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In vitro Antioxidant Activity and Free Radical Scavenging Effect of Roots of *Coleus forskohlii*

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Abstract—*Antioxidant potential of tuberous roots of Coleus forskohlii was studied by using 1,1-diphenyl-2-picryl hydrazyl (DPPH), nitric oxide, superoxide, hydroxyl radical scavenging activity of the methanolic extract of the tuberous roots. All the radical scavenging activities increased with increasing concentration of the plant extract. The extract showed a maximum scavenging effect of (49%) superoxide radical followed by inhibition of DPPH (34%), hydroxyl radical scavenging (44%) and scavenging of nitric oxide (47%) with the concentration ranging from 10 to 50 mg/ml. These findings indicated the antioxidant activity of the methanolic extract of tuberous roots of Coleus forskohlii.*

Keywords: *Coleus forskohlii, free radical scavenging, antioxidants*

INTRODUCTION

The role of free radicals and active oxygen in the pathogenesis of human diseases including cancer, aging and atherosclerosis has been recognized. Electron acceptors, such as molecular oxygen, react rapidly with free radicals to become radicals themselves, also referred to as reactive oxygen species (ROS). The ROS include superoxide anions ($O_2^{\cdot}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($^{\cdot}OH$) (Mathew and Abraham, 2006).

Considerable attention has recently been focused on the interrelationship of free radical related reactions and the development of a variety of pathological events. It is well established that lipid peroxidation is the deleterious result of free radical reaction, leading to disruption of biomembranes and dysfunction of cells and tissues (Cho *et al.*, 2003).

Cells have developed antioxidant mechanisms to quench the free radicals but when the generation of free radicals exceeds the scavenging capacity of the cell, the excess free radicals seek stability through electron pairing with biological macromolecules such as proteins, lipids and DNA in healthy human cells resulting in the induction of lipid peroxidation which leads to cancer, atherosclerosis, cardiovascular diseases, ageing and inflammatory diseases (Hazra *et al.*, 2008). Antioxidants offer resistance against the oxidative stress by scavenging the free radicals, inhibiting the lipid peroxidation and by other mechanisms and thus prevent disease. The free radicals are known to be scavenged by synthetic antioxidants, but due to their adverse side effects leading to carcinogenicity; the search for effective and natural antioxidants has become crucial (Adeolu *et al.*,2009).

There has been a global trend towards focusing on the area of natural phytochemicals present in herbs and functional foods as antioxidants. Since ancient times, humans have derived many benefits from natural plants and compounds. It has been recognized that traditional medicines and the polyphenols isolated from plants have potential therapeutic roles in the prevention and the treatment

of many human diseases related to excessive oxidative stress (Harnafi and Amrani, 2008).

The first step in the discovery of new natural product drugs from plant is the selection of the plant species to be investigated (Adou *et al.*, 2010). Plants belonging to the family Lamiaceae such as Rosemary, Sage, Thyme and Coleus are being extensively used in traditional medicinal practices in India. The genus Coleus has 300 species of which *C.amboinicus, C.forskohlii, C.spicatus* and *C.aromaticus* are naturally occurring species (Mohammed *et al., 2006*). Many *coleus* species such as *C.aromaticus, C.barbatus, C.blumei and C.sctellaroides, C.parvifolius* were reported for their constituents and biological activities viz., cytotoxicity, antimicrobial and antioxidant activities (Yuenyongsawad, 2005).

The tuberous roots of the plant *Coleus forskohlii* produces the labdane diterpenoid, forskolin. It is distributed over the subtropical warm temperature climatic zone on the mountains of India, Nepal, Myanmer, Srilanka, Thailand, and Africa. In India it is reported to be distributed in the dry hills of Western Uttar Pradesh, Gujarat, parts of Orissa, Western ghats and in kitchen gardens in Northern Karnataka (Belgaum and Dharward districts) for its carrots like tubers, which are used as condiment in the preparation of pickles (Reddy *et al.*, 2005) *Coleus forskohlii* is reported to be a potent medicinal herb for novel bioactive compounds with high free radical scavenging activity (Rasineni *et al.*, 2008).

Several methods are used to measure the antioxidant activity of a biological material. The most commonly involving chromogen compounds of radical nature that stimulate the reductive oxygen species. These methods are popular due to their ease, speed and sensitivity. The presence of antioxidants leads to the disappearance of these radical chromogens (Ali *et al.*, 2008). The present study has been undertaken to investigate the antioxidant activity of the tuberous roots of *Coleus forskohlii* using different *in vitro* models.

Materials and Methods

All chemicals and solvents were of analytical grade and were obtained from Himedia chemicals, Mumbai, India.

Sample Preparation

The plant material *Coleus forskohlii* was collected from Tamil Nadu Agricultural University, Coimbatore and was duly authenticated by Dr.G.V.S.Murthy, Joint Director, Botanical Survey of India, Southern Regional Centre, Tamil Nadu Agricultural University, Coimbatore. The roots of the plants were chopped into pieces and dried under shade for a week. The shade dried roots were coarsely powdered and extracted with 70% methanol using soxhlet apparatus. The extract was concentrated to near dryness in a rotary evaporator under reduced pressure and controlled temperature (40-50°C). The crude extracts yielded a dark brown solid, weighing 40g. The extracts were preserved in a refrigerator at 4°C for further use.

DPPH Radical Scavenging Activity

The DPPH (1, 1-diphenyl 2-dipicrylhydrazyl) radical scavenging activity was measured by the spectrophotometric method according to (Sreejayan *et al.*, 1996). To a methanolic solution of DPPH (200μM), 0.05 ml of the test compounds dissolved in methanol were added at different concentration. An equal amount of methanol was added to the control. After 20 min, the decrease in the absorbance of test mixture (due to quenching of DPPH free radicals) was read at 517 nm and the percentage inhibition calculated by using the standard formula (Prasanthkumar *et al.*, 2006).

$$\text{Inhibition \%} = \frac{(\text{Control-test})}{\text{Control}} \times 100$$

Hydroxyl Radical Scavenging Activity

Hydroxyl radical scavenging activity was measured according to the method of Kunchandy and Rao. (1990) by studying the competition between deoxyribose and test extract for hydroxyl radical generated by Fentons's reaction. The reaction mixture contained deoxyribose (2.8mM), Fecl3(0.1mM), EDTA (0.1mM), H_2O_2 (1mM), Ascorbate (0.1mM), KH_2PO_4- KOH buffer(20mM,pH 7.4) and various concentrations of the sample extracts in a final volume of 1.0 ml. The reaction mixture was incubated for 1 hour at 37°C. Deoxyribose degradation was measured as thiobarbituric acid reacting substances (TBARS) and the percentage inhibition was calculated.

Superoxide Radical Scavenging Activity

The Superoxide radical scavenging activity was measured in terms of inhibition of generation of O_2 (Sanchez,2002). The reaction mixture consisted of phosphate buffer (50mM, pH 7.6), riboflavin (20µg/0.2 ml), EDTA (12mM), NBT (0.1mg/3ml//0 and sodium cyanide (3 µg/0.2ml). Test compounds of various concentrations (10 - 50 mg/ml were added to a total volume of 3 ml. The absorbance was read at 530 nm before and after illumination under UV lamp for 15 min against a control instead of sample. The percentage inhibition was calculated by using the same formula as given above.

Nitric Oxide Radical Scavenging Activity

Nitric oxide was generated from sodium nitroprusside and measured by Griess' reactions. Sodium nitroprusside (5mM) in standard phosphate buffer solution was incubated with different concentration (10 -50 mg/ml of the methanolic extract dissolved in phosphate buffer (0.025 M, pH 7.4) and the tubes were incubated at 25°C for 5 hours. Control without extracts, but with equivalent amounts of buffer were prepared in an identical manner. After 5 hr 0.5 ml of incubation solution was removed and diluted with 0.5ml of Griess' reagent (1% sulphanilamide, 2% O-Phosphoric acid and 0.1% napthylethylene diamine and read at 546 nm.

All the experiments were repeated with sufficient replicas and mean of the data is presented.

Statistical Analysis

Linear regression analysis was used to calculate the IC_{50} values.

Results and Discussion

DPPH radical is scavenged by antioxidants through the donation of proton forming the reduced DPPH. The color changes from purple to yellow after reduction which can be quantified by the decrease in absorbance at wavelength of 517nm. DPPH Radical scavenging activity increased with increasing concentration of the plant extract with IC_{50} value of 34 percent. As given in the Table 1, the hydroxyl radical scavenging or inhibiting activity of extract of *Coleus forskohlii* shows percent inhibition from 5 to 60 corresponding to 10 to 50 mg/ml with the IC_{50} value of 34 percent

Table 1: DPPH and Hydroxyl Radical Scavenging Activity of Root Mehanolic Extract of *Coleus Forskohlii*.

Concentration in mg/ml	Percent inhibition	
	DPPH radical	Hydroxy radical
10	27	5
20	39	15
30	43	12
40	59	44
50	65	60
IC_{50}	34	44

associated with several diseases. Oxygen reacts with the nitrite anions, which act as free radicals (Baskar *et al.*, 2005). In the present study the methanolic extract of *Coleus forskohlii* root showed better activity in competing with oxygen to react with nitric oxide and thus inhibit the generation of anions.

CONCLUSION

The results of the present study indicates a strong *in vitro* antioxidant activity of the methanolic extract of roots of *Coleus forskohlii*. It may be concluded that the methanolic extract of tuberous roots of *Coleus forskohlii* can protect cells from oxidative damage and toxic effects of ROS and has potential role in control of several diseases.

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