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Full Length Research Paper

Antioxidant potential of ethanolic extract of aerial parts of *Coleus spicatus* Benth

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The objective of this investigation was to evaluate the antioxidant potential of ethanolic extract of the aerial parts of C*oleus spicatus*. Antioxidant activity of ethanolic extract of C*. spicatus* was assessed by three different *in-vitro* model of measuring antioxidant profile; total antioxidant activity, ferric reducing ability of plasma (FRAP) assay and estimation of total flavonoid. Significant total antioxidant activity was found in ethanolic extract of C*. spicatus*. The IC_{50} values of the ethanolic extract of *C. spicatus* and ascorbate were found to be 380 µg/ml and 410 µg/ml, respectively. The ethanolic extract of C*. spicatus* also showed significant result in FRAP assay method. High flavonoid content was found in ethanolic extract of C*. spicatus.* The high antioxidant capacity observed for ethanolic extract of C*. spicatus* suggests that this plant could be used as an additive in the food industry providing good protection against oxidative damage.

Key words: *Coleus spicatus,* total antioxidant activity, ferric reducing ability of plasma (FRAP) assay, total flavonoids.

INTRODUCTION

Antioxidants may be defined as compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. Antioxidants can also protect the human body from free radicals and reactive oxygen species (ROS) effects (Gulcin et al., 2010). Free radical is a chemical compound which contains an unpaired electron spinning on the peripheral layer around the nucleus. The family of free radicals generated from the oxygen is called ROS which cause damage to other molecules by extracting electrons from them in order to attain stability. ROS are ions, atoms or molecules that have the ability to oxidize reduced molecules. The large generation of free radicals, particularly ROS and their high activity plays an important role in the progression of a great number of pathological disturbances like inflammation, atherosclerosis, stroke, heart disease, diabetes mellitus, multiple sclerosis, cancer, Parkinson's disease, Alzheimer's disease, etc (Mensor et al., 2001; Parejo et al., 2002; Hou et al., 2003; Orhan et al., 2003; Tepe et al., 2005; Ozgen et al., 2006).

Plants constitute an important source of active natural products which differ widely in terms of structures, biological properties and mechanisms of actions. Natural antioxidants tend to be safer and also possess anti-viral, anti-inflammatory, anti-cancer, antimutagenic, anti-tumour, and hepatoprotective properties. The source of natural antioxidants may be all or any part of plants such as fruits, vegetables, nuts, seeds, leaves, roots, barks, peels, plant, etc. (Baravalia et al., 2009; Kaneria et al., 2009; Locatelli et al., 2010). However, it has been reported that generally leaves are selected for antioxidant studies (Chanda and Dave, 2009). Recently there is a growing interest on the discovery of natural antioxidants, mainly for two reasons:(I) there are epidemical and clinical evidences suggesting that consumption of vegetables and fruits reduces the risk of developing chronic disease such as cancer; (II) phytochemicals are

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Abbreviations: FRAP, Ferric reducing ability of plasma; **ROS,** reactive oxygen species; **TPTZ,** 2, 4, 6-tripyridyl-S-triazine; **$FeCl_3.6H_20$,** iron(III) chloride hexahydride; **HCl,** hydrochloric acid.

Table 1. Total antioxidant activity of ethanolic extract of *C. spicatus*

Concentration (µg/ml)	Percentage of activity (±SEM)*	
	Sample (ethanolic extract)	Standard (ascorbate)
125	32.25 ± 0.051	26.87 ± 0.08
250	48.61 ± 0.029	30.30 ± 0.05
500	52.03 ± 0.031	60.64 ± 0.02
1000	64.37 ± 0.019	55.23 ± 0.01
	IC_{50} = 380 µg/ml	IC_{50} = 410 µg/ml

*All values are expressed as mean ± SEM for three determinations.

generally safer than synthetic chemicals (Dasmalchi et al., 2007). Therefore, the great interest has been recently focused on the natural foods, medicinal plants and phytocostituents due to their well-known abilities to scavenge free radicals (antioxidant power) (Hou et al., 2003; Galvez et al., 2005; Kukic et al., 2006). Therefore, the importance of search for natural antioxidants has increased in recent years so many researchers focused the same (Jayaprakasha et al., 2003).

Coleus spicatus belongs to the family Labiatae; has shown cytotoxic properties, antitumor activity, and diuretic activity (Annual report, 1978). It has been reported that the isolation of diterpenes of abietan series is from the leaves of *C. spicatus* (Arihara et al., 1977). Hence, the objective of this study was to evaluate the *in-vitro* antioxidant activity of ethanolic extract of aerial parts of *C. spicatus*; were evaluated by three *in vitro* free radical scavenging models.

MATERIALS AND METHODS

Collection and identification of plant materials

The aerial parts of *C. spicatus* were collected from Hosur, Dharmapuri District of Tamil Nadu, India. Taxonomic identification was made from botanical survey of medical plants unit Siddha, government of India, Palayamkottai. The aerial parts of *C. spicatus,* were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

Preparation of extracts

The above powdered materials were successively extracted with ethanol by hot continuous percolation method in Soxhlet apparatus (Harborne, 1984) for 24 h. The extracts were concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till the dry powder was obtained.

Determination of antioxidant activity

Total antioxidant activity (Phosphomolybdic acid method)

The antioxidant activityof the sample was evaluated by the transformation of Mo (VI) to Mo (V) to form phosphomolybdenum complex (Prieto et al., 1999). An aliquot of 0.4 ml of sample solution was combined in a vial with 4 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The vials were capped and incubated in a water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The antioxidant activity was expressed relative to that of ascorbic acid.

Ferric reducing ability of plasma (FRAP) assay

A modified method of Benzie and Strain (1996) was adopted for the FRAP assay. The stock solutions included 300 mM acetate buffer at pH 3.6, 10 mM 2, 4, 6-tripyridyl-S-triazine (TPTZ) solution in 40 mM hydrochloric acid (HCl) and 20 mM iron (III) chloride hexahydride ($FeCl_3.6H_2O$). 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°C before using. Plant extracts (0.15 ml) were allowed to react with 2.85 ml of FRAP solution for 30 min in the dark condition. Readings of the colored product (Ferrous tripyridyltriazine complex) were taken at 593 nm. The standard curve was linear between 200 and 1000 µM $FeSO_4$. Results were expressed in µM (Fe (II) /g) dry mass and compared with that of ascorbic acid.

Estimation of total flavonoids (Cameron et al., 1943)

0.2g of the plant material was ground with ethanol-water in two different ratios namely 9:1 and 1:1, respectively. The homogenate was filtered and these two ratios were combined. This was evaporated to dryness until most of the ethanol was removed. The resultant aqueous extract was extracted in a separating funnel with hexane or chloroform. The solvent extracted aqueous layer was concentrated 0.5 ml of aliquot of extract was pipetted-out in a test tube. 4 ml of the vanillin reagent (1% vanillin in 70% conc. H_2SO_4) was added and kept in a boiling water bath for 15 min. The absorbance was read at 360 nm. A standard was run by using catechol (110 µg/ml).

RESULTS AND DISCUSSION

Determination of total antioxidant activity by phosphomolybdic acid method

The percentage of total antioxidant activity of ethanolic extract of *C. spicatus* is presented in Table 1. The ethanolic extract of *C. spicatus* exhibited a maximum total antioxidant activity of 64.37% at 1000 µg/ml whereas for ascorbate (standard), it was found to be 55.23% at 1000

Table 2. FRAP assay of ethanolic extract of *C. spicatus.*

Concentration (µg/ml)	% of activity(±SEM)*	
	Sample (ethanolic extract)	Standard (ascorbate)
125	49.42 ± 0.015	72.04 ± 0.01
250	57.58 ± 0.029	82.05 ± 0.03
500	66.60 ± 0.032	86.04 ± 0.02
1000	79.31 ± 0.028	98.07 ± 0.04
	IC_{50} = 130 µg/ml	IC_{50} = 50 µg/ml

*All values are expressed as mean ± SEM for three determinations.

Table 3. The total flavonoids content of ethanolic extract of *C. spicatus*.

Extract	Total flavonoids content (mg/g) (±SEM)*
Ethanolic extract of *C. spicatus*	5.56 ± 0.11

*All values are expressed as mean ± SEM for three determinations.

µg/ml. The IC_{50} values of the ethanolic extract of *C. spicatus* and ascorbate were found to be 380 µg/ml and 410 µg/ml, respectively.

FRAP assay

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Table 2 shows the FRAP values of the ethanolic extract of *C. spicatus* and ascorbate at various concentrations (125, 250, 500, 1000 µg/ml). The maximum reducing ability at 1000 µg/ml for ethanolic extract of *C. spicatus* and ascorbate were found to be 79.31 and 98.07%, respectively. The IC_{50} values of ethanolic extract of *C. spicatus* and ascorbate were recorded as 130 and 50 µg/ml, respectively.

Total flavonoids

Plants are conceived as sources of antioxidants due to the presence of polyphenols and flavonoids which possess wide biological properties (Durgas Jr et al., 2006). Recent studies show that many flavonoids and related polyphenols contribute significantly to the total antioxidant activity of many plants (Luo et al., 2002). The total amount of flavonoids content of ethanolic extract of *C. spicatus* is presented in Table 3. The ethanolic extract of *C. spicatus* was found to contain high amounts of flavonoids.

Conclusion

The results of the above investigation indicate that ethanolic extract of *C. spicatus* showed strong antioxidant activity. Therefore, further work should be performed on the isolation and identification of the antioxidant components in *C. spicatus*.

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