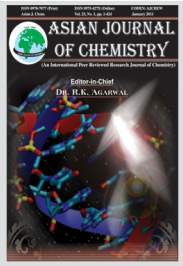
Asian Journal of Chemistry; Vol. 23, No. 11 (2011), 5099-5101

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in



In Vitro Antioxidant Activity of Ethanolic Extract of Aerial Parts of *Coleus spicatus*. Benth

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(*Received*: 7 February 2011; *Accepted*: 27 July 2011) AJC-10220

The present study is to evaluate *in vitro* antioxidant activities of ethanolic extract of aerial parts of *Coleus spicatus*. Benth by hydroxyl, nitric oxide radical and superoxide anion scavenging activity. Ascorbate was used as the reference compound for hydroxyl, nitric oxide and quercetin was used for superoxide anion scavenging activity. The antioxidant activity of ethanolic extract of aerial parts of *Coleus spicatus*. Benth was on a concentration dependent manner. The ethanolic extract of *Coleus spicatus*. Benth was found to have significant antioxidant activity when compared to standard.

Key Words: *Coleus spicatus*, Hydroxyl radical scavenging activity, Nitric oxide radical activity, Superoxide anion scavenging activity.

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[1]. 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 reactive oxygen species, which cause damage to other molecules by extracting electrons from them in order to attain stability. Reactive oxygen species are ions, atoms or molecules that have the ability to oxidize reduced molecules. Oxidative damages caused by free radicals to living cells mediate the pathogenesis of many chronic diseases, such as Parkinson's disease, Alzheimer's disease[2], cancers, aging, coronary, heart ailments cardiovascular diseases, atherosclerosis, cataracts and chronic inflammatory diseases and other degenerative diseases[3].

Reactive oxygen species can be produced from both endogenous and exogenous substances. Potential endogenous sources include mitochondria, cytochrome P450 metabolism, peroxisomes and inflammatory cell activation[4]. Exogenous sources of free radicals include tobacco, smoke, ionizing radiation, certain pollutants, organic solvents and pesticides. There are many synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), which are commonly used, but they are reported to have side effects and are carcinogenic[5]. Therefore, there is an increased interest in the use of natural antioxidants due to their presumed safety, nutritional and therapeutic value[6]. This may explain the interest in examining plant extracts as a source of cheaper and effective antioxidants and the growing interest in nutraceuticals.

Coleus spicatus. belongs to the family Labiatae, has shown cytotoxic properties, antitumor activity and diuretic activity[7]. Arihara *et al.*[8] reported the isolation of diterpenes of abietan series from the leaves of *Coleus spicatus*. Hence, the objective of the present study is to evaluate *in vitro* antioxidant activity of ethanolic extract of aerial parts of *Coleus spicatus*. The activity was evaluated by *in vitro* free radical scavenging models *i.e.*, hydroxyl radical scavenging activity, nitric oxide radical activity, superoxide anion scavenging activity.

EXPERIMENTAL

Collection and identification of plant material: The aerial parts of *Coleus spicatus*. were collected from Hosur, Dharmaburi 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 *Coleus 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[9] for 24 h. The extracts

were concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

Determination of Antioxidant activity

Determination of hydroxyl radical scavenging activity: This was assayed as described by Elizabeth and Rao[10]. The assay is based on quantification of degradation product of 2-deoxy ribose by condensation with TBA. Hydroxyl radical was generated by the Fe^{3+} -ascorbate-EDTA-H_2O_2 system (Fenton reaction). The reaction mixture contained 0.1 mL deoxyribose (2.8 mM), 0.1 mL EDTA (0.1 mM), 0.1 mL H_2O_2 (1 mM), 0.1 mL ascorbate (0.1 mM), 0.1 mL KH_2PO_4-KOH buffer, pH 7.4 (20 mM) and various concentrations of plant extract in a final volume of 1 mL. The reaction mixture was incubated for 1 h at 37 ºC. Deoxyribose degradation was measured as TBARS and the percentage inhibition was calculated.

Determination of nitric oxide radical scavenging activity[11]: Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which were measured by the method of Garrat[11]. The reaction mixture (3 mL) containing 2 mL of sodium nitroprusside (10 mM), 0.5 mL of phosphate buffer saline (1 M) were incubated at 25 ºC for 2.5 h. After incubation, 0.5 mL of the reaction mixture containing nitrite was pipetted and mixed with 1 mL of sulphanilic acid reagent (0.33 %) and allowed to stand for 5 min for completing diazotization.

Then 1 mL of naphthylethylene diamine dihydrochloride (1 % NEDA) was added, mixed and allowed to stand for 0.5 h. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be estimated by the use of Griess Illosvery reaction at 540 nm.

Determination of superoxide anion radical scavenging activity[12]: Superoxide radical (O_2^-) was generated from the photoreduction of riboflavin and was deducted by nitro blue tetrazolium dye (NBT) reduction method. Measurement of superoxide anion scavenging activity was performed based on the method described by Winterbourne *et al.*[12]. The assay mixture contained sample with 0.1 mL of nitro blue tetrazolium (1.5 mM NBT) solution, 0.2 mL of EDTA (0.1 M EDTA), 0.05 mL riboflavin (0.12 mM) and 2.55 mL of phosphate buffer (0.067 M phosphate buffer). The control tubes were also set up where in DMSO was added instead of sample. The reaction mixture was illuminated for 0.5 h and the absorbance at 560 nm was measured against the control samples. Ascorbate was used as the reference compound. All the tests were performed in triplicate and the results averaged. The percentage inhibition was calculated by comparing the results of control and test samples.

RESULTS AND DISCUSSION

Inhibition of hydroxyl radical: The percentage of hydroxyl radical scavenging activity of ethanolic extract of aerial parts of *Coleus spicatus* is presented in Table-1. The ethanolic extract of aerial parts of *Coleus spicatus* exhibited a maximum degradation of deoxy-ribose mediated by hydroxyl radical scavenging activity of 75.51 % at 1000 µg/mL whereas for ascorbate (standard) it was found to be 55.23 % at 1000 µg/mL. The IC_{50} values of ethanolic extract of aerial parts of *Coleus spicatus* and ascorbate were found to be 365 µg/mL and 410 µg/mL respectively.

TABLE-1
HYDROXYL RADICAL SCAVENGING ACTIVITY OF ETHANOLIC EXTRACT OF AERIAL PARTS OF *Coleus spicatus*.

S. No.	Concentration (µg/mL)	% of activity (± SEM)*	
		Sample (Ethanolic extract)	Standard (Ascorbate)
1	125	25.72 ± 1.12	26.87 ± 0.07
2	250	37.06 ± 1.51	30.30 ± 0.05
3	500	62.71 ± 1.01	60.64 ± 0.02
4	1000	75.51 ± 0.77	55.23 ± 0.01
		IC_{50} = 365 µg/mL	IC_{50} = 410 µg/mL

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

Inhibition of nitric oxide radical: Table-2 summarizes the scavenging of nitric oxide radical by the ethanolic extract of aerial parts of *Coleus spicatus* and ascorbate. The maximum scavenging activity is ng 64.86 and 55.23 % respectively at 1000 µg/mL ethanolic extract and ascorbate. The concentration required for 50 % inhibition of ethanolic extract and ascorbate was recorded as 250 and 410 µg/mL respectively.

TABLE-2
NITRIC OXIDE RADICAL SCAVENGING ACTIVITY OF ETHANOLIC EXTRACT OF AERIAL PARTS OF *Coleus spicatus.*

S. No.	Concentration (µg/mL)	% of activity (± SEM)*	
		Sample (Ethanolic extract)	Standard (Ascorbate)
1	125	41.75 ± 0.35	26.87 ± 0.07
2	250	50.01 ± 0.21	30.30 ± 0.05
3	500	58.59 ± 0.19	60.64 ± 0.02
4	1000	64.86 ± 0.46	55.23 ± 0.01
		IC_{50} = 250 µg/mL	IC_{50} = 410 µg/mL

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

Inhibition of superoxide anion radical: The superoxide anion radical scavenging activity of ethanolic extract of aerial parts of *Coleus spicatus*. is illustrated in Table-3. The maximum scavenging activity of ethanolic extract and quercetin at 1000 µg/mL was found to be 75.92 and 98.01 % respectively. The IC_{50} value of plant extract and quercetin was recorded as 410 and 60 µg/mL respectively.

TABLE-3
EFFECT OF ETHANOLIC EXTRACT OF AERIAL PARTS OF *Coleus spicatus* ON SUPEROXIDE ANION SCAVENGING ACTIVITY METHOD

S. No.	Concentration (µg/mL)	% of activity(± SEM)*	
		Sample (Ethanolic extract)	Standard (Quercetin)
1	125	29.51 ± 0.53	73.81 ± 0.01
2	250	35.30 ± 0.59	91.31 ± 0.01
3	500	58.63 ± 0.67	92.99 ± 0.02
4	1000	75.92±0.93	98.01 ± 0.01
		IC_{50} = 410 µg/mL	IC_{50} = 60 µg/mL

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

Based on the results obtained from the present study, the ethanolic extract of aerial parts of *Coleus spicatus* was found

to have significant antioxidant activity when compared to the reference standard ascorbate and quercetin respectively.

Conclusion

In present work, the high antioxidant capacity observed for ethanolic extract of aerial parts of *Coleus spicatus* suggests that it may play a role in preventing human diseases in which free radicals are involved, such as cancer, ageing and cardiovascular diseases. Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the plant extract.

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