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Free radical quenching activity and polyphenols in three species of *Coleus*

Girish K Rasineni[1], Dayananda Siddavattam[2] and Attipalli R Reddy[1]*

[1]Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad - 500 046, India.
[2]Department of Animal Sciences, School of Life Sciences, University of Hyderabad, Hyderabad - 500 046, India.

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***Coleus* is an important aromatic herb of the family Lamiaceae which is routinely grown as a traditional medicinal herb in India. We examined the total content of polyphenols, tannins, flavones and flavonols, their antioxidant and lipid peroxidation inhibition properties in leaf and stem tissues of three species of *Coleus* (*Coleus forskholii Briq., Coleus aromaticus Benth.* and *Coleus zeylanicus Benth.*). Plant extracts of *C. forskholii* exhibited high amounts of polyphenols and higher antioxidant activity in the tissues compared to *C. aromaticus* and *C. zeylanicus.* The leaf extracts of *C. forskholii* showed significantly high amounts of total polyphenols (23.46 mg g^{-1} fw), flavones and flavonols (250.8 µg g^{-1} fw) and high antioxidant activity (12.29 mM g^{-1}fw). HPLC profiling of leaf and stem tissues showed the presence of standard antioxidative polyphenols and more potent antioxidative polyphenols. Our results demonstrate that *C. forskholii* could be used as an important source of phenolic compounds with significantly high antioxidant activity.**

Key words: Antioxidants, *Coleus forskholii*, *Coleus aromaticus*, *Coleus zeylanica*, polyphenols.

INTRODUCTION

Several medicinal plants are traditionally noted for their bio-medicinal properties often exhibiting a wide range of biological and pharmacological activities, such as anti-inflammatory, anti-bacterial and anti-fungal properties. The extracts from these plant tissues especially from the leaves, roots, barks, seeds and fruits were used in traditional medicinal practices. The active constituents contributing to these protective effects are the naturally occurring phytochemicals, vitamins and minerals which give plants their colour and flavor. The alkaloids, tannins, flavonoids and phenolic compounds play a major role in preventing number of chronic diseases by a definite physiological action on the human body like anti-inflammatory, antithrombotic, antioxidant and anticarcinogenic activities (Craig, 1999).

Polyphenols are commonly present in certain edible and non-edible plant tissues and have arouse a great deal of interest recently. Numerous studies of plant extracts on the animal models showed various effects pertaining to cancer, cardiovascular diseases, neurodegenerative diseases, diabetes and osteoporosis (Augustin et al., 2005). Among the diverse roles of polyphenols, they protect the cell constituents against destructive oxidative damage thus limiting the risk of various degenerative diseases, associated with oxidative stress (Villano et al., 2005). Phenolics are chemical compounds characterized by the presence of at least one aromatic ring (C6) with one or more hydroxyl groups (Sakihama et al., 2002). These compounds tend to be potent free radical scavengers and their abilities to act as antioxidants depend on their chemical structure, capability to donate/accept electrons, thus delocalizing the unpaired electron within the aromatic structure and the Polyphenols are broadly classified into two categories, flavonoids and phenolic acids (Augustin et al., 2005). Flavonoids is a large family consisting of more than 4000 ubiquitous secondary plant metabolites, which are further divided into five subclasses namely flavonols, flavones, anthocyanins, catechins and flavonones (Merken and Beecher, 2000). These flavornoid compounds have a common diphenylpropane structure (C6C3C6) with different degrees of hydroxylation, oxidation and substitution and these compounds commonly occur as glycosides in plants (Pietta, 2000). As antioxidants, flavonoids have been reported to be able to interfere with the activities of enzymes involved in reactive oxygen species generation, quenching free radicals,

*Corresponding author. E-mail: arreddy@yahoo.com. Tel: +91-40-23134508. Fax: +91-40-23010120.

chelating transition metals and rendering them redox inactive in the Fenton reaction (Heim et al., 2002). Anti-oxidant compounds presented in plant extracts are therefore multi-functional and their activity and action mechanism would largely depend on the composition and conditions of the test system

Flavonols such as quercetin, myricetin, isorhamnetin, kaempferol and corresponding flavones, apigenin and luteolin have been well established as potent antioxidants that prevent oxidation of low-density lipoprotein and inhibit lipid peroxidation (Formica and Regelson, 1995; Hertog and Hollman, 1996; Shahidi and Wanasundara, 1992). Other phytophenolics such as phenolic acids, stilbenes, tannins, lignans and lignins are especially common in leaves, flowering tissues and woody parts such as stems and barks for growth, development and defense mechanism in plants (Larson, 1988). The anti-oxidant properties of phytophenolics depend mainly upon factors such as metal-reducing potential, chelating behavior, pH and solubility characteristics (Decker, 1997).

The other important phyto-constituents are tannins, water-soluble polyphenols which tend to reduce the mutagenic activity and also oxygen-free radicals. The anticarcinogenic and antimutagenic potentials of tannins may be related to their antioxidative capability like protecting against cellular oxidative damage including lipid peroxidation and the inhibition of generation of superoxide radicals (King-Thom et al., 1998). Free radicals induce cellular damage and are also involved in several human diseases such as cancer, atherosclerosis, inflamamatory disorders and in aging processes (Halliwell, 1994; Aviram, 2000).

Synthetic antioxidants, such as propyl gallate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butylhydroquinone have been widely used to control lipid oxidation. However, use of these synthetic antioxidants has been questioned due to their potential health risks and toxicity (Kahl and Kappus, 1993). The search for antioxidants from natural sources has received much attention to identify compounds that act as suitable antioxidants and replace the synthetic ones. In addition, these naturally-occurring antioxidants can be formulated to give neutraceuticals that can help to prevent oxidative damage from occurring in the body

Although a variety of medicinal herbs are known to be potent sources of phenolic compounds, studies dealing in isolating polyphenols, evaluating their antioxidative effects and their synergistic effects with other bioactive compounds in certain indigenous medicinal plants are little known or scarce. *Coleus* plants have been used traditionally as folk medicine in India, to treat various diseases including congestive heart failures. Forskholin from *Coleus forskholii* has been identified as a potent activator of adenylate cyclase, leading to an increase in levels of cAMP affecting cardiac muscle contraction, blood and intraocular pressure, cancer, eczema, rheumatism and obesity (Duke and Maryl, 2002). However, studies on polyphenolic compounds and their properties in different *Coleus* species are little known. Our objectives in the present study were to determine: 1.The contents of total polyphenols, flavanoids and tannins in the plant extracts of three *Coleus* species; 2.To characterize the free radical scavenging and lipid peroxidation inhibition activities.

MATERIALS AND METHODS

Plant material

C. forskholii was obtained from Acharya N. G. Ranga Agricultural University, Hyderabad, Andhra Pradesh. *Coleus aromaticus* and *Coleus zeylanicus* were from Pinchandikulum Bioresource Center, Auroville, Tamilnadu, India. The three species of *Coleus* were grown in 30 cm pots under natural photoperiod in the Uni-versity of Hyderabad Botanical Gardens, Hyderabad, India. The daily average maximum and minimum air temperatures during the growth were 33 and 24^{o}C, respectively. The plants (n = 4) were well watered and periodically fertilized with Hoagland nutrient solution. Tissues from three month-old- plants were used for all the experiments in the present study.

Total phenolics estimation

Total phenolic content in the tissue extracts were determined using Folin-ciocalteau reagent according to the method of Malick and Singh (1980) using gallic acid as standard. 500 mg fresh weight of the different tissues of three *Coleus* species were homogenized in 80% ethanol using mortar and pestle and the homogenate was centrifuged at 10,000 x g for 20 min. The supernatant was used for estimation of total polyphenols in different species of *Coleus* tissue extracts. Folin-ciocalteau reagent (0.5 ml) was added to 3 ml of ethanolic plant extract and then 2 ml 20% sodium carbonate was added. The contents were incubated for 5 min at room temperature and the absorbance of blue colour was read at 650 nm. A calibration curve was prepared using gallic acid standards at concentrations of 100 µg to 1 mg L^{-1}. Total content of phenolic compounds in plant extracts was calculated as gallic acid equivalents (GAE) using the formula:

$$C = \frac{c \times V}{M'}$$

Where,
C-Total content of phenolic compounds (mg/g) in plant extract expressed in GAE
c-The concentration of gallic acid (mg/ml)
V-The volume of plant extract (ml)
M´-The weight of tissue sample

Estimation of flavones and flavonols

Flavones and flavonols were expressed in quercetine equivalent per gram fresh weight tissue.1g of different tissues of three *Coleus* species were homogenized in 95% ethanol using mortar and pestle and were kept at 37°C for 24 h. The homogenate was filtered and adjusted to 25 ml with 80% ethanol (v/v). 0.5 ml of ethanol extract was mixed with 1.5 ml 95% ethanol (v/v), 0.1 ml 10% aluminum chloride (m/v), 0.1 ml of 1 $molL^{-1}$ potassium acetate and 2.8 ml water. The content was incubated for 30 min at room temperature and the absorbance was measured at 415 nm. A calibration curve was prepared using quercetine standards at concentrations of 12.5, 25.0, 50.0, 80.0 and 100.0 μgml^{-1}. Total content of flavones and flavonols in plant extracts of different tissues of three *Coleus* species was calculated in quercetine equivalents (Ivan et al., 2004).

Estimation of tannins

Tannin content in different tissues of three *Coleus* species was determined by Folin-Denis reagent according to the method of Schanderl (1970) using tannic acid as standard. 0.5 g of different tissues of three *Coleus* species were taken in a conical flask containing 25 ml of distilled water and boiled for 30 min. The solution was centrifuged at 1,000 x g for 20 min and supernatant was collected. To this supernatant (1 ml), 1 ml of Folin-Denis reagent and 2 ml of sodium carbonate solution were added. The solution was made up to 5 ml with distilled water, incubated for 30 min at room temperature and the absorbance was read at 700 nm. A calibration curve was prepared using tannic acid concentrations ranging from 0 - 100 µg. The values were expressed in micrograms (µg) of tannic acid equivalents per gram fresh weight.

Extraction and estimation of total antioxidants

The total antioxidant content in different tissues of the three *Coleus* species was estimated according to Miller et al., (1993) using antioxidant assay kit (ABTS method) obtained from Cayman Chemical Company (Genetix Bio-Asia, India). ABTS (2, 2'-Azino-bis-[3-ehthylbenzthiazoline sulphonate]) is a chromogen which changes into a coloured mono-cation radical form ($ABTS^{\cdot+}$) by an oxidetive agent metmyoglobin and the $ABTS^{\cdot+}$ has an absorption peak at 750 nm. Addition of antioxidants to above mixture will reduce $ABTS^{\cdot+}$ into colourless form and the extent of decolourization corresponds to the percentage reduction of $ABTS^{\cdot+}$, which corresponds to function of concentration and is calculated with relative to the reactivity of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a water soluble analog of Vitamin E. The antioxidant activity was expressed as millimolar of Trolox equivalents per gram fresh weight 500 mg of different tissues (stems and leaves) of three *Coleus spe*-species were homogenized in a mortar and pestle using 2 ml of cold assay buffer (5 mM potassium phosphate (pH 7.4) containing 0.9% sodium chloride and 0.1% glucose).The homogenate was centrifuged at 10,000 x g for 15 min at 4°C and the supernatant was used in all assays. 10 µl of Trolox standards (of different concentrations), 10 µl of metmyoglobin and 150 µl of chromogen were added in a micro-plate, which served as standard. 10 µl of extract, 10 µl of metmyoglobin and 150 µl of chromogen were added into sample wells and the reaction was initiated by adding 40 µl of H_2O_2 in all the wells. The mixtures were incubated for 5 min at room temperature with intermittent shaking and the absorbance was measured at 750 nm using ELISA reader (Multiscope lab systems, Finland). Antioxidant concentrations in the plant extracts were calculated using the equation obtained with the linear regression from a standard curve ($Y= -0.7313X +0.3534$, $R^2 = 0.998$) and by substituting the average absorbance values for each sample into the equation.

$$\text{Antioxidant content (mM)} = \frac{\text{Sample average absorbance} - \text{y-intercept}}{\text{Slope}} \times \text{dilution}$$

Y-intercept = 0.3534; Slope = - 0.7313

Lipid peroxidation assay

Lipid peroxidation inhibition assay was estimated according to Dasgupta and De (2007). Lipid peroxidation in egg yolk was induced by FeSO4. Malondialdehyde (MDA), produced by the oxidation of polyunsaturated fatty acids, reacts with two molecules of thiobarbituric acid (TBA) yielding a pinkish red chromogen which was measured at 532 nm. Percentage inhibition of lipid peroxidation by different *Coleus* tissue extract was calculated according to the formula;

(1-E/C) x 100

C = Absorbance value of the fully oxidized control
E = Absorbance in presence of extracts

Qualitative analysis by HPLC

Standards

Polyphenolic standards including chlorogenic acid, caffeic acid, syrengic acid, coumaric acid, ferulic acid, myrecitin and quercetin were purchased from Sigma – Aldrich. All the standards were dissolved in methanol to concentration of 1 mg/ml and were stored in darkness.

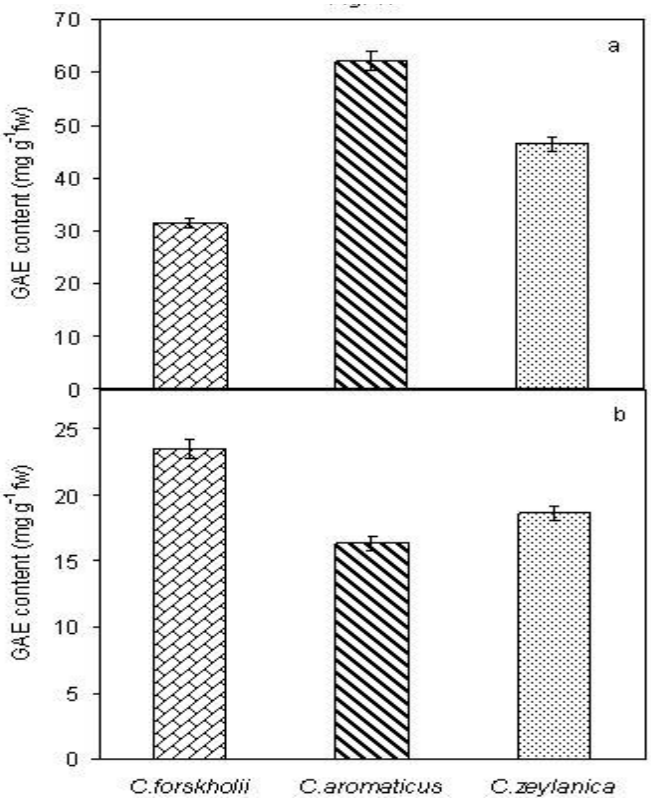

Figure 1. Total phenolic (GAE) content in different tissues of three *Coleus* species. a: GAE content in stem tissues. b: GAE content in leaf tissues. Values are average of three independent experiments ± SE.

Preparation of samples

Two hundred and fifty mg plant material was sonicated with 25 ml of methanol: water (1:4) in an ultrasonic bath for 20 min. After centrifugation at 7600 x g for 10 min, the supernatant was adjusted to 25 ml in a measuring flask. Samples were quantified immediately after the extraction in order to avoid possible chemical alterations.

High-performance liquid chromatography

HPLC analysis was carried out according to modified method of Irene et al. (2004) in a Shimadzu SPD – 10 AVP isocratic system. Acetonitrile and water (7:3) with 0.1% formic acid was used as solvent system at 1ml min^{-1} flow rate. Luna 5 µ C_{18} (2) 100 A column (250 x 4.6 mm) was used for separation and peaks were detected at 330 nm.

Statistical analysis

Regression analysis of antioxidant activity versus the total phenolic content was done using the EXCEL program. All experiments were done in triplicate. Results are given as mean arithmetic values ± SE (* $p < 0.05$).

RESULTS AND DISCUSSION

Studies on dietary and medicinal plants have shown that polyphenols inhibit oxidative stress (Manach et al., 2004; Rice-Evans et al., 1996). Antioxidant activity of leaf extracts from medicinal plants (Zainol et al., 2003) and fruits (Banerjee et al., 2005) found a direct linear relationship between the total phenolic content and total antioxidant activity, indicating that the phenolic compounds might be the major contributors to the antioxidant activities of these extracts (Martina et al., 2007)

Plants belonging to the family Lamiaceae such as Rosemary, Sage, Thyme and Coleus are being extensively used in traditional medicinal practices in India. *C. forskohlii*, the Indian herb, produces the labdane diterpenoid, forskolin in its tuberous roots (Shah et al., 1980), which has been shown to be a hypotensive agent with spasmolytic, cardiotonic and platelet aggregation inhibitory activity, antiglaucomic in nature and a potent stimulator of the enzyme adenylate cyclase. Decoction of *C. aromaticus* leaves was used to administer to patients suffering from chronic cough and asthma (CSIR, 1986, 1992). It is considered to be antispasmodic, stimulant and stomachic and is used for the treatment of headache, fever, epilepsy and dyspepsia (Khory and Katrak, 1999).

Studies on the phenolic compounds, flavonoids, tannins and their antioxidative activities in the plant extracts of *Coleus* were not properly documented till now. The present study showed that the total phenolic content in different tissues of the three species of *Coleus* varied from 16.32 to 62.12 mg g^{-1} fw. Stems of *C. aromaticus* showed higher content of total polyphenols (62.12 mg g^{-1} fw) compared with *C. forskholii* (31.32 mg g^{-1}fw) and *C. zeylanicus* (48.5 mg g^{-1} fw) (Figure 1a). Among the three *Coleus* species, highest total polyphenolic content was observed in *C. forskholii* leaves (23.46 mg g^{-1} fw) compared to *C. aromaticus* (16.32 mg g^{-1} fw) and *C. zeylanicus* (18.8 mg g^{-1} fw) (Figure 1b). Phenolics are known as radical scavengers or radical-chain breakers, extinguishing strongly the different oxidative free radicals. The antioxidative property of the phenolics has been predicted mainly due to their redox potential (Rice-Evans et al., 1995).

Flavonoids are the naturally occurring polyphenolic compounds representing one of the most prevalent classes of compounds in medical herbs such as *Silybum marianum*, *Alpina officinarum*, *Hypericum perforatum* and also in vegetables, nuts, fruits and beverages such as coffee, tea and red wine (Hertog et al., 1993). Some of the most commonly present flavonoids are quercetin – a flavonol abundant in onion, tea, and apple; catechin - a flavanol found in tea and several fruits; hesperetin - a flavanone present in citrus fruits; cyanidin - an anthocya-

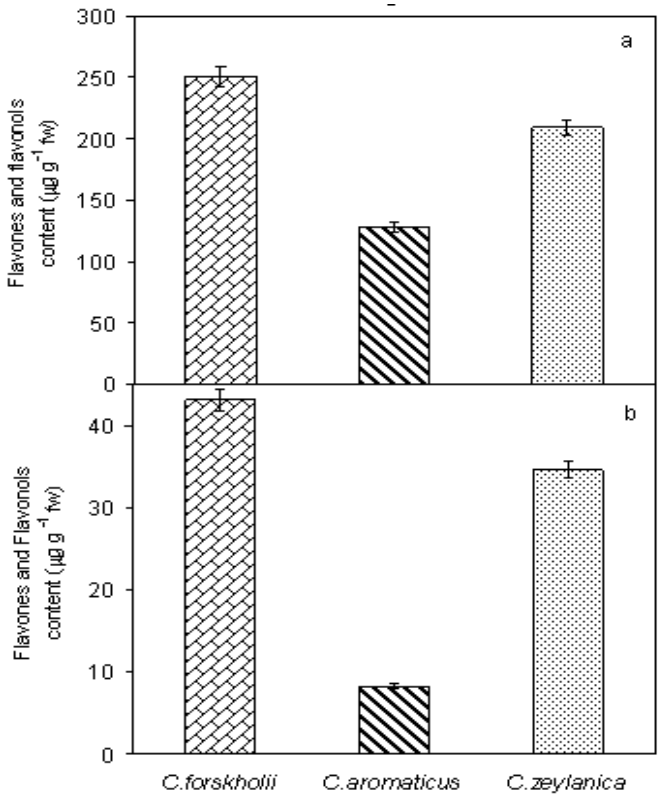

Figure 2. Flavones and flavonol contents in the different tissues of three *Coleus* species. a: Flavones and flavonol content in leaf tissues. b: Flavones and flavonols content in stem tissues. Values are average of three independent experiments ± SE.

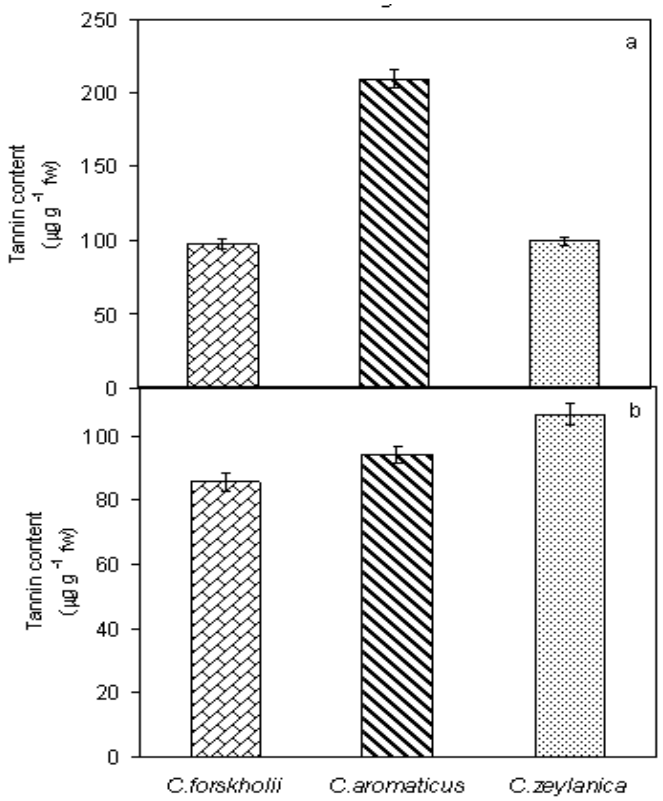

Figure 3. Tannin contents in different tissues of three *Coleus* species. a: tannin content in leaf tissues. b: tannin content in stem tissues. Values are average of three independent experiments ± SE.

nin giving color to many red fruits like blackcurrant, raspberry, strawberry, etc. Our results showed varied amounts of flavones and flavonols ranging between 8.25 to 250.8µg g^{-1} in the different extracts of the three *Coleus* species. Stem tissues of *C. forskholii* showed higher content of flavones and flavonols (43.12 µg g^{-1} fw) compared with *C. aromaticus* (8.25 µg g^{-1} fw) and *C. zeylanicus* (34.65 µg g^{-1} fw) (Figure 2b). Also, the leaf tissues *C. forskholii* showed highest amount of flavones and flavonols (250.8 µg g^{-1} fw) compared with *C. aromaticus* (127.38 µg g^{-1} fw) and *C. zeylanicus* (209.0µg g^{-1} fw) (Figure 2a). Epidemiological studies have shown the protective role of flavonoids against various cancers and more particularly hormone related cancers (Messina et al., 1994)

Our data on the tannin content in different tissues of three species of *Coleus* varied from 85.14 µg g^{-1} fw to 209.82 µg g^{-1} fw. Stem tissues of *C. zeylanicus* showed higher tannins content (106.60µg g^{-1}fw) when compared with *C. forskholii* (85.54 µg g^{-1} fw) and *C. aromaticus* (94.12 µg g^{-1} fw) (Figure 3b). Among the three Coleus species, highest tannin content was noticed in *C. aromaticus* leaves (209.82 µg g^{-1} fw) compared to *C. zeylanicus* (99.58 µg g^{-1} fw) and *C. forskholii* (97.24 µg g^{-1} fw) (Figure 3a). Tannins have been known to be anticarcinogenic to a certain concentration range and tend to defend the tumor-promoting activities, whereas at higher concentrations, they are virtually shown to inhibit the digestive enzymes and reduce the bioavailability of iron and vitamin B_{12} (King-Thom et al., 1998; Shahidi andWanasundara, 1992; Butler, 1989). Tannins have also been reported to exert serious physiological effects like damage to mucosal lining of gastrointestinal tract and alteration of excretion of certain cations at higher concentrations (Leiner, 1980).

Plant extracts from three species of *Coleus* showed antioxidant activities proving their capacity to scavenge the $ABTS^{\bullet+}$ radical-cation. The antioxidant activity in ethanol extracts of stem and leaf tissues of three *Coleus* species were expressed in Trolox Equivalent Antioxidant Capacity (TEAC). *C. forskholii* stem tissue had relatively higher antioxidant content (12.6 mM g^{-1} fw) compared to *C. aromaticus* (11.6 mM g^{-1}fw) and *C. zeylanicus* (11.8 mM g^{-1} fw) (Figure 4a). Total antioxidant content in the leaves of the three species of *Coleus* showed that *C. zeylanicus* had slightly higher antioxidant content (12.40 mM g^{-1} fw) compared to *C. forskholii* (12.29 mM g^{-1} fw)

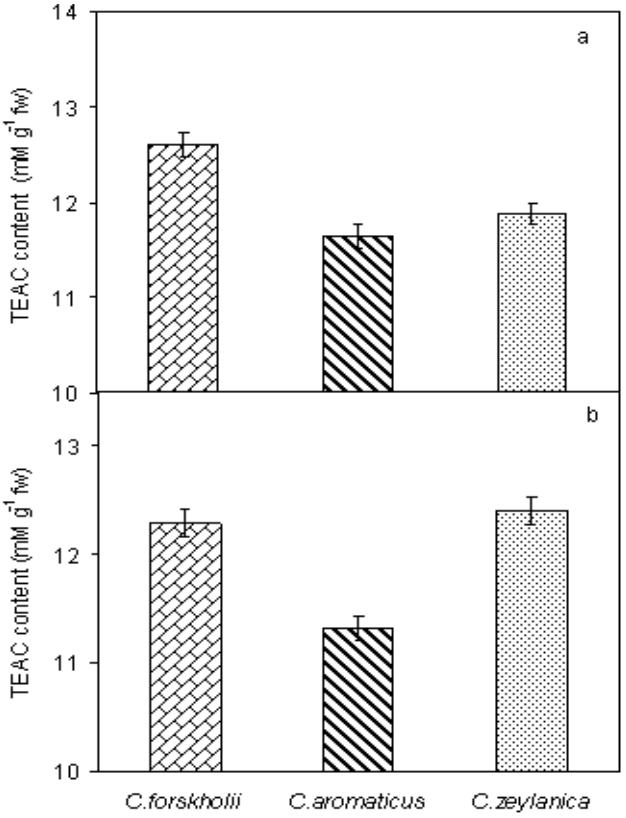

Figure 4. Total antioxidant (TEAC) content in the different tissues of three *Coleus* species. a: TEAC content in stem tissues. b: TEAC content in leaf tissues. Values are average of three independent experiments ± SE.

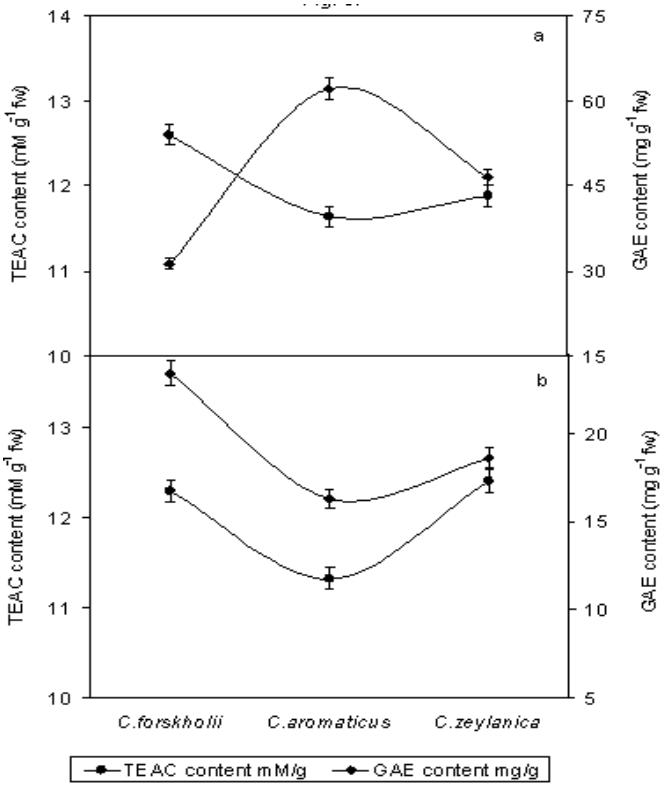

Figure 5. Regression analysis between total phenolic (GAE) and antioxidant (TEAC) contents in different tissues of three *Coleus* species. a: Leaf tissue (R^2 = 0.23). b: Stem tissue (R^2 = 0.95). Values are average of three independent experiments ± SE.

and *C. aromaticus* (11.32 mM g^{-1} fw) (Figure 4b).

The regression coefficient between TEAC and total phenolic contents of stem tissues of three *Coleus* species was R^2=0.95 (Figure 5b), whereas the regression in leaf tissues was R^2 = 0.23 (Figure 5a). These regression values showed that the antioxidant activity of *Coleus* plant extracts was not due to the result of total polyphenols but may be related to the presence of some individual active phenolic compounds. The unclear relationship between the antioxidant activity and the total phenolics indicate that the total phenolics content does not incurporate all the antioxidants. It is known that the synergism between the antioxidants in the mixture makes the antioxidant activity not only dependant on the concentration but also might be due to the structure and the interaction among the different antioxidants (VanderJagt et al., 2002)

Egg yolk lipids undergo rapid non-enzymatic peroxidetion when incubated in the presence of ferrous sulphate. Lipid peroxides are likely involved in numerous pathological events, including inflammation, metabolic disorders and cellular aging (Ames et al., 1993; Wiseman et al., 1996). The percentage inhibition of non-enzymatic peroxidation of the three species of *Coleus* stem extracts are shown in Figure 6b *C. forskholii* stem extracts showed slightly high percentage of inhibited lipid peroxidation (54.43%) than *C. aromaticus* (48.65%) and *C. zeylanicus* (47.83%). Also, the leaf tissues of *C.forskholii* showed higher percentage of lipid peroxidation (62.06%) than that of *C. aromaticus* (49.27%) and *C. zeylanicus* (47.62%) (Figure 6a) Comparatively high percentage inhibition of lipid peroxidation in *C. forskholii* leaves suggest they may afford better cytoprotective effect.

Qualitative analysis of three *Coleus* species extracts were carried out using reverse phase HPLC and the chromatographic profiles were compared with the retention times of reference standards. The UV- spectra of the eluted compounds revealed that the most abundant phenolics in methanol extracts of *Coleus* tissues were hydroxylcinnamic acid derivatives. The chromatographic profile of *C. forskholii* leaf extracts showed the peaks corresponding to standard antioxidative polyphenols like chlorogenic acid, caffeic acid, coumaric acid, quercetin and ferulic acid. The leaf extracts of *C. aromaticus* showed chlorogenic acid, caffeic acid and coumaric acid and *C. zeylanicus* showed peaks corresponding to caffeic acid and coumaric acid (Figure 7). Chlorogenic acid is an extremely wide spread plant metabolite which appears to

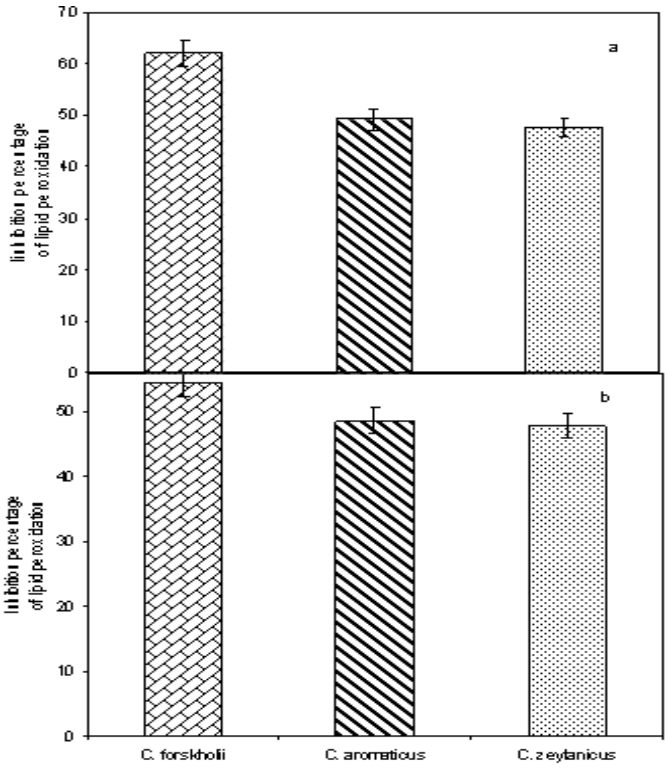

Figure 6. Lipid peroxidation inhibition assays using different tissue extracts of three *Coleus* species. a: Leaf tissues. B: Stem tissues. Values are average of three independent experiments ± SE.

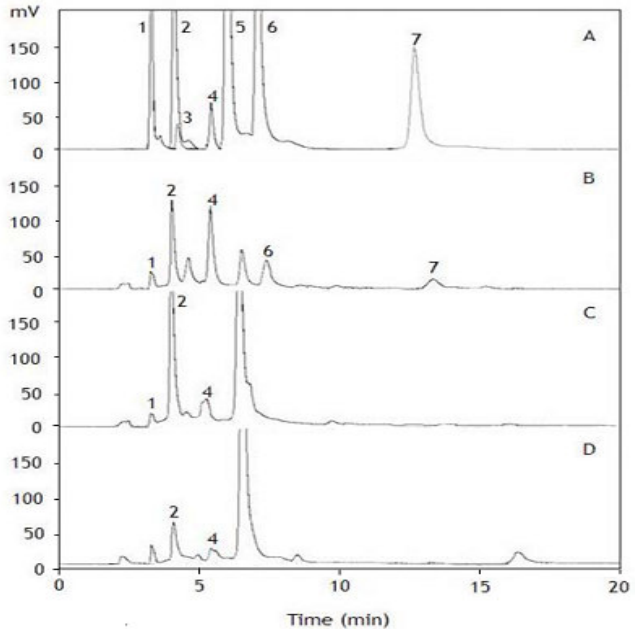

Figure 7. HPLC - separation of phenolic standards (A) and HPLC profiles in leaf tissue of the three *Coleus* species analysed: *Coleus forskholii* (B), *Coleus zeylanicus* (C), *Coleus aromaticus* (D). Peaks: 1- Chlorogenic acid; 2- Caffeic acid; 3- Syringic acid; 4- Coumaric acid; 5- Ferulic acid; 6- Myrecitin; 7- Quercetin.

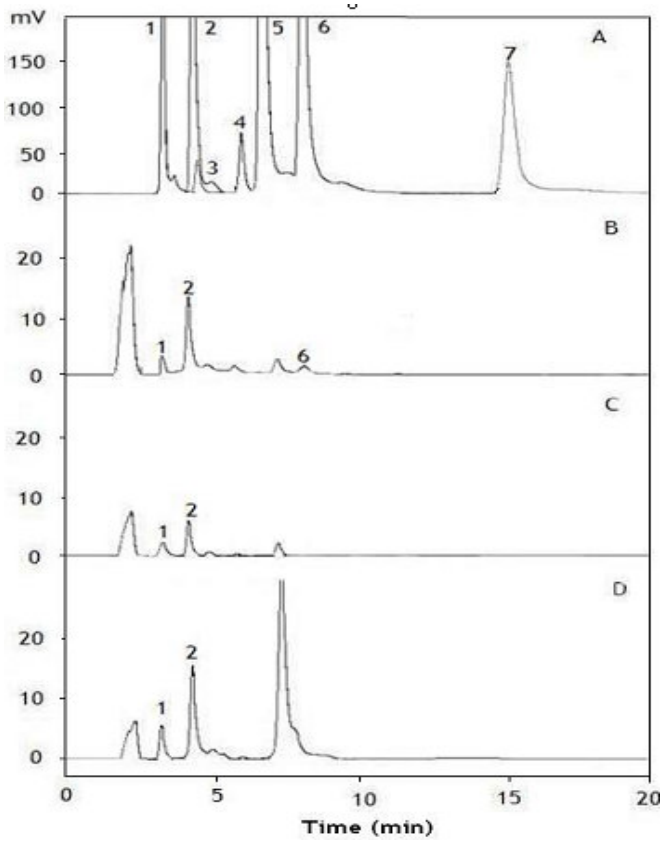

Figure 8. HPLC separation of phenolic standards (A) and HPLC profiles in the stem tissue of the three *Coleus* species; *Coleus forskholii* (B), *Coleus zeylanicus* (C), *Coleus aromaticus* (D). Peaks: 1- Chlorogenic acid; 2- Caffeic acid; 3- Syringic acid; 4- coumaric acid; 5- Ferulic acid; 6- Myrecitin; 7- Quercetin.

provide protection against certain form of oxidative stress (Grace and Logan, 2000). Recently quercetin rich-onions consumption showed increased resistance of lymphocytic DNA to *ex-vivo* induced oxidation (Scalbert et al., 2005). There are quantitative differences in the levels of phenolic compounds between leaf and stem samples, but chromatographic profile of stem tissues showed the presence of antioxidative polyphenols like chlorogenic acid, caffeic acid and myrecitin (Figure 8). The quanti-tative differrences of polyphenols in methanolic extracts of leaf and stem tissue may be due to presence of polyphe-nols at epicuticular levels In leaf but in stem tissues they are bound to lignified cells and can only be extracted through alkaline hydrolysis (Witzell et al., 2003).

The antioxidant capacity of phenolic compounds in different tissues of three species of *Coleus* showed higher polyphenolics content and antioxidant activity in all three species demonstrating that the *Coleus* species are the potent source of novel bioactive compounds with wide range of medicinal properties in particular the high free radical scavenging activity. Among the three *Coleus*

species, higher contents of polyphenols, flavones and flavonols and antioxidant properties in *C. forskholii* can be attributed to its wide medicinal properties like adrenergic, antiaggregant, anticancer, antidepressant, antiglaucomic, hypotensive and bronchodilator. In addition, the diterpene 'Forskolin' is also known for its cardiotonic and adenylate cyclase activation properties. Low amounts of tannins in *C. forskholii* can be attributed to its high antioxidative property with fewer side effects. Our data in the present study clearly demonstrates that among the three species of *Coleus, C. forskholii* is superior species which can be used as potent medicinal herb for novel bioactive compounds with high free radical scavenging activity.

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