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IN VITRO ANTIOXIDANT ACTIVITY OF THE METHANOLIC ROOT, STEM AND LEAF EXTRACTS OF *Ophiorrhiza mungo* L.

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ABSTRACT

In vitro antioxidant activity of methanolic root, stem and leaf extract of *Ophiorrhiza mungo* (Rubiaceae) were evaluated. The root extract of *O. mungo* had shown good DPPH radical scavenging activity when compared to stem and leaf extracts, the ascorbic acid was used as standard antioxidant and positive control. The DPPH radical scavenging activity of extract increased with the increasing concentration. The reducing power of extracts was carried out with ascorbic acid as a standard reducing agent. The root extracts showed higher reducing power than stem and leaf extracts. The total antioxidant activity of the extracts was evaluated using the phosphomolybdate method which was expressed as ascorbic acid equivalent. The present study revealed that the high antioxidant activity of root may be due to the presence of various phytochemicals and other active compounds which need to be investigated further.

Keywords: DPPH, Phosphomolybdate, Free Radicals

INTRODUCTION

Antioxidants are radical scavengers which protect the human body against free radicals **[1]**. Free radicals are continuously produced by the body's normal use of oxygen such as respiration and some cell mediated immune functions. The cause of the majority of disease conditions like arthrosclerosis, hypertension, Alzheimer's disease, Parkinson disease, cancer, diabetes mellitus and inflammatory conditions are primarily due to the imbalance between the free radical generated in the body and the antioxidants scavenge them **[2]**. Antioxidant principles from natural

resources provide enormous scope in correcting the imbalance.

Antioxidants are considered as possible protecting agents reducing oxidative damage of human body from free radicals and retard the progress of many chronic diseases **[3]**. Therefore, there is a growing interest in the substances exhibiting antioxidant properties. Since the most commonly used synthetic antioxidants such as butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT) and Tert. Butylhydroquinone are suspected of being responsible for liver damage and carcinogenesis **[4]** and has restricted its use, has caused an increased interest towards natural antioxidant substance **[5]**. Therefore, the importance of searching for and exploiting natural antioxidants especially of plant orgin has increased greatly in recent years.

Ophiorrhiza is a genus of flowering plants in the family Rubiaceae, the species of the genus *O. prostrata* and *O. pumila* has been reported for the presence of a cytotoxic quinoline alkaloid camptothecin (CPT) an anti cancerous compound in its roots, recently *O. mungo* has been reported for the CPT from its root at 0.04 % g dry weight **[6]**. *Ophiorrhiza mungo* is a suffrutescent herb, 45-60 cm high distributed in Khashi hills and Western Ghats of India. Leaves and stems contain traces of hydrocyanic acid. Roots contain small amount of a bitter amorphous alkaloid *β stiosterol*, 5 α-ergot - 7- en 3 β-ol and 5 α - ergot -8-en-3 β-ol. Traditionally roots are used against snake bites, and the root bark have sedative and laxative properties **[7, 8]**.

The main objectives of the present study were to study the antioxidant potential of methanolic extracts of root, stem and leaf extracts of *O. mungo* in different *in vitro* antioxidant assay including 1,1-diphenyl-2-picryl hydrazyl free radical scavenging, total antioxidant activity, reducing power etc.

MATERIALS AND METHODS

Chemicals

All chemicals used were of analytical grade. 1,1-Diphenyl-2-picryl hydrazyl (DPPH) was obtained from Sigma Chemicals, USA. Ascorbic acid, Folin-Ciocalteu reagent, Sodium carbonate, aluminium chloride, potassium acetate, potassium ferricyanide, trichloroacetic acid (TCA), ferric chloride and all solvents were obtained from SISCO Research Laboratories Pvt, Ltd. Mumbai, India.

Plant materials

Samples of *O.mungo* were collected from Ponmudi in the Western Ghats forests of Kerala, India and were maintained in the greenhouse of the Department of Botany, University of Kerala, Karyavattom. The plant material was dried under shade and then powdered in a mechanical grinder. The powdered material was extracted with methanol using Soxhlet apparatus. The

methanol extract was concentrated in a rotary evaporator (Super fit Rotavap, India) and stored in the screw cap vials.

Total Phenolic content

The total phenolic content was determined using Folin-Ciocalteau reagent as previously described **[9]**. To the 0.5 ml of extract solutions in acetone, 2.5 ml of Folin-Ciocalteus reagent was added and the contents mixed thoroughly. After 4 minutes, 2 ml of 7.5 % sodium carbonate was added and then the mixture was incubated at 45^0C for 15 minutes. The absorbance was measured at 765 nm. The total phenolic contents were expressed as chlorogenic acid equivalent in milligram per gram of the extract using a standard curve generated with chlorogenic acid.

Total Flavanoid Content

The total flavanoid contents in the root, stem and leaf extracts was determined by using the aluminium chloride colorimetric method as previously described **[10]**. 0.2 ml of extract solution in a test tube was mixed with 0.1 ml of 10 % aluminium chloride, 0.1 ml of 1 M potassium acetate and 4.6 ml of distilled water. The absorbance was measured at 415 nm after incubation at room temperature for 45 minutes. The total flavanoid contents were expressed as qucertin equivalent in µg/mg of extract using a standard curve generated with qucertin.

Reducing Power

Reductive potential of the extracts was carried out by the method as described earlier **[11]**. Different concentrations of the methanolic root, stem and leaf extracts of *O. mungo* (20 µg – 100 µg /ml) were prepared, 1ml of extracts was mixed with 0.2 M phosphate buffer (2.5 ml, pH 6.6) and 1 % potassium ferricyanide (2.5 ml). The mixture was incubated at 50^0C for 20 minutes and 10% TCA (2.5 ml) was added to the mixture and centrifugation was carried out at 3000 rpm for 10 minutes. To 2.5 ml of the supernatant 2.5 ml of distilled water was added. To this 0.5 ml of ferric chloride (0.1%) was added and the absorbance was measured at 700 nm in a spectrophotometer (Shimadzu UV-1700, Japan). Higher absorbance of the reaction mixture indicates higher reductive potential. Ascorbic acid was used as standard. The entire test was performed in triplicates.

DPPH Radical Scavenging Activity

The ability of the methanolic root, stem and leaf extracts to scavenge DPPH radical was determined according to method described earlier **[12]** with some modification. Different concentrations of the extracts (10 – 100 µg / ml) were prepared in 100 % methanol, 2 ml of DPPH (100 µM) methanol solution was added to 1 ml solution of the extract or standard and allowed to react at room temperature for 30 minutes and the absorbance was measured

at 517 nm using a spectrophotometer. The percentage scavenging activity of different concentrations was determined and IC 50 value of extracts was compared with that of ascorbic acid which was used as standard.

Total Antioxidant Activity

Total antioxidant capacity was measured by phosphomolybdate using ascorbic acid as the standard **[13]**. An aliquot of 0.1 ml of the extract (20 µg - 100 µg / ml) was combined with 1 ml of reagent (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated on a boiling water bath at 95^0C for 90 min. After the sample had cooled to room temperature, the absorbance was measured at 695 nm. The total antioxidant was expressed as µg equivalents of ascorbic acid by using the standard ascorbic acid graph.

RESULTS

DPPH Assay

The root and leaf extract showed H-donor activity. The highest DPPH radical scavenging activity was detected in the root extract (IC_{50} = 0.023 mg/ml) followed by leaf extract (IC_{50} = 0.068 mg /ml) but the stem extract showed only 33.33% inhibition in 100 µg / ml. Thus IC 50 value could not be determined for stem extract **(Table 1, Figure 1)**. The activity was compared to that of ascorbic acid.

Reducing Power

Table 2 shows the reductive capabilities of root, stem and leaf extracts of *O. mungo* when compared to the standard ascorbic acid. The reducing power increased with increasing concentration of sample extracts. The root extracts showed highest reducing ability than the leaf and stem extracts. However, the activity was less than the standard ascorbic acid.

Total Antioxidant Capacity

Total antioxidant capacity of the root, stem and leaf extracts of *O. mungo*, were expressed as the number of gram equivalent of ascorbic acid, which is 55 µg, 51 µg, 51 µg ascorbic acid equivalent / 100 µg respectively **(Table 3)**.

Total Phenolic and Flavonoid content

Total phenolic content was estimated by using Folin-ciocalteu reagent. Total phenolic content of the root, stem and leaf extracts of *O. mungo* were expressed as chlorogenic acid equivalent. Leaf extract showed the maximum phenolic content of 54 mg followed by root and stem which is 34 mg and 24.5 mg chlorogenic acid equivalent respectively. The total flavonoid content in the fractions was expressed as µg quercetin equivalent. The leaf extract showed maximum flavonoid content of 56 µg /.1mg followed by root and stem which is 23 µg /.1 mg and 18 µg /.1 mg respectively.

Table 1: Free Radical Scavenging Activity of *O. mungo* By DPPH Radical Inhibition

Concentration (µg /ml)	Percentage Inhibition		
	Root Extract	Stem Extract	Leaf Extract
20	40.9 ± .004	4.54 ± .0028	40.9 ± .0020
40	57.57 ± .001	13.63 ± .004	46.9 ± .005
60	62.12 ± .002	27.27 ± .002	48.4 ± .005
80	66.66 ± .002	28.78 ± .002	53.03 ± .025
100	65.15 ± .002	33.33 ± .002	57.57 ± .001

Values are Mean ± SE of 3 Replicates

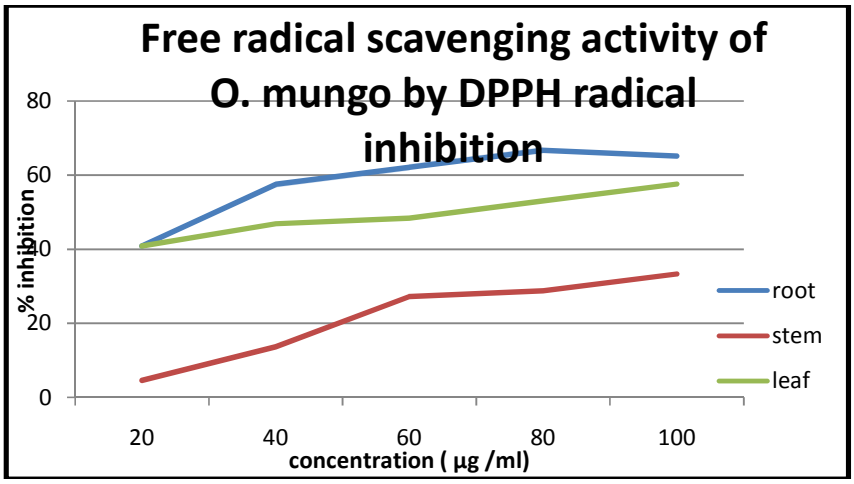

Figure 1: Free Radical Scavenging Activity of *O. mungo* By DPPH Radical Inhibition

Table 2 – Reducing Power of Root, Stem and Leaf Extracts of *O. mungo*

Concentration (µg/ml)	Absorbance at 700 nm inhibition		
	Root Extract	Stem Extract	Leaf Extract
20	.438 ± .011	.406 ± .010	.427 ± .012
40	.443 ± .004	.429 ± .009	.437 ± .001
60	.457 ± .001	.429 ± .003	.451 ± .001
80	.465 ± .003	.431 ± .004	.452 ± .002
100	.483 ± .008	.435 ± .005	.460 ± .001

Values are Mean ± SE of 3 Replicates

Table 3 – Total Antioxidant Activity of Root, Stem and Leaf Extracts of *O. mungo*

Concentration µg/ml	Ascorbic Acid Equivallent		
	Root Extract	Stem Extract	Leaf Extract
20	1.542 ± .001	1.473 ± .001	1.499 ± .001
40	1.558 ± .002	1.452 ± .002	1.515 ± .001
60	1.571 ± .0005	1.525 ± .001	1.527 ± .002
80	1.584 ± .002	1.539 ± .003	1.542 ± .002
100	1.589 ± .005	1.560 ± .005	1.56 ± .001

Values are Mean ± SE of 3 Replicates

DISCUSSION

Free radicals are chemical entities that can exist separately with one or more unpaired electrons. It has been implicated in the pathology of many diseases and conditions including diabetes, cardiovascular diseases, inflammatory conditions, cancer and aging **[12]**. Antioxidants may offer resistance against the oxidative stress by scavenging free radicals inhibiting lipid peroxidation and by many other mechanisms and thus prevent disease **[13]**.

DPPH is a relatively stable free radical and the assay determines the ability of extracts to reduce DPPH radical to the corresponding hydrazine by converting the unpaired electrons to paired one. Antioxidants can act by converting the unpaired electrons to paired ones. The dose dependent inhibition of DPPH radical **(Figure: 1)** indicates that root, stem and leaf extracts of *O. mungo* causes reduction of DPPH radical in a stoichiometric manner **[l4, 15]**.

The transformation of Fe^{3+} into Fe^{2+} in the presence of different extracts was measured to determine the reducing power ability. The reducing ability of a compound generally depends on the presence of antioxidants which exerts the antioxidant activity by breaking the free radical chain and donating a hydrogen atom **[16]**. The reducing property of extracts of *O .mungo* **(Table 2)** implies that the antioxidant principles present in the extract may be capable of donating hydrogen atom in a dose dependent manner.

The total antioxidant capacity of the extracts was evaluated using the phosphomolybdate method **[17]**. In the presence of the extracts, the Mo (V1) is reduced to Mo (V) and form a phosphomolybdenum V complex which

shows maximum absorbance at 695 nm. All extracts possessed antioxidant activity.

CONCLUTION

Based on the results obtained it may be concluded that the methanolic root extract of *O. mungo* showed strong antioxidant activity, reducing power ability, free radical scavenging activity than the stem and leaf when compared to standard like ascorbic acid. As the three extracts of *O. mungo* exhibited different antioxidant activities, there may be different percentages of phytochemical constituents present in 3 extracts. Further studies to evaluate more *in vitro* antioxidant assays and the isolation and identification of the antioxidant principles in the root of *O. mungo* are to be carried out.

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