Full Length Research Paper

Antioxidant activity of the medicinal plant *Coleus forskohlii* Briq.

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Antioxidant status of different parts of *Coleus forskohlii* including roots, stem, leaves and tubers was analyzed. For the enzymatic antioxidant properties, the activities of superoxide dismutase, peroxidase, polyphenol oxidase and catalase were significantly higher ($P < 0.05$) in tubers than in the leaves, roots and stem. Among the non-enzymatic antioxidants, except for the chlorophyll and lycopene content, the reducing power and chelating abilities on Fe^{2+}, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity, total phenol, flavonoids and β-carotene were significantly higher ($P < 0.05$) in tubers than in the leaves, roots and stem, respectively. The tubers possessed significantly rich sources of both enzymatic and non-enzymatic antioxidants besides their medicinal properties.

Key words: *Coleus forskohlii,* roots, stem, leaves, tubers, enzymatic antioxidants, non enzymatic antioxidants.

INTRODUCTION

Plants and plant-derived products are part of the health-care system since ancient human civilization. Herbal medicines have been used for many years (Kareru et al., 2007). Generally, they use plants for nourishment and medical purposes (Cakilcioglu and Turkoglu, 2010). As in the case in the other countries of the world, in recent years, the plants used traditionally for curative purposes have attracted the attention of researchers (Saya et al., 2000; Etkin and Elisabetsky, 2005; Kargloğlu et al., 2008; Uğurlu and Seçmen, 2008; Pieroni and Giusti, 2009; Leonti, 2011).

Oxidative stress can arise from an imbalance between the generation and elimination of reactive oxygen species (ROS), leading to excess ROS levels, that inflicts indiscriminate damage to virtually all biomolecules, leading, in turn, to various diseases and cell death (Scandalios, 2005). Reactive species can be eliminated by a number of enzymatic and non-enzymatic antioxidant defence mechanisms (Boullier et al., 2001). Despite the presence of the antioxidant defence system in the cell to counteract oxidation from ROS, radical-related damage of DNA and proteins has been proposed to play a key role in the development of age dependent diseases such as cancer, atherosclerosis, arthritis, Alzheimer's disease, other neurodegenerative disorder and other such conditions (Collins, 2005). The use of traditional medicine is wide spread and plants are still a large source of natural antioxidants that might serve as leads for the development of novel drugs (Linn and Huang, 2002). *Coleus forskohlii* (Willd.) Briq. (synonym *C. barbatus* (Andr.) Benth.), a member of the family Lamiaceae, is an ancient root drug in Ayurvedic materia medica (Shah, 1996). The morphology, phytochemistry and pharmacological aspects of *C. forskohlii* has been reported (Kavitha et al., 2010). Forskolin, a labdane diterpene (7β-Acetoxy-8,13-epoxy-1α, 6β, 9α-trihydroxy-labd-14-ene-11-one) isolated from *C. forskohlii* (Bhat et al., 1977; Saleem et al., 2005), was reported to activate adenyl cyclases resulting in an increase in cAMP (Seamon and Daly, 1981). The mechanisms of interaction of forskolin were studied in detail (Zhang et al., 1997; Tesmer et al., 1997; Tang and Gilman, 1995). Forskolin showed positive effects against a wide range of conditions such as asthma (Lichey et al., 1984), glaucoma (Caprioli and Sears, 1983), hypertension (Dubey et al., 1981), cancer (Agarwal and Parks, 1983), heart diseases (Kramer et al., 1987), diabetes (Ammon and Müller, 1984) and obesity (Allen et al., 1986). It also showed inhibition of platelet activating

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factor (Nourshargh and Hoult, 1986) and increase in the rate of sensory nerve regeneration in freeze-lesioned sciatic nerves (Kilmer and Carlsen, 1984). Its foliage is also employed in treating intestinal disorders and is used as a condiment. However, the antioxidant status of different parts of the plant has not been reported so far which prompted the analysis of the antioxidant potential of the different parts of *C. forskohlii.*

MATERIALS AND METHODS

Sampling

The different parts of *C. forskohlii* including roots, stem, leaves and tubers were screened for their antioxidant potential. The samples were collected from the experimental garden of the department. The roots, stem, leaves and tubers of the plants were properly washed in tap water and then rinsed in distilled water. They were cut into small pieces, dried overnight in hot air oven at 40°C, ground to a particle size 40 mesh by using mortar and pestle and stored at -4°C in air tight container until used.

Enzymatic antioxidants

The enzymatic antioxidants assayed were superoxide dismutase, peroxidase, polyphenol oxidase and catalase.

Superoxide dismutase

Superoxide dismutase activity was estimated following the method of Kakkar et al. (1984) using na-pyrophosphate buffer (0.052 M, pH 8.3). The assay was based on chromogen production using phenozinemethosulphate and nitrobluetetrazolium in the of presence of SOD enzyme. One unit of SOD activity was defined as the amount of enzyme that inhibits the rate of reaction by 50% under specified conditions. The enzyme activity was expressed as units/g dry tissue.

Peroxidase

Peroxidase activity was estimated in the supernatant following the method of Mahadevan and Sridhar (1982) using freshly prepared pyrogallol reagent. Enzyme activity was recorded as the change in absorbance per minute at 430 nm immediately after the addition of the substrate and was expressed as changes in absorbance in min/g dry tissue.

Polyphenol oxidase

The polyphenol oxidase activity was measured by the method of Mahadevan and Sridhar (1982) using catechol as a substrate. The reaction mixture contained 3.0 ml of phosphate buffer, 1.0 ml of 0.01 M catechol in phosphate buffer and 2.0 ml of the enzyme source. Changes in absorbance were recorded in a UV Vis spectrophotometer (Systronics-117) at 495 nm for 3 min at an interval of 1 min. The enzyme activity was expressed as changes in absorbance in min/g dry tissue.

Catalase

100 mg of the dry roots, stem, leaves and tubers powder was crushed separately with phosphate buffer (0.1 M, pH 7.0) at 4°C and centrifuged. The supernatant was used for the assay. Catalase activity was estimated following the method of Luck (1963). The enzyme activity was expressed as units/g dry tissue.

Non enzymatic antioxidants

Chlorophyll, lycopene, β-carotene, reducing power and chelating abilities on Fe^{2+}, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity, total phenol and flavonoid content were the non-enzymatic antioxidants analysed in different parts of *C. forskohlii.*

Chlorophyll

Chlorophyll was estimated following the method of Arnon (1949). 100 mg of the dry roots, stem, leaves and tubers were immersed in 5 ml acetone and kept at low temperature in a deep freeze for 24 h. The supernatant was decanted off and the plant tissues were rinsed repeatedly with a little volume of acetone until they were completely free from green colour. The final volume was measured at 665 nm in UV Vis spectrophotometer (Systronics-117). The chlorophyll content was expressed as mg/g dry tissue.

Lycopene and β-carotene

Lycopene and β-carotene were determined according to procedures of Barros et al. (2007). 100 mg of the dry roots, stem, leaves and tubers powder was extracted with 10 ml of 1% metaphosphoric acid for 45 min at room temperature and filtered through Whatman no. 1 filter paper. The absorbance of the filtrate was measured at 453, 505 and 663 nm, respectively. Contents of lycopene and β-carotene were calculated according to the following:

lycopene (mg/100 ml) = -0.0458 × A663 × A505 - 0.0806 × A453

β-Carotene (mg/100 ml) = 0.216 × A663 - 0.304 × A505 + 0.452 × A453

The results were expressed as µg carotenoid/g dry tissue.

Estimation of reducing power and chelating activity on the Fe^{2+}

Extraction

100 mg of the dry powder of roots, stem, leaves and tubers was stirred with 50 ml of ethanol and water (3:1, v/v) at 75°C and centrifuged at 3,000 rpm for 1 h. Each extract was then filtered through filter paper; the filtrate was collected and dried by a rotary evaporator at 40°C, filled in a plastic bottle and stored at -4°C until used. The reducing power of the ethanol extract was measured according to the method of Oyaizu (1986) using potassium ferricyanide. The presence of reductants (antioxidants) in the tested samples would result in reducing Fe^{3+}/ferricyanide complex to the ferrous form (Fe^{2+}). The Fe^{2+} can be monitored by measuring the formation of the Perl's Prussian blue at 700 nm (Chung et al., 2002).

The chelating activity of the ethanolic extracts on Fe^{2+} was measured following the method of Decker and Welch (1990) using ferrozine reagent. Aliquot of 1 ml of the ethanolic extracts (1 mg/ ml) was mixed with 2.5 ml of de-ionized water. The mixture was left for reaction with $FeCl_2$ (2 mM, 0.1 ml) and ferrozine (5 mM, 0.2 ml) for 10 min at room temperature. The absorbance was measured at 562 nm in a UV Vis spectrophotometer (Systronics - 117). A lower

Table 1. Enzymatic antioxidants in the different parts of *C. forskohlii.*

Enzymes	Root	Stem	Leaf	Tuber
Superoxide dismutase (units/g dw)	145.34±10.52[c,d]	108.5±0[d]	91.25±4.82[a,d]	257.18±45.16[a,b,c]
Peroxidase ($\triangle$A min/g dw)	42.33±3.10[b,c,d]	34.31±3.49[a,c,d]	23.25±2.06[a,b,d]	56.16±3.30[a,b,c]
Polyphenol oxidase($\triangle$A min/g dw)	14.17±0.74[b,d]	11.06±0.37[a,d]	12.57±0.04[d]	25.85±2.17[a,b,c]
Catalase (units/g dw)	2258.28±4.59[b,c,d]	1118.52±4.51[a,d]	1131.40±2.32[a,d]	5586.06±31.31[a,b,c]

Each value is expressed as mean ± standard deviation (n = 5). Means with different superscripts within the same column are significantly different (P < 0.05). [a]P < 0.05 compared with roots; [b]P < 0.05 compared with stem; [c]P < 0.05 compared with leaves; [d]P < 0.05 compared with tubers. $\triangle$, change.

absorbance indicates a higher chelating power. Chelating activity was calculated following the equation:

$$\text{Chelating activity (1\%)} = 1 - \frac{\text{Absorbance of sample at 562 nm}}{\text{Absorbance of control at 562 nm}} \times 100$$

DPPH radical-scavenging activity (RSA)

0.3 ml of the roots, stem, leaves and tubers extracts (1 mg/ ml) was mixed separately with 2.7 ml of methanolic solution containing DPPH radicals (200 μM) (Hatano et al., 1988). The mixture was shaken vigorously and left to stand for 60 min in the dark (until stable absorption values were obtained). The reduction of the DPPH radical was determined by measuring the absorption at 517 nm. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discolouration using the equation:

$$\%RSA = [(A_{DPPH} - A_S) / A_{DPPH}] \times 100$$

Where, A_S is the absorption of the solution when the sample extract was added at a particular level and A_{DPPH} is the absorbance of the DPPH solution.

Extraction and estimation of total phenol content

100 mg of the dry powder of the roots, stem, leaves and tubers was kept separately in 5 to 10 ml of 80% ethyl alcohol and allowed to boil for 5 to 10 min in hot water bath and then cooled in a pan of cold water. The tissues were crushed in a mortar and pestle for 5 to 10 min and passed through a double layered cloth. The ground tissue was again extracted in boiling 80% alcohol, cooled and then again passed through Whatman no. 1 filter paper. Total phenol was estimated following the method of Mahadevan and Sridhar (1982) using Folin-Ciaocalteu reagent. Catechol was used as the standard. The amount of phenolics was expressed as μg catechol equivalents/g dry tissue.

Extraction and estimation of total flavonoid content

Total flavonoid was estimated spectrophotometrically based on the modified method of Zhishen et al. (1999). 100 mg of the dry roots, stem, leaves and tubers powder was dissolved in 10 ml of ethanol and kept in a shaker overnight. The obtained extract was filtered with Whatman no.1 filter paper and the filtrate was collected. The ethanol was then removed under room temperature at 30 to 32°C to obtain the concentrated extract. To 0.1 ml of each sample extract in a 10 ml volumetric flask, distilled water was added to make the volume to 5 ml and 0.3 ml of 5% NaNO$_2$ was added to this. 3 ml of 10% AlCl$_3$ was added 5 min later. After 6 min, 2 ml of 1 M NaOH was added and the absorbance was measured at 510 nm. Rutin was used as a standard. The amount of flavonoids was expressed as mg rutin equivalents/g dry tissue.

Statistical analysis

Data were expressed as mean ± standard deviation. Significant differences among the means were determined by Fisher's least significant difference test after one way ANOVA. Significance of between-treatment means was tested at 0.05 level of probability. Correlations between content of components and antioxidant attribute were determined by linear regression analysis using Stat plus version 5.8 (2009) software.

RESULTS AND DISCUSSION

Enzymatic antioxidants

The activities of the enzymatic and non-enzymatic antioxidants in the different parts of *C. forskohlii* are presented in Tables 1 and 2. For the enzymatic antioxidant properties (Table 1), superoxide dismutase activity was found to be significantly higher (P < 0.05) in tubers (257.18 units/g dry tissue) than in roots (145.34 units/g dry tissue), stem (108.5 units/ g dry tissue) and leaves (91.25 units/g dry tissue). Peroxidase activity was also found to be significantly higher (P < 0.05) in tubers (56.16 min/g dry tissue) than in the roots (42.33 min/g dry tissue), stem (34.31 min/g dry tissue) and leaves (23.25 min/g dry tissue). Polyphenol oxidase activity too, remained significantly higher (P < 0.05) in tubers (25.85 min/g dry tissue) followed by roots (14.17 min/g dry tissue), leaves (12.57 min/g dry tissue) and stem (11.06 min/g dry tissue), respectively. Catalase activity, on the other hand, was recorded to be significantly higher (P < 0.05) in tubers (5586.06 units/g dry tissue) followed by roots (2258.28 units/g dry tissue), leaves (1131.40 units/g dry tissue) and stem (1118.52 units/g dry tissue), respectively. Enzy-matic antioxidants serve as an intrinsic defense tool to resist oxidative damage in plants (Verma and Dubey, 2003). One of the mechanisms *in vivo* is improving the endogenous cellular antioxidants mechanisms, such as up-regulation of the activity superoxide dismutase (Halliwell, 2008).

Table 2. Levels of non-enzymatic antioxidants of *C. forskohlii.*

Parameter	Root	Stem	Leaf	Tuber
Reducing power (OD at 700nm)	$1.15\pm0.01^{b,c,d}$	$0.61\pm0.05^{a,c,d}$	$2.19\pm0.09^{a,b,d}$	$2.31\pm0.07^{a,b,c}$
Chelating abilities (%)	$86.71\pm0.45^{b,c,d}$	$76.87\pm0.56^{a,c,d}$	$88.84\pm0.92^{a,b,d}$	$90.75\pm0.62^{a,b,c}$
DPPH radical-scavenging activity (%)	$85.27\pm0.14^{b,c,d}$	$69.40\pm0.07^{a,c,d}$	$87.34\pm0.12^{a,b,d}$	$90.32\pm0.16^{a,b,c}$
Total phenol(µg catechol/g dw)	$24.22\pm0.52^{b,c,d}$	$21.26\pm0.45^{a,d}$	$20.44\pm0.55^{a,d}$	$27.05\pm0.71^{a,b,c}$
Flavonoids (mg rutin/g dw)	$3.66\pm0.56^{b,d}$	$2.89\pm0.35^{a,c,d}$	4.15 ± 0.15^{b}	$4.37\pm0.35^{a,b}$
Chlorophyll(mg/g dw)	$0.37\pm0.06^{b,c}$	$1.31\pm0.18^{a,c,d}$	$37.73\pm0.36^{a,b,d}$	$0.22\pm0.01^{b,c}$
Lycopene (µg carotenoid /g dw)	$2.52\pm0.15^{b,c}$	$4.50\pm0.21^{a,c,d}$	$11.36\pm0.16^{a,b,d}$	$2.47\pm0.25^{b,c}$
β-carotene(µg carotenoid/g dw)	$12.37\pm0.13^{b,c,d}$	$5.05\pm0.55^{a,c,d}$	$11.52\pm0.20^{a,b,d}$	$33.25\pm0.13^{a,b,c}$

Each value is expressed as mean ± standard deviation (n = 5). Means with different superscripts within the same column are significantly different (P < 0.05). [a]P < 0.05 compared when roots; [b]P < 0.05 compared when stem; [c]P < 0.05 compared with leaves; [d]P < 0.05 compared with tubers.

Non-enzymatic antioxidants

Reducing power

Table 2 shows the reducing power of the different parts of *C. forskohlii*. The presence of reducers (the antioxidants) causes the conversion of the Fe^{3+} /ferricyanide complex to the ferrous form. The formation of Perl's Prussian blue at 700 nm indicates a higher reducing power. The reducing power of tubers (2.31 A_{700} at 1.0 mg/ml) was found to be significantly higher (P < 0.05) than leaves (2.19 A_{700} at 1.0 mg/ml), roots (1.15 A_{700} at 1.0 mg/ml) and stem (0.61 A_{700} at 1.0 mg/ml), respectively. It was reported that, the reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (Shimada et al., 1992). Accordingly, the tubers of *C. forskohlii* might contain higher amount of reductone, which could react with free radicals to stabilise and block radical chain reactions.

Chelating activity on the Fe^{2+}

The chelation of Fe^{2+} ions was estimated by the method of Decker and Welch (1990) in which ferrozine quantitatively forms complexes with Fe^{2+}. In the presence of chelating agents, the formation of this complex is disrupted and therefore impends the formation of the red colour imparted by the complex as well. Measurement of this colour change, therefore, allows for the estimation of the chelating activity of the coexisting chelator. The formation of Fe^{2+} - ferrozine complex was not completed in the presence of the extract, indicating that the different parts of *C. forskohlii* chelated the iron (Table 2). However, the chelating activity of the tubers (90.75% at 1.0 mg/ ml) was found to be significantly higher (P < 0.05) than that of the leaves (88.84% at 1.0 mg/ ml), roots (86.71% at 1.0 mg/ ml) and stem (76.87% at 1.0 mg/ ml), respectively. Metal chelating agents reduce the concen-

tration of catalyzing transition metal in lipid peroxidation by forming sigma bonds with metals and reducing the redox potential, thereby stabilizing the oxidized form of the metal ion (Elmatas et al,. 2006).

DPPH radical-scavenging activity (RSA)

The free radical DPPH possess a characteristic absorption at 517 nm (purple in colour), which decreases significantly on exposure to radical-scavengers by providing hydrogen atoms or by electron donation. A lower absorbance at 517 nm indicates a higher radical-scavenging activity of the extract. Free radical-scavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation (Amarowicz et al., 2004). From the analysis (Table 2), the radical-scavenging activity of the tubers (90.32% at 1.0 mg/ml) was found to be significantly higher (P < 0.05) followed by leaves (87.34% at 1.0 mg/ml), roots (85.34% at 1.0 mg/ml) and stem (69.40% at 1.0 mg/ml), respectively.

Total flavonoid and phenol content

Recent studies have shown that, many flavonoids and related polyphenols contribute significantly to the total antioxidant activity of many plants (Luo et al., 2002). The total flavonoid in the tubers (4.37 mg rutin equivalents/g dry tissue) was found to be significantly higher (P < 0.05) than those of the other parts of this plant. The total phenol content (Table 2) was found to be significantly higher (P < 0.05) in the tubers (27.05 µg catechol equivalents/g dry tissue) than in the roots (24.22 µg catechol equivalents/ g dry tissue) and stem (21.26 µg catechol equivalents/g dry tissue). The antioxidant property of the compounds was well correlated with the content of their phenolic compounds (Velioglu et al., 1998). Phenols contain good antioxidant, antimutagenic and anticancer properties (Ahmad and Mukhtar, 1999). Therefore, the highest content of total phenol in tubers is held respon-

sible for the better antioxidant properties.

Chlorophyll, lycopene and β-Carotene

Chlorophyll (33.73 mg/g dry tissue) and lycopene (11.36 µg carotenoid/g dry tissue) of the leaves were significantly higher ($P < 0.05$) than those of the other parts of the plant. The β-Carotene of the tubers (33.25 µg carotenoid/g dry tissue) was significantly higher ($P < 0.05$) than those of the other parts of this plant.

Crude extracts of the various parts (leaves, fruits, roots, stem and trunk bark) of *Garcina atroviridis* showed strong antioxidant activity exceeding that of the standard vitamin E (Mackeen et al., 2000). Aqueous extracts from the different parts of the four medicinal plants, *Momordica charantia, Glycyrrhiza glabra, Acacia catechu* and *Terminalia chebula* were found to be rich sources of enzymatic and non-enzymatic antioxidants (Naik et al., 2005). The roots, stem, leaves and tubers of *Withania somnifera* were found to be rich sources of both enzymatic and non-enzymatic antioxidants (Sumathi and Padma, 2008).

Among the enzymatic antioxidants and antioxidative attributes, a positive correlation was found between the chelating abilities on Fe^{2+} with superoxide dismutase ($r = 0.881$, $P < 0.05$), reducing power on Fe^{2+} with polyphenol oxidase ($r = 0.685$, $P < 0.05$), catalase ($r = 0.684$, $P < 0.05$), DPPH radical-scavenging activity with peroxidase ($r = 0.753$, $P < 0.05$) and polyphenol oxidase ($r = 0.707$, $P < 0.05$). Moreover, among the non-enzymatic antioxidants and antioxidative attributes, a positive correlation was found between the reducing power on Fe^{2+} with flavonoids ($r = 0.817$, $P < 0.05$), chelating abilities on Fe^{2+} with chlorophyll ($r = 0.785$, $P < 0.05$), β-Carotene ($r = 0.682$, $P < 0.05$) and DPPH radical-scavenging activity with total phenols ($r = 0.448$, $P < 0.05$).

Conclusion

The tubers possessed significant potential of both enzymatic and non-enzymatic antioxidants that could protect against oxidant and free radical injuries, in addition to having their medicinal properties. Thus, the effective source of *C. forskohlii* could be employed in all medicinal preparations to combat myriad diseases associated with oxidative stress, including cancer and related disorders.

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