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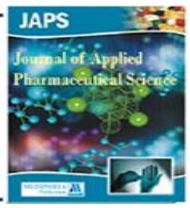

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In-Vitro Antioxidant potentials in leaves of *Coleus aromaticus* Benth and rhizomes of *Zingiber zerumbet* (L.) SM.

Om Prakash Rout, Rabinarayan Acharya and Sagar Kumar Mishra

Om Prakash Rout
Dept of Dravyaguna,
Rajiv Lochan Ayurvedic Medical College, Chandkhuri, Durg, Chhatisgarh.

Rabinarayan Acharya
Dept. of Dravyaguna,
I.P.G.T & R.A.,
Gujurat Ayurved University, Jamnagar, Gujurat, India.

Sagar Kumar Mishra
Pharmacognosy & Phytochemistry Division, University Dept. of Pharmaceutical Sciences, Utkal University, Vani Vihar, Bhubaneswar, Odisha, India.

For Correspondence
Dr. Om Prakash Rout
Lecturer
Dept of Dravyaguna,
Rajiv Lochan Ayurvedic Medical College & Hospital,
Chandkhuri, Gundardehi Road, Durg, Chhatisgarh.
Phone 91-9437283110

ABSTRACT

Antioxidant activity of aqueous extracts of two common plants, Parnayavani- leaves of *Coleus aromaticus* Benth and Sthulagranthi- rhizomes of *Zingiber zerumbet* (L.) Sm. was investigated by adopting various *in vitro* models such as DPPH assay and Nitric oxide radical scavenging assay. The results of the study show that both plants possesses significant free radical scavenging properties and a clear correlation exists between the antioxidant activity.

Keywords: Antioxidant activity, *Coleus aromaticus, Zingiber zerumbet,* DPPH radical scavenging assay, Nitric oxide radical scavenging assay

INTRODUCTION

Recently, there has been a surge in research on the potential role of antioxidants in the treatment of atherosclerosis, heart failure, liver dysfunction, neurodegenerative disorders, cancer, and diabetes mellitus (Ajitha et al, 2001). The main characteristic of an antioxidant is its ability to trap free radicals. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases (Kumaraswamy et al, 2008). Oxygen is essential for survival however, its univalent reduction generates several harmful reactive oxygen species (ROS), inevitable to living cells and highly associated with the wide range of pathogenesis such as diabetes, liver damage, inflammation, aging, neurological disorders and cancer. In spite of comprehensive network of cellular defensive antioxidants, many ROS still escape this surveillance inflicting serious anomalies favouring such diseases states (Esterbauer et al, 1996; Halliwell et al, 1993; Sies, 1997). Though synthetic antioxidants, BHT, BHA and radioprotector, Warfarin are being used widely, however, due to their potential health hazards, they are under strict regulation (Satio et al, 2003; Rades et al, 2004). Antioxidant principles from natural resources are multifaceted in their multitude/magnitude of activities and provide enormous scope in correcting the imbalance through regular intake of proper diet. Therefore, in the recent years, the interest is centered on antioxidants derived from herbal medicine in view of their medicinal benefits (Kamat , 2007; Kamat et al, 2004; Umadevi et al, 1995; Winston, 1999; Arora et al, 2003). In view of this, we selected Parnayavani- leaves of *Coleus aromaticus* Benth and Sthulagranthi- rhizomes of *Zingiber zerumbet* (L.) Sm. to assess the *in vitro* antioxidant activity.

MATERIALS AND METHODS

Plant materials and Chemicals

C. aromaticus leaves and *Z. zerumbet* rhizomes were collected from the field of Silviculture office, Ghatikia, Bhubaneswar, Odisha, identified and authenticated by Mr. Rashmi Ranjan Pani, HOD, Deptt. of Botany, Mangala Mahavidyalaya, Kakatpur, Puri, Odisha (Bot./Auth./03-08/C,A). The voucher specimen was preserved at UDPS, Utkal University for future references. The plants collected were shade dried and finely powdered. The powdered plant material was subjected to aqueous extraction with chloroform: water (1:1000) by maceration. The extract evaporated under vacuum gave a dry extract and was stored in dry desiccators.

All chemicals and solvents were of analytical grade and were obtained from Ranbaxy Fine Chemicals, Mumbai. 1,1-diphenyl,2-picryl hydrazyl (DPPH) and ascorbic acid were procured from Sigma Aldrich Co., St. Louis, USA and sodium nitroprusside (SNP), Griess reagent (1% sulphanilamide, 2% o-phosphoric acid and 0.1% napthylethylene dianmine dihydrochloride) and Rutin from Sisco Research Laboratories Pvt. Ltd., India. UV spectrophotometer (Shimadzu 1650), pH meter (Elico Ltd., India) were the instruments used during the study.

Evaluation of *in vitro* antioxidant activity

DPPH radical scavenging assay

DPPH scavenging activity was measured by the spectrophotometric method. To a methanolic solution of DPPH (200 µM), 0.05 ml of the test compound dissolved in water were added at different concentrations (20, 40, 100, 125 and 250 µg/ml). An equal amount of water 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 Ascorbic acid was taken as the standard. The percentage inhibition was calculated by using the formula:

% inhibition = Control - Test/Control × 100. The absorbance of the control was 0.720.

Nitric oxide radical scavenging assay

Nitric oxide was generated from sodium nitroprusside and measured by Griess reaction[5]. Sodium nitroprusside (5 mM) in standard phosphate buffer solution was incubated with different concentrations (20, 40, 100, 125 and 250 µg/ml) of the aqueous extract dissolved in phosphate buffer (0.025 M, pH 7.4) and the tubes were incubated at 25^0C for 5 hr. Control experiments without the test compounds, but with equivalent amounts of buffer were conducted in an identical manner. After 5 hr, 0.5 ml of incubation solution was removed and diluted with 0.5 ml of Griess reagent (1% sulphanilamide, 2% o-phosphoric acid and 0.1% napthylethylene diamine dihydrochloride). The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with napthylethylene diamine was read at 546 nm. Rutin was taken as the standard. The percentage inhibition was calculated by using the same formula as has been given for DPPH radical scavenging assay. The absorbance of the control was 0.752.

RESULTS AND DISCUSSIONS

DPPH radical scavenging assay

The DPPH radical scavenging assay is an easy, rapid and sensitive method for the antioxidant screening of plant extracts**.** A number of methods are available for the determination of free radical scavenging activity but the assay employing the stable 2, 2-diphenyl-1-picryl-hydrazyl radical (DPPH) has received the maximum attention owing to its ease of use and its convenience. The results are expressed as percentage (%) of inhibition exhibited by the test substances and the standard drug. (Table 1-3 and Figure 1-4). IC_{50} value was calculated in each cases.

Table1. DPPH scavenging activity of the aqueous leaf extract of C. *aromaticus.*

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.590	18.05
40	0.398	45.97
100	0.343	52.36
125	0.218	69.72
250	0.192	73.33
IC_{50}	97.78 µg/ml	

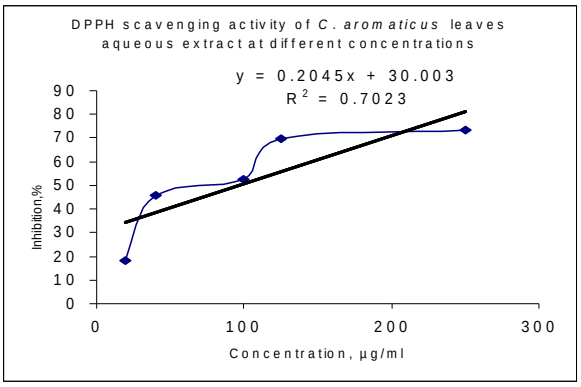

Fig 1. DPPH scavenging activity of the aqueous leaf extract of *C. aromaticus* at different concentrations.

Table 2. DPPH scavenging activity of the aqueous rhizome extract of *Z. zerumbet*.

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.602	16.38
40	0.412	42.77
100	0.368	48.88
125	0.256	64.44
250	0.214	70.27
IC_{50}	114.25 µg/ml	

Table 3. DPPH scavenging activity of Ascorbic acid.

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.471	34.58
40	0.364	49.44
100	0.241	66.52
125	0.157	78.19
250	0.128	82.22
IC_{50}	44.15 µg/ml	

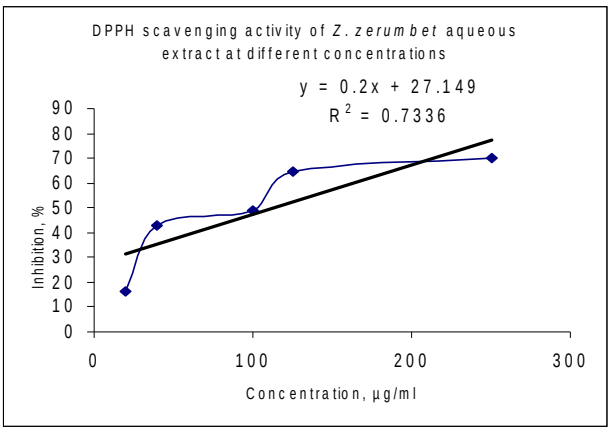

Fig 2. DPPH scavenging activity of the aqueous rhizome extract of *Z. zerumbet* at different concentrations.

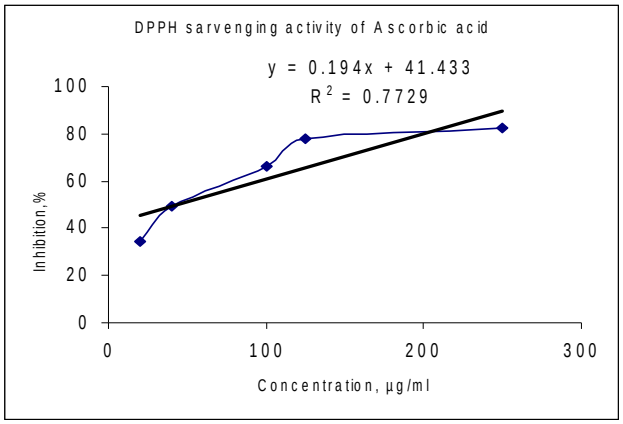

Fig 3. DPPH scavenging activity of Ascorbic acid

The aqueous extracts of *C. aromaticus* leaves and *Z. zerumbet* rhizomes exhibited DPPH scavenging activity in the tested concentrations. It was observed that, the percentages inhibition was increased with the increase in concentration of the extracts. IC_{50} value for scavenging of DPPH by the aqueous extracts of *C. aromaticus* and *Z. zerumbet* was found to be 97.78 and 114.25 µg/ml, respectively, while for Ascorbic acid it was 44.15 µg/ ml.

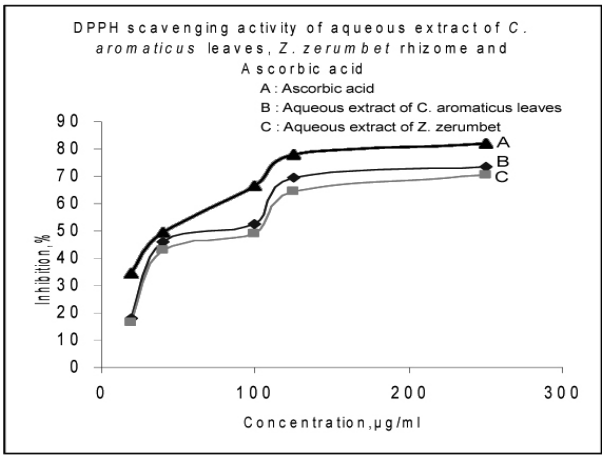

Fig 4. DPPH scavenging activity of the aqueous extracts of *C. aromaticus* leaves and *Z. zerumbet* rhizomes with the standard, Ascorbic acid.

The 1, 1-diphenyl -2-picryl hydrazyl (DPPH) radical was widely used as the model system to investigate the scavenging activities of several natural compounds such as phenolic and anthocyanins or crude mixture such as aqueous extract of plants. DPPH radical is scavenged by antioxidants through the donation of proton forming the reduced DPPH. The colour changes from purple to yellow after reduction at wavelength 517 nm. Radical scavenging activity increased with increasing percentage of the free radical inhibition.

Antioxidant activity will aid in the interpretation of clinical results of various products which are tested in biological models for chronic diseases. It is reasonable to expect that high antioxidant activity have greater potential to reduce free radicals in the body. Thus it is important to know the antioxidant potential of the medicinally important plant species.

Nitric oxide radical scavenging assay

Nitric oxide is a diffusible free radical which plays many roles as an effector molecule in diverse biological systems including neuronal messenger, vasodilation and antimicrobial antitumor activities. NO generated from sodium nitroprusside in aqueous solution at physiological pH reacts with oxygen to form nitrite ion. Aqueous extract of both the plants inhibited nitrite formation in concentration dependent manner. This may be due to the presence of antioxidant principles in the extract, which complete with oxygen to react with nitric oxide. The results are expressed as percentage (%) inhibition exhibited by the test substances and the standard drug (Table 4-6 and Figure 5-8). IC_{50} value was calculated in each case.

Table 4. Nitric oxide scavenging activity of the aqueous leaf extract of C. *aromaticus.*

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.552	26.59
40	0.460	38.82
100	0.403	46.40
125	0.349	53.59
250	0.289	61.56
IC_{50}	140.48 µg/ml	

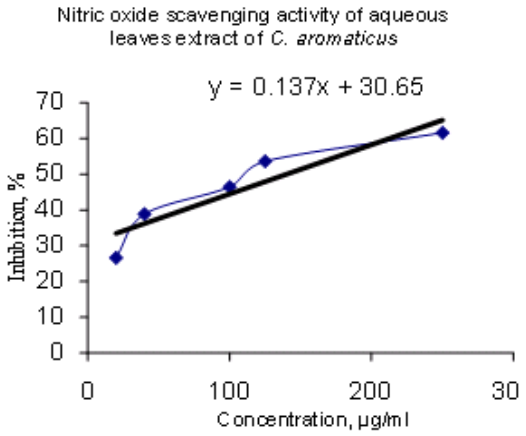

Fig 5. Nitric oxide scavenging activity of the aqueous leaf extract of C. *aromaticus.*

Table 5. Nitric oxide scavenging activity of the aqueous rhizome extract of *Z. zerumbet.*

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.543	27.79
40	0.486	35.37
100	0.336	55.31
125	0.281	62.63
250	0.266	64.62
IC_{50}	112.45 µg/ml	

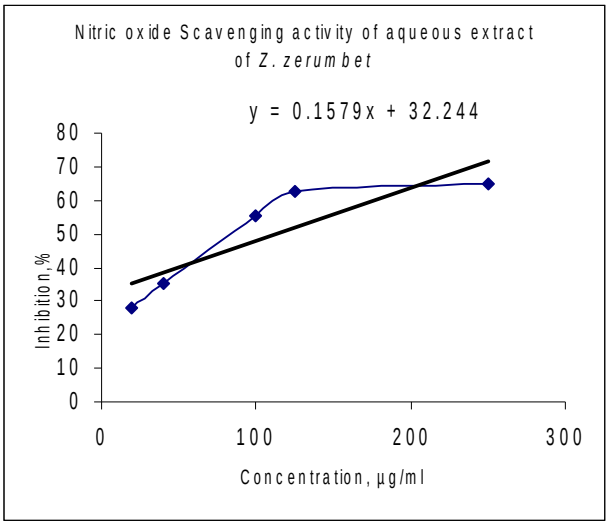

Fig 6. Nitric oxide scavenging activity of the aqueous rhizome extract of *Z. zerumbet* at different concentrations.

Table 6. Nitric oxide scavenging activity of Rutin

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.519	30.98
40	0.413	45.07
100	0.343	54.38
125	0.228	69.68
250	0.171	77.26
IC_{50}	77.99 µg/ml	

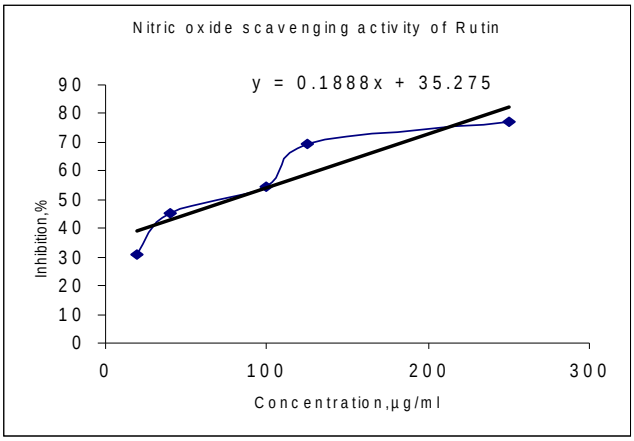

Fig 7. Nitric oxide scavenging activity of Rutin

The aqueous extracts of *C. aromaticus* leaves and *Z. zerumbet* rhizomes exhibited Nitric oxide scavenging activity in the tested concentrations. It was observed that, the percentages inhibition was increased with the increase in concentration of the extracts. IC_{50} value for scavenging of Nitric oxide by the aqueous extracts of *C. aromaticus* and *Z. zerumbet* was found to be 140.48 and 112.45 µg/ml, respectively, while for Rutin it was 77.99 µg/ml.

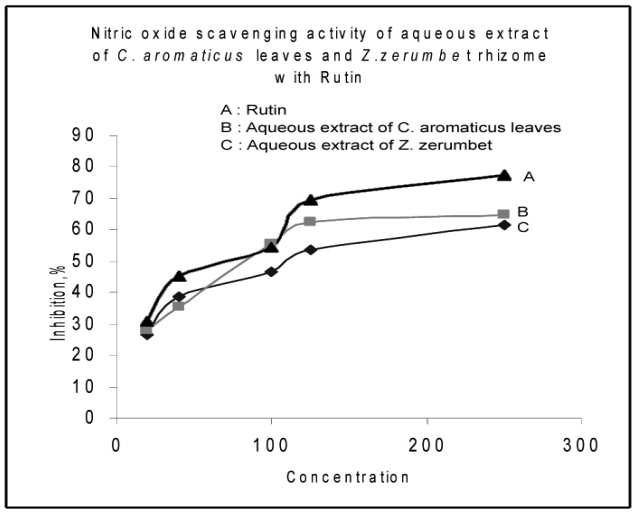

Fig 8. Nitric oxide scavenging activity of the aqueous extracts of *C. aromaticus* leaves and Z. zerumbet rhizomes with the standard, Rutin.

Nitric oxide is a free radical generated by endothelial cells, macrophage, neurons etc., and involved in the regulation of various physiological process. Excess concentration of NO is associated with several diseases. Oxygen reacts with the excess nitric oxide to generate nitrite and peroxy nitrite anions, which act as free radicals.

CONCLUSIONS

The aqueous extracts of *C. aromaticus* leaves and *Z. zerumbet* rhizome exhibited antioxidant activity. Since reactive oxygen species are important contributors to several serious ailments, the observed Nitric oxide scavenging activity of the extracts might be helpful for its consideration for treatment of different chronic diseases.

The antioxidant activity provides an indication of the therapeutic importance of each of the extracts. Antioxidant activity will aid in the interpretation of clinical results of various products which are tested in biological models for chronic diseases. It is reasonable to expect that high antioxidant activity have greater potential to reduce the free radicals in the body. Thus it is important to find out the antioxidant potential of plant species. Recently, there has been a surge in research on the potential role of antioxidants in the treatment of atherosclerosis, heart failure, liver dysfunction, neurodegenerative disorders, cancer, and diabetes mellitus. The main characteristic of an antioxidant is its ability to trap free radicals. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. Free radicals which have one or more unpaired electrons are produced during normal and pathological cell metabolism. Reactive oxygen species (ROS) react easily with free radicals to become radicals themselves. ROS are various forms of activated oxygen, which

include free radicals such as superoxide anion radicals (O2.-) and hydroxyl radicals (OH.), as well as (O2). Antioxidants provide protection to living organisms from damage caused by uncontrolled production of ROS and concomitant lipid peroxidation, protein damage and DNA stand breaking. Ayurvedic literature contains a large number of plants that can be used against diseases, in which reactive oxygen species and free radicals plays important role.

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