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Studies on in vitro evaluation of antioxidant activity of *Tinospora Cordifolia* stem extract.

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Abstract

The present investigation was undertaken to determine the evaluation of antioxidant activity of *Tinospora Cordifolia* stem extract.

Based on the extensive literature review it was observed that stem extract of *Tinospora cordifolia* was used for antioxidant studies. Hence, we have carried out antioxidant activity studies of the plant by taking only the extracting them in different solvents. the extract were tested for antioxidant activity in various *in vitro* antioxidant models *viz*., Ferrous reducing antioxidant power assay, Total reducing power, 1,1-diphenyl-2- picrylhydrazyl (DPPH) scavenging activity, Lipid peroxidation inhibitory activity and Superoxide scavenging activity in order to choose a solvent extract which shows potent antioxidant activity.

Keywords: *Estimation of total phenol and flavonoid content, Antioxidant activity assays, Total reducing power, DPPH radical scavenging activity, Lipid peroxidation inhibitory activity, Superoxide radical scavenging activity.*

Introduction

A large number of plants are being used in medicine for therapeutic or prophylactic purposes. The therapeutic properties of medicinal plants are attributed owing to the presence of active substances such as phenol and flavonoids. These natural compounds physiologically affect the body of human beings, interact with the pathogens and interrupt their growth at different stages of development and make the body disease free.

Ayurveda and other traditional medicines have gained momentum due to their totalitarian approach. Potential application of *Tinospora cordifolia* stem in Ayurveda and other traditional medicines to reduce oxidative stress along with other health benefits is well documented.

Several synthetic antioxidants such as Butylated Hydroxyanisole (BHA) and Butylated Hydroxytoluene (BHT) have restricted use in foods as they are suspected to be carcinogenic (Basniwal *et al*., 2009). In this context, natural, multifunctional, stable, non-toxic and natural compounds from plants which are pharmacologically effective or with low or no side effects are preferred for use in preventive Ayurvedic medicine and in food industry (Sati *et al*., 2010).

Antioxidant activity of stem extract of *Tinospora Cordifolia* (Willd.) studies were carried out by using various *in vitro* methods *viz.*, Ferrous reducing antioxidant power assay, Total reducing power, 1,1-Diphenyl-2-picrylhydrazyl scavenging method, Lipid peroxidation inhibitory method and Superoxide scavenging method. The results from the antioxidant studies showed that ethanol stem extract of the plant possessed significant antioxidant activity. Among the plants tested, *Tinospora cordifolia* was more potent in inhibiting lipid peroxidation and also scavenging superoxide radicals.

Present investigation encompasses successful attempts in proving the antioxidant activities of *Tinospora cordifolia* Plant stem extract. These aspects, which I believe has commercial applications for design and development of new generation drugs. The presence of active phytochemical substances also provide substantial basis for the use of the plant in ethnomedicine.

Materials and Methods

Plant material

Fresh plant of *Tinospora cordifolia* (Willd.) were collected from G.B.Pant University of Agricultural & Technology, Pantnagar, (U.K). It was identified by Dr.Anju Pal, Scientist, G.B. Pant University of Agriculture And Technology, Pantnagar, Uttarakhand

Chemicals

Solvents *viz*., chloroform, hexane, methanol and ethanol, BHT, BHA, quercetin, rutin and DPPH, Trichloroacetic acid, potassium ferricyanide, Tris HCl, L- ascorbic acid, 2, 4, 6-tripyridyl-s-tri-azine (TPTZ), HCl, ferric chloride and thiobarbutyric acid, Nitroblue tetrazolium (NBT), Nicotinamide adenine dinucleotide (NADH), Phenazine methosulphate (PMS), phenol reagent, sodium carbonate, gallic acid and potassium acetate were from Merck Uttarakhand, (India).

Preparation of Plant Material

Fresh Plant was washed thoroughly in distilled water and the surface water was removed by air drying under shade. The stem were subsequently dried in a hot air oven at 40°C for 48 h, powdered to 100-120 mesh in an apex grinder [Optics Technology, India] and used for extraction.

Preparation of aqueous extract

Fifty grams powdered of *Tinospora cordifolia* were 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 rpm for 30 min. The supernatant was filtered through Whatman No.1 filter paper and heat sterilized at 120°C for 30 min. The extract was preserved aseptically in brown bottles at 4°C until further use.

Estimation of total phenol content

Total soluble phenol content was estimated by Folin-Ciocalteau method based on the procedure of Malick and Singh (1980). Different aliquots of plant extract from the stock solution (1 g/ml) were mixed with distilled water to reach a final concentration of 3 ml. To this, 0.5 ml of freshly prepared Folin-Ciocalteau reagent was added and allowed to stand for 3 min at room temperature after which 2 ml of 20% sodium carbonate was added. The mixture was kept in boiling water for a minute, cooled and checked for absorbance at 650 nm in a spectrophotometer against a reagent blank. The concentration of the total phenol compounds in the extract was obtained by extrapolating the absorbance of gallic acid on standard gallic acid graph. The concentration of total phenols was expressed as mg/g of dry extract.

Estimation of total flavonoid content

The total soluble flavonoid content was estimated according to the method of Woisky and Salatino (1998). Stock solution of 0.5 ml (1g/ml) of the extract, 1.5 ml methanol, 0.1 ml potassium acetate (1M) was added to reaction 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 a standard curve of rutin. The total flavonoid content was expressed as equivalent to rutin in mg/g of the extract.

Antioxidant activity assays

Ferrous reducing antioxidant power assay (Total antioxidant activity assay)

The FRAP assay was carried out according to the method of Benzie and Strain (1996) with slight modifications. The FRAP solution contained 25 ml acetate buffer (300 mM, pH 3.6), 2.5 ml 2, 4, 6-tripyridyl-s-tri-azine (10 mM solution in 40 mM HCl) and 2.5 ml ferric chloride (20 mM). The temperature of the solution was raisedto 37°C before use. Stock solution of 0.15 ml (1g/ml) of the extract was allowed to react with 2.85 ml of the FRAP solution for 30 min in dark condition. Readings

of the coloured product (ferrous tripyridyltriazine complex) were taken at 593 nm in a spectrophotometer [UV-160A, Optics Technology, India]. Results were expressed as µM Fe (II)/g dry mass using the standard curve constructed for different concentrations of ascorbic acid.

Total reducing power

The determination of reducing power was performed as described by Yen and Duh (1993). Test samples at different concentrations (0.1 - 0.9 mg/ml) were mixed with 0.5 ml of phosphate buffer (20 mM, pH 6.6) and 0.5 ml of 1% potassium ferricyanide and incubated at 50°C for 20 min. To these mixtures 0.5 ml of 10% trichloro acetic acid was added and centrifuged at 2500 rpm for 10 min. The supernatant was mixed with 1.5 ml of distilled water and 0.3 ml of 0.1% ferric chloride and the absorbance was read at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

DPPH radical scavenging activity

The DPPH scavenging assay was a modification of the procedure of Moon and Terao (1998). Briefly, 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) and 1 ml of DPPH (500 µM in ethanol). The mixture was shaken vigorously and left to stand for 30 min. The absorbance of the resulting solution was measured at 517 nm and compared with that of BHA. Radical scavenging potential was expressed as IC_{50} values, which represents the sample concentration at which 50% of the radicals are scavenged. The percentage of DPPH scavenging was calculated using the following formula:

Per cent scavenging = $[(A_{control} - (A_{sample} - A_{sample\ blank}) / A_{control}] \times 100$

Lipid peroxidation inhibitory activity

The lipid peroxidation inhibitory activity was determined according to the method of Duh and Yen (1997). In brief, egg lecithin (3 mg/ml phosphate buffer, pH 7.4) was sonicated using ultraschallprozessor [Optics Technology, India] for 10 min to ensure proper liposome formation. Test samples (0.1 ml) at different concentrations (0.1 - 0.9 mg/ml) were added to l ml of liposome mixture and the control was without the test sample. Lipid peroxidation was induced by adding 10 µl of ferric chloride (400 mM) and 10 µl of L-ascorbic acid (200 mM). After incubation for 1 h at 37°C the reaction was stopped by adding 2 ml of hydrochloric acid containing 15% trichloroacetic acid and 0.375% of thiobarbutyric acid. 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. Inhibitory activity was expressed as IC_{50} value, which represents the sample concentration at which 50% lipid peroxidation inhibition takes place. Percentage of lipid peroxidation inhibition was calculated using the following formula:

Per cent inhibition = $[(A_{control} - (A_{sample} - A_{sample\ blank}) / A_{control}] \times 100$

Superoxide radical scavenging activity

The superoxide scavenging ability was assessed according to the method of Nishikimi *et al*. (1972) with slight modifications. The reaction mixture contained nitroblue tetrazolium (0.1 mM) and nicotinamide adenine dinucleotide (0.1 mM) with or without sample to be assayed in a total volume of 1 ml of tris-HCl buffer (0.02 M, pH 8.3). The reaction was started by adding phenazine methosulphate (10 µM) to the mixture, and change in the absorbance was recorded at 560 nm every 30 sec for 2 min. The per cent scavenging was calculated against a control without test sample. Radical scavenging potential was expressed as IC_{50} value, which represents the sample concentration at which 50% of the radicals are scavenged. The results were compared with that of quercetin. The percentage scavenging of superoxide anion was calculated using the following formula:

Per cent scavenging = $[(A_{control} - (A_{sample} - A_{sample\ blank}) / A_{control}] \times 100$

Statistical analysis

The experimental results are expressed as mean ± standard deviation (SD) of triplicate measurements. The data was subjected to One Way Analysis of Variance (ANOVA) and the significance of differences between the sample means was calculated by Turkeys test. Data was considered statistically significant at P value ≤ 0.05. Statistical analysis was performed using Graph Pad statistical software.

Results and discussion

Total phenol and flavonoid content of stem extract

The result of the total phenol and flavonoid contents of *T. cordifolia* stem extract in different solvents is given in Table 1.1. The present study revealed that the level of polyphenols in the ethanol extract (5.1 mg/g) was higher when compared to methanol, hexane, chloroform and aqueous extract. Ethanol extract of the stem had a flavonoid content of 0.52 mg/g which was followed by methanol (0.42 mg/g). The flavonoid content of other extract tested was lower than the ethanol and methanol extract. Aqueous extract had the least polyphenol and flavonoid contents.

Table 1.1 : Polyphenol and flavonoid content of *Tinospora cordifolia* plant extract in different solvent Extract.

Solvents	Total phenols (mg/g)	Flavonoid content (mg/g)
Aqueous	1.13 ± 0.05a	0.12 ± 0.02a
Ethanol	5.10 ± 0.20d	0.52 ± 0.02d
Methanol	4.36 ± 0.15c	0.42 ± 0.02c
Hexane	2.13 ± 0.25b	0.23 ± 0.01b
Chloroform	1.66 ± 0.20b	0.21 ± 0.03b

Values are means of three independent replicates
Mean values with different superscripts are significantly different from each other as indicated by Turkey's HSD ($P \leq 0.05$)

Medicinal plants are an important source of antioxidants (Rice-Evans, 2004). Natural antioxidants increase the antioxidant capacity of the plasma and reduce the risk of certain diseases (Prior and Cao, 2000). Natural antioxidants mainly come from plants in the form of polyphenols such as flavonoids, phenolic acids and tocopherols (Ali *et al*., 2008; Demiray *et al*., 2009). The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites that possess an aromatic ring bearing one or more hydroxyl constituents (Singh *et al*., 2007). Phenolic compounds are widely found in the secondary products of medicinal plants, as well as in many edible plants (Hagerman *et al*., 1998). A number of studies have focussed on the biological activities of phenolic compounds, which are the potential antioxidants and free radical scavengers (Rice-Evans *et al*., 1995; Cespedes *et al*., 2008; Chanda and Dave, 2009; Annegowda *et al*., 2010). It is reported that the phenols are responsible for the variation in the antioxidant activity of the plant (Cai *et al*., 2004). Many flavonoids are found to be strong antioxidants capable of effectively scavenging the reactive oxygen species because of their phenolic hydroxyl groups (Cao *et al*., 1997). The antioxidant activity of phenolic compounds is mainly due to their redox properties, which play an important role as free radical scavengers, reducing agents, quenchers of singlet oxygen and complexes of pro-oxidant metals (Mohan *et al*., 2008; Butkhup and Samappito, 2011). They exhibit antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydroperoxides into free radicals (Pokorny 2001; Pitchaon *et al*., 2007). The key role of phenolic compounds as scavengers of free radicals is emphasized in several reports (Moller *et al*., 1999; Aziz *et al*., 2007; Chinedu *et al*., 2011). Polyphenolic compounds have an important role in stabilizing lipid oxidation and are associated with antioxidant activity (Cakir *et al*., 2003). Due to the important physiological functions of phenolic compounds, the total polyphenol content and flavonoid content of the stem of *T. cordifolia* was measured in different solvent extract.

The result from the present study indicates that the phenolic compounds and flavonoids are better extracted with ethanol than with other solvents. The result obtained from the estimation of total polyphenol and flavonoid contents in our study is in agreement with the study by Tsao and Deng (2004) which showed that phenolic acids and flavonoids are generally better extracted using alcohols, water or a mixture of water and alcohols. Eventhough, methanol has been reported by various workers as the most suitable solvent for the extraction of polyphenolic compounds from plant tissues (Siddhuraju and Becker, 2003; Yao *et al.*, 2004; Suhaj, 2006), the result of our study is contrary to the earlier done studies. The result from our study is in agreement with Karadeniz *et al*. (2005) and Koffi *et al*. (2010) who showed that ethanol is preferred for the extraction of antioxidant compounds mainly because of its low toxicity. The results obtained from our study vary from the earlier reports on *T. cordifolia* stem extract, which have shown higher concentrations of phenols and flavonoids (Singh *et al*., 2010; Vaghasiya *et al*., 2011).

Ferrous reducing antioxidant assay

The FRAP assay results of *T. cordifolia* is shown in Table 1.2. Among the extract tested, ethanol extract had a total antioxidant activity of 41.13 µM Fe (II)/g followed by methanol 33.36 µM Fe (II)/g. Aqueous extract had the least reducing ability of 4.96 µM Fe (II)/g.

Table 1.2 : Total antioxidant activity assay of *Tinospora cordifolia* stem extract.

Solvents	**Total antioxidant activity (µM Fe (II)/g)**
Aqueous	4.96 ± 0.15a
Ethanol	41.13 ± 1.38e
Methanol	33.36 ± 1.85d
Hexane	20.96 ± 1.15c
Chloroform	11.36 ± 1.58b

Values are means of three independent replicates
Mean values with different superscripts are significantly different from each other as indicated by Turkey's HSD ($P \leq 0.05$)

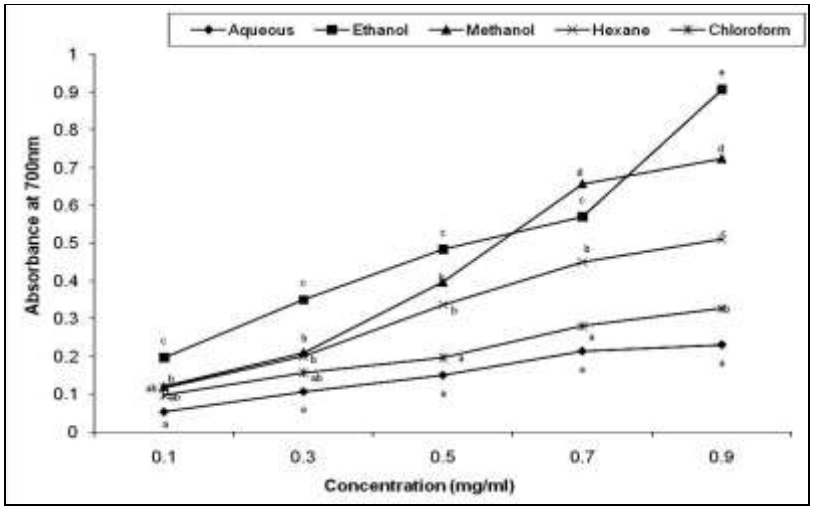
FRAP assay is based on the ability of antioxidants to reduce ferric (Fe3+) to ferrous (Fe2+) in the presence of 2, 4, 6-tri (2-pyridyl)-s-triazine (TPTZ) forming an intense blue Fe2+ -TPTZ complex with an absorption maximum at 593 nm. This reaction is pH dependent (optimum pH 3.6). From the results obtained, it was found that the ethanol stem extract of *T. cordifolia* had the highest antioxidant activity which may be due to the increased concentration of polyphenols. The antioxidant activity was proportional to the polyphenol content of the solvent extract. Antioxidant compounds such as polyphenols are more efficient reducing agents for ferric iron (Wong *et al*., 2005). According to Oktay *et al.* (2003), a highly

positive relationship between total phenols and antioxidant activity appears to be the trend in many plant species. It is generally believed that plants which are having more phenolic content show good antioxidant activity and there is a direct correlation between total phenols and antioxidant activity (Velioglu *et al*., 1998; Brighente *et al*., 2007; Biglari *et al*., 2008; Salazar *et al*., 2008; Saravana *et al*., 2008). Differences in antioxidant activities of the extract could be due to the different polarities of the solvents and thus different extractability of the antioxidative compounds (Maisuthisakul *et al*., 2007). Antioxidant properties of single compounds within a group can vary remarkably, so that the same levels of phenolics do not necessarily correspond to the same antioxidant responses (Parejo *et al*., 2002).

Total reducing power

The reducing power of different solvent extract of *T. cordifolia* stem using the potassium ferricyanide method is shown in Figure 1.1. The result indicates that the reducing ability of the extract increased with the concentration. Among all the extract tested for their reducing abilities, ethanol extract showed better reducing power with an increase in the absorbance at 700 nm from 0.19 at 0.1 mg/ml to 0.90 at 0.9 mg/ml which was followed by methanol.

Fig. 1.1 : Total reducing power of *Tinospora cordifolia* stem extract



Values are means of three independent replicates
Mean values with different superscripts are significantly different from each other as indicated by Turkey's HSD ($P \leq 0.05$)

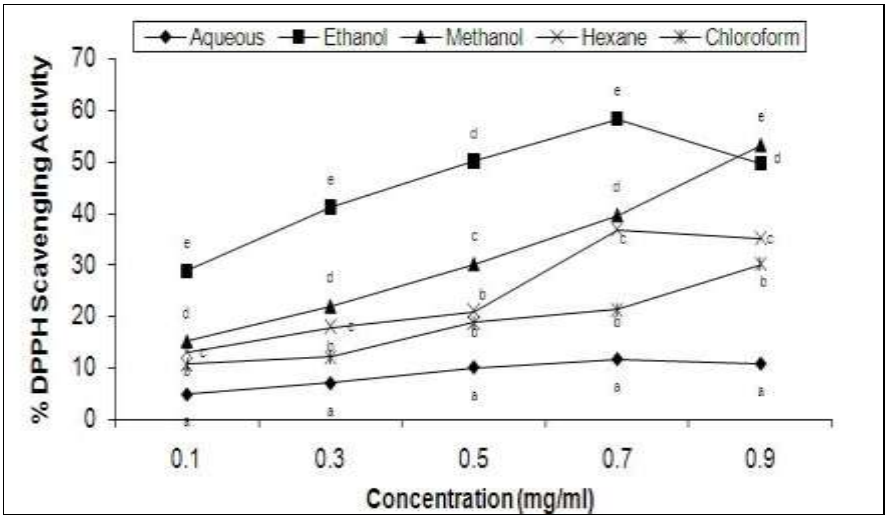
The reducing capacity of a compound from Fe3+ to Fe2+ form may serve as a significant indicator of its antioxidant capacity (Meir *et al*., 1995; Oktay *et al*., 2003;Hazra *et al*., 2008; Rachh *et al*., 2009). The stem extract of *T. cordifolia* exhibited concentration dependent activity and showed a positive correlation with the polyphenol content. Accordingly, ethanol extract of all the three plants showed better reducing ability when compared to other solvent extract. The presence of compounds with hydroxyl groups in the extract may be responsible for reducing power (Policegoudra *et al*., 2007; Vedpriya and Yadav, 2011). It

appears that antioxidant activity may have a mutual correlation with the reducing effect. The reducing properties are generally associated with the presence of reductones. Gordan (1990) reported that the antioxidant activity of reductones is believed to break radical chains by donation of a hydrogen atom, indicating that the antioxidative properties are concomitant with the development of the reducing power. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants (Yen and Chen, 1995).

DPPH radical scavenging activity

The DPPH radical scavenging activity of *T. cordifolia* stem extract is shown in Figure 1.2. Among the extract tested, ethanol extract had better scavenging activity of 50.23% (IC50 value of 0.5 mg/ml). Methanol had a scavenging activity of 53.43% (IC50 value of 0.9 mg/ml). The IC50 values could be reached at a lower concentration with ethanol extract as compared to methanol. As with other extract tested IC50 values could not be reached even at higher concentrations.

Fig. 1.2 : DPPH radical scavenging activity of *Tinospora cordifolia* stem extract.



Values are means of three independent replicates
Mean values with different superscripts are significantly different from each other as indicated by Turkey's HSD ($P \leq 0.05$)

The stable radical DPPH has been used widely for the determination of primary antioxidant activity (Brand-Williams *et al*., 1995; Katalinic *et al*., 2004; Goncalves *et al*., 2005). DPPH is one of the compounds that possess a proton free radical with a characteristic absorption, which decreases significantly on exposure to proton radical scavengers. The reaction of DPPH is measured by degree of decrease of absorbance at 517 nm, by an antioxidant (Brand-Williams *et al*., 1995; Conforti *et al*., 2002). The DPPH antioxidant assay is based on the ability of DPPH a stable free radical to decolourize in the

presence of antioxidants (Singh *et al*., 2002; Ara and Nur, 2009). Antioxidants react with DPPH which is a stable free radical and convert it to 1, 1-diphenyl-2-picryl hydrazine.

It is well accepted that the DPPH radical scavenging by antioxidants is due to their hydrogen donating ability (Nikolaos *et al*., 2003; Singh and Rajini, 2004; Goncalves *et al*., 2005; Hou *et al*., 2005; Negi *et al*., 2005). The antioxidant activity may be directly correlated to the phenolic content in different solvent extract (Chu *et al*., 2000; Singh *et al*., 2002; Cai *et al*., 2004; Kulkarni *et al*.,

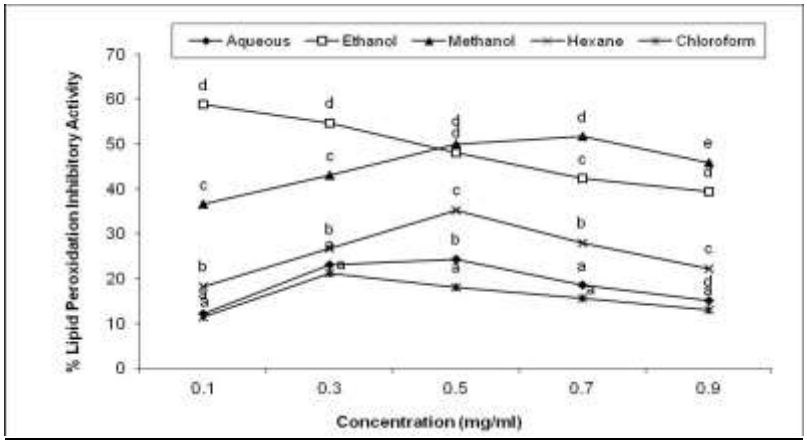
2004; Tawaha *et al*., 2007; Chinedu *et al*., 2011). The ethanol stem extract of *T. cordifolia* recorded the highest phenol and flavonoid contents and also had the highest DPPH scavenging activity. This suggests that the phenolic compounds contributed significantly to the antioxidant capacity of the investigated plant species.

The amount of sample required to decrease the initial DPPH concentration (IC50) by 50% is a parameter widely used to measure the antioxidant activity. The lower the IC50 value, the higher the antioxidant activity. DPPH radical scavenging activity of *T. cordifolia* stem part with an IC50 value of 0.02 mg/ml. The difference in the IC50 value can be attributed to the distribution of secondary metabolites that may fluctuate between different plant organs (Lisiewska *et al*., 2006).

Lipid peroxidation inhibitory activity

The lipid peroxidation inhibitory activity of *T. cordifolia* stem extract is shown in Figure 1.3. Ethanol extract had an IC50 value of 0.1 mg/ml which showed a lipid peroxidation inhibition of 57.5%, whereas methanol extract showed an inhibition of 50.43% at a higher concentration of 0.7 mg/ml (IC50 value). As with other extract, 50% inhibition could not be achieved even at 0.9 mg/ml.

Fig. 1.3 : Lipid peroxidation inhibitory activity of *Tinospora cordifolia* stem extract



Values are means of three independent replicates

Mean values with different superscripts are significantly different from each other as indicated by Turkey's HSD ($P \leq 0.05$)

To evaluate the lipid peroxidation inhibitory activity of the stem extract, the liposome model system was used. Thiobarbutyric acid reacts with malondialdehyde (MDA) to form a diadduct, a pink chromogen, which can be detected spectrophotometrically at 532 nm. MDA is a major product of lipid peroxidation and is used to study the lipid peroxidation process (Singh *et al*., 2002; Dok-Go *et al*., 2003).

Polyunsaturated fatty acids (PUFA) present in cell membranes are oxidized by both enzymatic and by free radical chain reactions (Torel *et al*., 1986). An over abundance of free radicals can lead to uncontrolled chain reactions of lipid peroxidation resulting in pathological conditions that may include liver injury, atherosclerosis, kidney damage, ageing and susceptibility to cancer (Rice-Evans and Burdon, 1993). Lipid peroxidation proceeds in three stages: initiation, propagation and termination. In the initiation stage of lipid peroxidation, free radicals obstruct hydrogen from PUFA to form the lipid radical. In the propagation stage, the lipid radical breaks down to generate more free radicals thus maintaining the chain of reactions. In the termination stage, the free radical species react together or with antioxidants to form inert products. Lipid peroxidation can be suppressed by enzymatic inactivation of free radicals by antioxidants that inhibit the initiation stage
and/or accelerate the termination stage (Shimada *et al*., 1992). Thus, lipid peroxidation can be prevented at the initiation stage by free radical scavengers and singlet oxygen quenchers, and peroxyl-radical scavengers which break propagation of chain reaction (Cook and Samman, 1996).

The result of lipid peroxidation inhibitory activity of different solvent extract of *T. cordifolia* showed that ethanol stem extract of all the three plants showed the highest inhibition of lipid peroxidation. Lipid peroxidation inhibitory activity mainly depends on the solubility and hydrophobicity of the compounds present in the respective extract (Son and Lewis, 2002). The highest lipid peroxidation inhibition of ethanol extract may be due to the presence of high amounts of polyphenols and flavonoids. Flavonoids are known for their antioxidant effect on lipid peroxidation as a result of scavenging of hydroxyl radicals at the stage of initiation and termination of peroxyl radicals (Hussain *et al*., 1987).

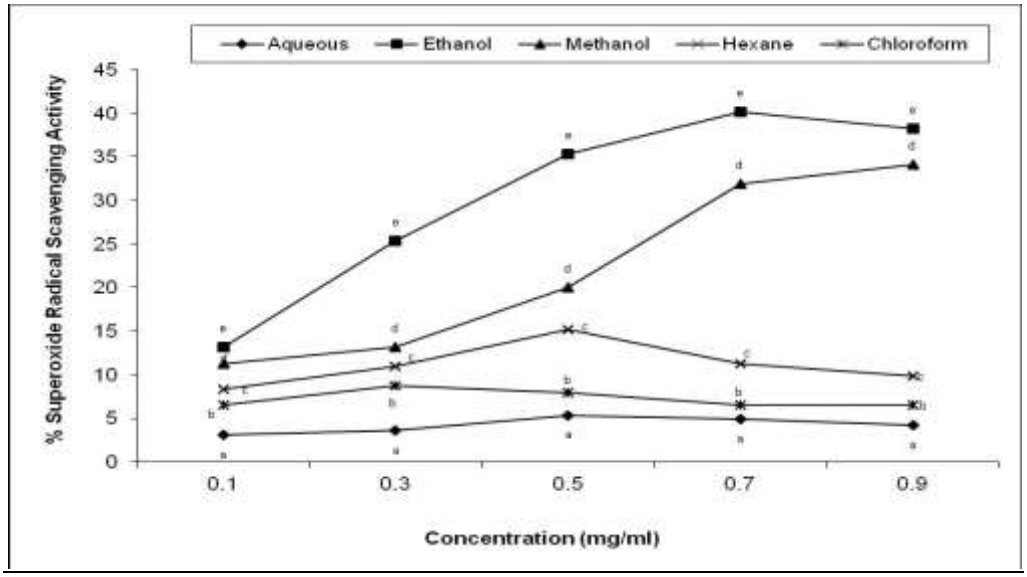
They may donate hydrogen atoms forming a flavonoid radical and flavonoid radical in turn are able to react with free radicals there by terminating the radical chain reaction (Baderschneider and Winterhalter, 2001). It is reported that lipid peroxidation can be inhibited by flavonoids, possibly through their activity as strong oxygen scavengers and singlet oxygen quenchers (Baumann *et al*., 1980; Bergman *et al*., 2003).

Superoxide scavenging activity

The superoxide radical generated from dissolved oxygen by PMS-NADH coupling can be measured by their ability to reduce NBT. Figure 1.4 represents the superoxide quenching ability of *T. cordifolia* stem extract. The superoxide scavenging ability was generally low with all the solvent and aqueous extract. As

with ethanol extract, even at 0.7 mg/ml concentration the percentage radical scavenging was 40.13%. Per cent radical scavenging abilities of other extract were lower than the ethanol extract.

Fig. 1.4 : Superoxide scavenging activity of *Tinospora cordifolia* stem extract



Values are means of three independent replicates
Mean values with different superscripts are significantly different from each other as indicated by Turkey's HSD ($P \leq 0.05$)

Superoxide radicals are generated during the normal physiological process, mainly in mitochondria. Glucose oxidation is believed to be the main source of free radicals. In its enediol form, glucose is oxidized in a transition-metal dependent reaction to an enediol radical anion that is converted into reactive ketoaldehydes and to superoxide anion radicals. The superoxide anion radicals undergo dismutation to hydrogen peroxide, which if not degraded can lead to production of extremely reactive hydroxyl radicals (Wolff and Dean, 1987; Jiang *et al*., 1990). Although, superoxide anion is by itself a weak oxidant, it gives rise to the powerful and dangerous hydroxyl radicals as well as singlet oxygen both of which contribute to oxidative stress (Dahl and Richardson, 1978; Halliwell and Gutteridge, 1990; Meyer and Isaksen, 1995; Pietta, 2000). Therefore superoxide radical scavenging by antioxidants has physiological implications.

This shows that the antioxidant activity of the extract was due to the presence of polyphenols and flavonoids in it. Robak and Gryglewski (1988) reported that flavonoids are effective antioxidants mainly because they scavengensuperoxide anions.

From the results it appeared that the superoxide scavenging activity of the plant extract is negligible compared to the standard quercetin. It was shown that IC50 value could be reached only with ethanol stem extract of *T. cordifolia*, IC50 values could not be reached. Eventhough the IC50 values could not be reached, there was a positive correlation with flavonoid content and superoxide radical scavenging activity

of the extract. The result supports the earlier study by Mathew and Kuttan (1997) on *T. cordifolia*, which showed that the IC50 value for superoxide scavenging could be as high as 6 mg/ml.

Conclusion

T. cordifolia is very important medicinal plant which holds a special position in Ayurvedic system of medicines for prevention and treatments of various human ailments. Because of presence of various kinds of phytochemicals in Giloy, it has found applications in pharmaceutical chemistry due to its antioxidant properties.

Antioxidant activity assay using DPPH• model was performed on the different extract of the stem of *Tinospora Cordifolia* as well as two pure isolated phenols and flavonoids from the plant. The results revealed that *Tinospora Cordifolia* stem extract and fractions have very powerful antioxidant activity.

The study has shown that the hydro-ethanolic extract of the stem of *Tinospora Cordifolia* has antioxidant activities and thus gives credence to its use in Ayurvedic medicine.

Antioxidant manipulations in prevention or control of diseases. Natural products from dietary components such as Indian species and medicinal plants are known to possess antioxidant activity. Future approach include gene therapy to produce more antioxidant in the body, genetically engineered plant products with higher level of antioxidant, and the use of functional foods enriched with antioxidant.

Finally, it is concluded that phenols and flavonoids are responsible for antioxidant activity of *Tinospora Cordifolia* (Willd.) Stem extract.
So, it has immense scope as an effective source to develop drug for the treatment of antioxidant related diseases.

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