-105.
43. Yen GC, Duh PD. Antioxidative properties of methanolic extracts from peanut hulls. Journal of the American Oil Chemists Society 1993;70:383-386.
44. Yen GC, Chen HY. Antioxidant activity of various tea extracts in relation to their antimutagenecity. Journal of Agriculture and Food Chemistry 1995;43:27-32.
45. Zhang HY, Wang LF. Theorotical elucidation on structure- antioxidant activity relationships for indolinonic hydroxylamines. Bioorganic and Medicinal Chemistry Letters 2002;12:225-227.


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