

ANTIOXIDANT ACTIVITY AND PHARMACOLOGICAL SCREENING OF *TINOSPORA CORDIFOLIA* (THUNB.)

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ABSTRACT

The communication deals with the antioxidant activity of aqueous and methanolic extracts of *Tinospora cordifolia* (Thunb.) stem, along with the phytochemical, pharmacological and nutritive properties. Methanolic extract showed good antioxidant efficacy compare to the BHA standard. Reducing power of the extract showed concentration dependence. Lipid peroxidation inhibition was not significant. Methanolic extract showed significant DPPH radical scavenging activity ($EC_{50} = 0.5$ mg/mL), while the superoxide radical scavenging activity of the methanolic extract was mild and showed concentration dependence. The phytochemical analysis showed low amount of total ash content, i.e. 11.3 ± 1.4 mg/g. Total phenol, tannin and flavonoids contents were 17.3 ± 0.4 , 13.8 ± 0.5 and 6.5 ± 0.2 mg/g respectively which fall under moderate range. Crude fiber and moisture contents (231 ± 3.2 and 214 ± 5.3 mg/g respectively) as well as carbohydrate content (184.1 ± 5.2 mg/g) and nutritive value (1.46 ± 0.3 cal/g) were significantly high. *T. cordifolia* stem showed high swelling and foaming indices, $400 \pm 3.5\%$ and $111.12 \pm 2.1\%$ respectively, which is an indication of good drug release characteristics. Present findings suggest *Tinospora cordifolia* as potential source of natural antioxidant, fiber and nutrient content in food, fodder and pharmaceutical industries.

INTRODUCTION

The oxidative property of oxygen has a double-edged property, being essential for life; it also exacerbates the damage within the cell by oxidative events (Sen *et al.*, 2010) which is due to free oxygen radical (Gerschman *et al.*, 1954). Now it is established that free oxygen radicals or reactive oxygen species (ROS), as well as reactive nitrogen species (RNS), are products of normal cellular metabolism, and play dual role as in being beneficial at low/moderate concentrations by involvement in physiological roles such as cellular response to noxia, in defense against infectious agents and in the functioning of a number of cellular signaling pathways (Valko *et al.*, 2007). In higher quantities however, these free radicals react with membrane lipids, nucleic acids, proteins, enzymes and other bio-molecules, resulting in cellular damage (Shivaprasad *et al.*, 2005). These free radicals are generated within the body by various endogenous pathways, like consumption of O_2 by mitochondria during aerobic respiration, phagocytosis of infected cells, degradation of fatty acids and natural toxins; as well as the exogenous interferences, like exposure to sources of low-wavelength electromagnetic radiations, such as gamma-rays (Krishnaiah *et al.*, 2007).

Naturally occurring antioxidants in body are uric acid, some proteins, ascorbic acid and vitamin E, which contribute 58, 21, 14 and 7% respectively to plasma antioxidant capacity (Wayner *et al.*, 1987; Niki, 2010). When the rate of generation of free radicals surpasses their rate of neutralization by the endogenous antioxidants (Krishnaiah *et al.*, 2007), the condition is referred to as oxidative stress (Sen *et al.*, 2010). Oxidative stress have been reported to be involved in pathogenesis of various disorders and diseases (Niki, 2010)

which is counteracted by the natural antioxidants contained in food, fruits, beverages, spices and medicinal plants. Various synthetic antioxidants have also been prepared from pharmacological viewpoint (Niki, 2010).

The review of literature revealed that considerable contributions have been made on medicinal plants by many workers (Dadsena *et al.*, 2013; Dandapat *et al.*, 2013; Kullu *et al.*, 2013; Kumar *et al.*, 2013a; Mahato *et al.*, 2013; Tabassum *et al.*, 2013; Toppo *et al.*, 2013; Sahu *et al.*, 2013).

T. cordifolia (Menispermaceae) is a deciduous climbing shrub distributed throughout the tropical Indian subcontinent (Srivastava, 2011) and has been studied for its immunomodulatory, anti-allergic rhinitis, anti-ulcer, anti-hyperglycemic, cardioprotective, chemopreventive, hepatoprotective, hypolipidaemic, neuroprotective and radioprotective actions and against obstructive jaundice (Thatte, *et al.*, 1992; Dhuley, 1997; Grover *et al.*, 2000; Stanley *et al.*, 2000; Premanath and Lakshmidevi, 2010; Megraj *et al.*, 2011). The role of oxidative stress is quite evident in most of the disorders (Valko *et al.*, 2007). But there is paucity of information on antioxidant property of the plant. Apart from this, the scientific assessment of pharmacological parameters is also imperative for acceptance of the herbal health claims. With this background the antioxidant properties of *Tinospora cordifolia* stem extracts, along with its phytochemical, pharmacological and nutritive properties have been studied.

MATERIALS AND METHOD

Collection of plant material

The fresh mature parts of stem were collected, chopped, dried in shade under room temperature for six to seven days and then crushed into coarse powder using electric grinder. The powder was sieved to get fine powder using fine plastic sieve which was stored in air tight bottle in the laboratory until required.

Extract preparation

50g of the powder was subjected to extraction by soxhlet using methanol and distilled water separately. The extracts obtained were filtered, concentrated after dryness in rotary flash evaporator maintained at 45°C, percentage yield of each extract was calculated and the dried extracts were stored in air tight containers at room temperature for further studies.

Phytochemical analysis

Following WHO (1998), 2g of powder was incinerated at 500-600°C to free the sample from carbon. The percentage of ash was calculated with reference to air dried powder. The total ash obtained was boiled in 25mL of distilled water for 5min and the insoluble matter was collected in an ash-free filter paper and incinerated at temperature not exceeding 450°C. Subtracting the weight of the insoluble matter from the weight of the ash gives the percentage of water soluble ash. For the acid-insoluble ash, the total ash was boiled with 25mL of 2N HCl for 5min; the insoluble matter was collected, washed, dried and weighed.

The amount of crude fibre was determined using the method described by Watanables and Olsen (1965). The moisture content was determined in terms of the loss in weight of the plant material on overnight heating at 150°C (Sadasivam and Manickam, 1996).

Total phenol was determined by FolinCiocalteu reagent, following Ramamoorthy and Bono (2007). Tannins were quantified as stated in the Quality control methods for medicinal plant materials (1998). Aluminium chloride colorimetric method was used to determine flavonoid content (Lin and Tang, 2007).

Pharmacological properties

The swelling index and foaming index were calculated using 1g of dry power of the sample (WHO, 1998).

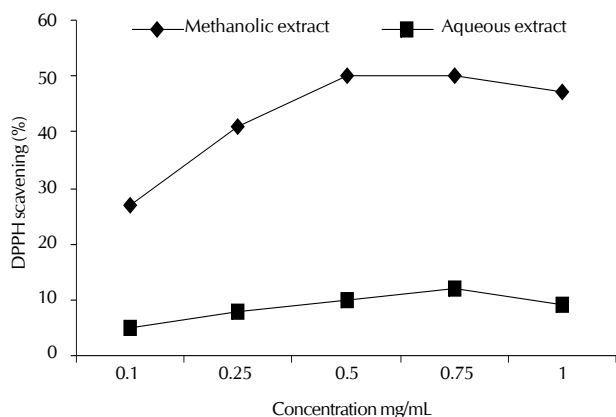


Figure 1: DPPH radical scavenging activity of *T. cordifolia* stem

Table 1: Quantitative analysis of phytochemical properties of the *T. cordifolia* stem ($M \pm SD$; $n=3$)

Sl. No.	Attributes	mg/g
1	Total ash	11.3 \pm 1.4
2	Water soluble ash	17.5 \pm 0.3
3	Acid Insoluble ash	1.9 \pm 0.7
4	Phenols	17.3 \pm 0.4
5	Tannins	13.8 \pm 0.5
6	Flavonoids	6.5 \pm 0.2
7	Crude fiber	231 \pm 3.2
8	Moisture content	214 \pm 5.3

Table 2: Pharmacological properties of *T. cordifolia* stem ($M \pm SD$; $n=3$)

Sl. No.	Attributes	%
1	Swelling Index	400 \pm 3.5
2	Foaming Index	111.12 \pm 2.1

Table 3: Nutritive properties of *T. cordifolia* stem ($M \pm SD$; $n=3$)

Sl. No.	Attributes	mg/g
1	Fat	60.4 \pm 6.2
2	Protein	45.2 \pm 2.5
3	Carbohydrate	184.1 \pm 5.2
4	Nutritive value (cal/g)	1.46 \pm 0.3

Nutritive value

Micro Kjeldahl method was used for the determination of protein. Crude fat, carbohydrate and nutritive value were calculated, following Nile and Khobragade (2009).

Antioxidant activity

The DPPH radical scavenging activity was assayed following Moon and Terao (1998) using a stable free radical, 1, 1-diphenyl-2-picryl hydrazyl (DPPH) and the superoxide anion scavenging activity following Fontana *et al.* (2001). Lipid peroxidation inhibitory activity was determined following Duh and Yen (1997). The reducing powers of *T. cordifolia* stem extracts were evaluated spectrophotometrically as they reduced potassium ferricyanide to potassium ferrocyanide. (Jayanthi and Lalitha, 2011). The total antioxidant capacity was determined by the spectrophotometric quantification of phosphomolybdate complex (Prieto *et al.*, 1999).

RESULTS AND DISCUSSION

Physicochemical analysis

The value obtained for the total ash content (Table 1) is somewhat lower than that reported by Vermani *et al.* (2010) and Nasreen *et al.* (2010) for *T. cordifolia* leaves and shoot, *i.e.* 7.2 \pm 2.1 and 7.5% respectively. However, the values obtained for water soluble and acid insoluble ash content (17.5 \pm 0.3 and 1.9 \pm 0.7) are comparable to their reports *i.e.* 25.42 \pm 4.36 and 2.41 \pm 0.16%; and 12.05 and 1.16% respectively. The amount and composition of ash remaining after combustion of plant material varies considerably with the part of plant, age, treatment etc. The constituents of ash also vary with time and from organ to organ since it mainly represents the inorganic part of the plant (Vermani *et al.*, 2010). Thus the reason for this variation might be that mature stem

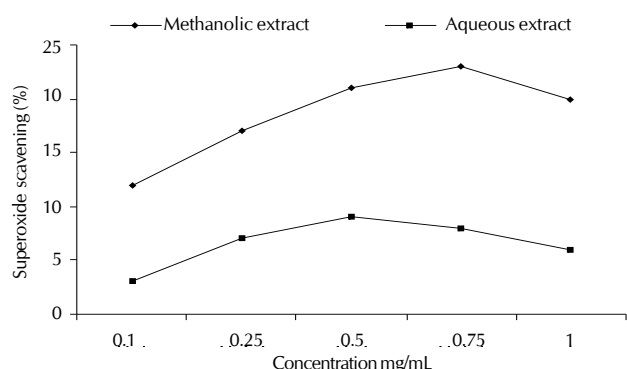


Figure 2: Superoxide radical scavenging activity of *T. cordifolia* stem

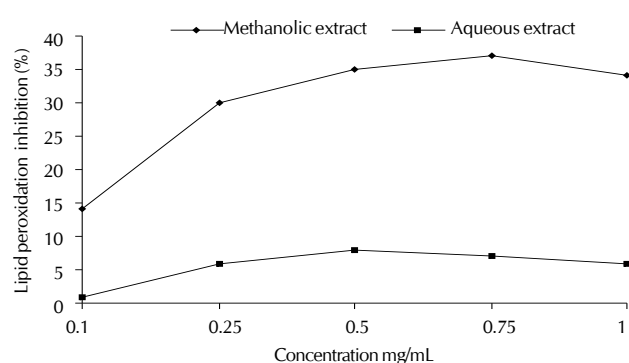


Figure 3: Lipid peroxidation activity of *T. cordifolia* stem

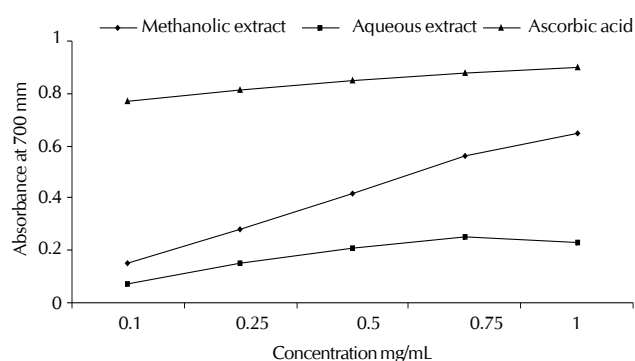


Figure 4: Reducing power of *T. cordifolia* stem

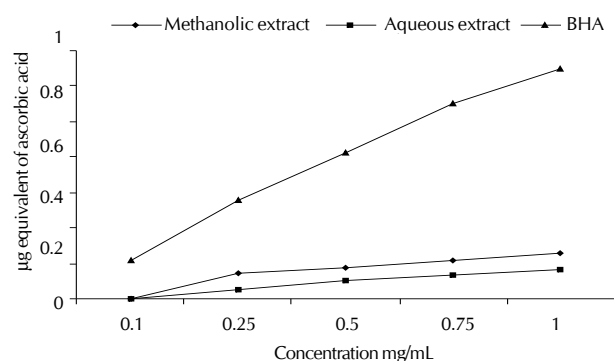


Figure 5: Total antioxidant capacity of *T. cordifolia* stem

samples contained less silicious materials than the leaves and shoot.

Dietary fiber is an imperative constituent of a balanced healthy diet (Trowel, 1978). Hoe and Siong (1999) have quantified the crude fiber content of several medicinally important plants like *Mangifera graifithii* (0.9%), *Solanum ferox* (1.9%), *Alternanthera sessilis* (2.7%) and *Gnetum gnemon* (4.7%). *T. cordifolia* can be considered a rich source of crude fiber, as it contained 231.0 ± 3.2 mg/g of the same (Table-1).

The total phenolic content was found to be 17.3 ± 0.4 mg/g and the flavonoid content of the sample was 6.5 ± 0.2 mg/g (Table 1), which are of moderate range. Phenolic compounds and flavonoids, found in the edible and inedible parts of plants portray antioxidant activity, and hence are of immense importance (Premanath and Lakshmidivi, 2010). The antioxidant capacity of phenols and flavonoids is mainly due to their redox properties, which allows them to cut as reducing agents, hydrogen donors, singlet oxygen quenchers or metal chelators (Kanimozi *et al.*, 2011).

T. cordifolia stem contained tannin in the range of 13.8 ± 0.5 mg/g which is comparable to that occurring in several common fruits and coffee beans. Tannins are major secondary metabolite of higher-order plants and these phytophenol-related chemicals are thought to be principal in molecular defense mechanism against herbivores and viruses. Also their antioxidant property is of immense importance, and for that green tea is taken all over the world (Beart *et al.*, 1985). The tannin content in tea has been reported as 37 ± 2.6 mg/g and the same in roasted coffee beans as 18 ± 1.7 mg/g (Savolainen,

1992). The tannin content in a number of tropical fruits ranged between 10 – 20 mg/g (Bagepalli and Rao, 1982).

Pharmacological analysis

The value of swelling index in present study (400%) is quite higher than standard polymers, like pectin (*i.e.* 55%) and xanthan (*i.e.* 44%). This signifies that the drug release rate of the plant is very high (Jain *et al.*, 2008). Swelling and foaming indices (Table 2) are considered indicators of drug release characteristics. The release of drug occurs as a result of complex interaction between diffusion, dissolution, and erosion mechanisms. On coming in contact with water, hydrophilic matrices undergo gel formation, and progressive phase transition from glassy to rubbery state occurs. This results in solvation of individual polymer chains. As the swelling continues, the swollen matrix retains more water until the shear forces in the dissolution medium disentangle the individual polymer chains from the matrix (Nayak *et al.*, 2011).

Medicinal plants are known to contain saponins that cause persistent foam when an aqueous decoction is shaken, which is indicated by the foaming index (WHO, 1998). Not much work has been done on foaming index and of those available, none has reported any significant value against the index. Thus, the foaming index of *T. cordifolia* can be considered high, which indicates a higher drug release rate (Table 2).

Nutraceutical properties

Under nutraceutical properties, amongst the investigated attributes, species is rich in carbohydrate content (Table 3). Comparing the results of the present study with that of various tropical and subtropical fruits and vegetables, as reported by

Hoe and Siong (1999), we may infer that *T. cordifolia*, with high fiber and carbohydrate, sufficient amounts of fat and protein, along with high nutritive value seems to be a good supplement for younger people, and to those suffering general weakness and anemia.

Antioxidant potential

Fig-1 shows the DPPH radical scavenging activity of the extracts. Comparing the results to BHA standard ($EC_{50} = 5.0 \mu\text{g/mL}$), methanolic extract showed significant DPPH radical scavenging activity (EC_{50} value obtained at 0.5 mg/mL), while aqueous extract could not achieve 50% inhibition even at 1 mg/mL concentration. EC_{50} value was reported at 0.5 mg/mL for ethanol and 0.9 mg/mL for methanol leaf extracts of *T. cordifolia* (Premanath and Lakshmidevi, 2010), which shows that ethanolic and methanolic leaf extracts are more efficient in DPPH radical scavenging than the aqueous stem extract. The superoxide radical scavenging activity is shown in Fig. 2. The quenching activity was quite low with the aqueous extract as compared to Quercetin standard ($EC_{50} = 150 \mu\text{g/mL}$), while that of the methanolic extract was mild and showed concentration dependence.

Lipid peroxidation inhibitory activity of stem extract of *T. cordifolia* is depicted in Fig 3. EC_{50} could not be achieved with the either of the extracts for the tested concentrations. The results were compared to BHA standard, which showed significant peroxidation inhibitory activity ($EC_{50} = 10 \mu\text{g/mL}$). It has been reported that EC_{50} was achieved with methanolic leaf extract of *T. cordifolia* at 0.7 mg/mL (Premanath and Lakshmidevi, 2010). It can hence be inferred that the leaf extracts of *T. cordifolia* are better lipid peroxidase inhibitors than the stem extracts.

Reducing power serves as a significant reflection of the antioxidant activity. The reducing power of the test samples are compared to the standard curve of ascorbic acid (Fig. 4), showing concentration-dependence. It is quite apparent that the methanolic extract possesses good reducing power as compared to the standard. 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 (Jayanthi and Lalitha, 2011).

Total antioxidant capacity (TAC) means the capacity of free radical scavenging by the bioactive constituents contained in the test sample (Niki, 2010). The comparative TAC of methanolic and aqueous stem extracts of *T. cordifolia* and BHA standard is depicted in Fig. 5.

Two active principles found in *T. cordifolia*, namely Tinocordifolin and Tinocordifolioside represent the two classes of antioxidants based on their mode of action, *i.e.* preventive antioxidants that inhibit oxidation by reducing the rate of chain initiation and by conversion of hydroperoxides to molecular products that are not potential sources of free radicals and chain termination antioxidants, that trap peroxy radicals (Brunton *et al.*, 1985; Singh *et al.*, 2003; Krishnaiah *et al.*, 2007).

On the basis of above studies, it can be concluded that *T. cordifolia* stem contains several beneficial compounds such as flavonoids, phenols and tannins, along with high crude

fiber content. The swelling and foaming indices, as a measure of pharmacological properties indicates good drug release characters. The sample with high carbohydrate, sufficient fat and protein contents and high nutritive value may serve as a good diet supplement. From the results of total antioxidant activity, reducing power and DPPH radical scavenging activity it can be concluded that even the crude extract of *T. cordifolia* stem can be used as a potential source of antioxidants.

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