Antioxidant, Total Phenolics, Free Radical Scavenging and Preliminary Cytotoxicity Studies of *Ophiorrhiza mungos*

Farhana Islam, Sharmin Reza Chowdhury, Tasdique Mohammad Quadery, Mohammad Abul Kaisar, Md. Gias Uddin and Mohammad A. Rashid*

Phytochemical Research Laboratory, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh

Abstract

The antioxidant activity of methanol extract of whole plant of *Ophiorrhiza mungos* (L) and its petroleum ether, carbon tetrachloride, dichloromethane and aqueous soluble partitionates was evaluated by analyzing the bleaching rate of 1,1-diphenyl-2-picrylhydrazyl (DPPH) and phosphomolybdenum total antioxidant assay using butylated hydroxytoluene (BHT) and ascorbic acid (ASA) as standard antioxidants. The total phenolic content was also determined by using Folin-Ciocalteau reagent and expressed in gallic acid equivalent (mg of GAE/g of sample). The result showed that the dichloromethane soluble fraction demonstrated the presence of highest amount of phenolic compounds (70.20±0.05 mg GAE/g of sample) and also revealed significant antioxidant activity (IC_{50} 8.50±0.85 µg/ml). A positive correlation was observed between the total phenolic content and total antioxidant capacity of *O. mungos* having correlation coefficient (R^2) of 0.9398. The general toxicity of the extractive was studied by brine shrimp lethality bioassay and from the results (LC_{50} 0.474 -2.204 µg/ml), it can be well predicted that the crude extract and the Kupchan partitionates possessed cytotoxic principles as demonstrated by considerable toxic potencies.

Key words: *Ophiorrhiza mungos*, total phenolic content, free radical scavenging activity, DPPH, brine shrimp.

Introduction

Ophiorrhiza mungos (L) belonging to the family Rubiaceae is a flowering plant, adapted to many environments. *O. mungos*, bengali name Ronjonkali, is an annual herb having 30 cm height and distributed all over Bangladesh. Traditionally, this plant is used in wound healing (Ayyanar and Ignacimuthu, 2009) and snake bites (Peter and Ibironke, 2002). In the present study, different partitionates of methanolic extract of the whole plants were used to investigate the antioxidant potential in terms of total phenolic content and free radical scavenging activity and cytotoxic properties of *O. mungos* for the first time.

Materials and Methods

Collection of plant material: Whole plants of *Ophiorrhiza mungos* (L) were collected in middle of 2010 from Dhaka University campus and a voucher specimen representing this collection has been deposited in Bangladesh National Herbarium (Accession number DACB- 35632), Mirpur, Dhaka.

The plant materials were chopped, dried and powdered and about 800 gm of the powdered material was soaked in 2.5 litres of methanol at room temperature for 7 days. The extract was filtered off using Whatman filter paper number 1 and concentrated with a rotary evaporator. An aliquot (9 gm) of the concentrated methanol extract was partitioned by modified Kupchan method (Vanwangen *et al.*, 1993) and evaporation of solvents afforded pet-ether (PSF 3.0 gm), carbon tetrachloride (CSF 3.5 gm), dichloromethane (DSF 1.5. gm) and aqueous (ASF 1.0 gm) soluble fractions. The residues were stored in a freezer for further studies.

Total phenolic content: The total phenolic content of the extracts was determined with the Folin-Ciocalteau reagent by using the method developed by Harbertson and Spayd (2006). To 0.50 ml of each sample (three replicates), 2.5 ml of 1/10 dilution of Folin-Ciocalteau reagent and 2.0 ml of sodium carbonate (7.5%, w/v) in water were added and incubated at 45 °C for 15 min. The absorbance of all samples was measured at 765 nm using a UV-Vis spectrophotometer and the results were expressed

*Correspondence to: Mohammad A. Rashid, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh. E-mail: rashidma@univdhaka.edu

as milligram of gallic acid equivalent per gram (mg GAE/g) of dry weight of extract.

DPPH free radical scavenging assay: The antioxidant activity of the methanolic extract and sub-fractions obtained from it was measured by evaluating the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical (Brand-Williams *et al*., 1995). For this, 2.0 ml of the different concentrations (500 µg/ml to 0.977 µg/ml) of the test samples were mixed with 3.0 ml of a methanol solution of DPPH (20 µg/ml) and after 30 minutes of reaction period at room temperature in dark, the absorbance was measured at 517 nm by a UV-vis spectrophotometer (Model No. UV 1700 Pharmaspec, Shimadzu). The inhibition of free radical DPPH in percent (%) was calculated as follows:

$$I\ \% = [1-(A_{sample}/A_{blank})] \times 100$$

where A_{blank} is the absorbance of the blank (containing all reagents except the test materials) and A_{sample} is the absorbance of the test material. The IC_{50} values (concentration of samples required to scavenge 50% of free radicals) were calculated from the regression equation, developed by plotting concentration of the samples versus percentage inhibition of free radical. Here, synthetic antioxidants, butylated hydroxytoluene (BHT) and L-ascorbic acid were used as positive controls.

Phosphomolybdenum antioxidant assay: The total antioxidant activity of the extract was evaluated by the phosphomolybdenum assay method (Prieto et al., 1999) which is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate-Mo (V) complex in acidic condition. The extract (2.0 mg/ml, 0.3 ml) was allowed to mix up with 3.0 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) and the reaction mixture was incubated at 95oC for 90 minutes. After cooling at room temperature, the absorbance of the solution was measured at 695 nm using a UV–Visible spectrophotometer against an appropriate blank. The antioxidant activity was expressed as the number of gram equivalents of ascorbic acid.

Table 1. Total antioxidant capacity, total phenolic content and free radical scavenging activity of different partitionates of *O. mungos.**

Sample	Total phenolic content (mg of GAE/gm of dried extract)	Free radical scavenging activity (IC_{50} µg/ml)	Total antioxidant capacity (mg of ascorbic acid/100 g of plant extract)
BHT	-	27.5 ± 0.54	-
ASA	-	5.8 ± 0.21	-
ME	30.45 ± 0.47	60.25 ± 0.29	0.337 ± 0.22
PSF	14.95 ± 0.73	125.65 ± 0.61	0.201 ± 0.75
CSF	45.50 ± 0.84	12.625 ± 0.35	0.480 ± 0.85
DSF	70.20 ± 0.05	8.50 ± 0.85	0.553 ± 0.32
ASF	40.20 ± 0.05	20.30 ± 0.52	0.297 ± 0.11

*The average values of three calculations are presented as mean ± S.D.
BHT= Butylated hydroxytolune; ASA= Ascorbic acid; ME = Methanol extract; PSF= Pet ether soluble fraction; CSF= Carbon tetrachloride soluble fraction; DSF= Dichloromethane soluble fraction; ASF= Aqueous soluble fraction; S.D. = Standard Deviation.

Brine shrimp lethality bioassay: For screening of pharmacological properties, the brine shrimp lethality bioassay was conducted which is considered to be a rapid general bioassay technique for the natural products. This method indicates cytotoxicity as well as a wide range of pharmacological activities e.g. anticancer, antiviral and pesticidal properties (Meyer *et al*., 1982). Brine shrimp eggs were hatched in simulated sea water to get nauplii. Test samples of different concentrations (400 µg/ml to 0.781 µg/ml) were prepared by dissolving in dimethylsulfoxide (DMSO). Ten nauplii were counted by visual inspection and were taken in vials containing 5 ml of simulated sea water. Then test samples were added to the pre-marked vials through a micropipette and after an incubation period of 24 hours, the survivors were counted. The LC_{50} (concentration required to kill half of the test

organisms) values of the test samples were calculated from the regression equation, prepared from the logarithm of sample concentration and percentage mortality of the shrimp nauplii.

Table 2. LC_{50} values of different extractives of *O. mungos* observed in brine shrimp lethality bioassay.

Samples	LC_{50} (µg/ml)
ME	0.934
PSF	1.251
CSF	0.826
DSF	0.474
ASF	2.204
Vincristine sulfate	0.451

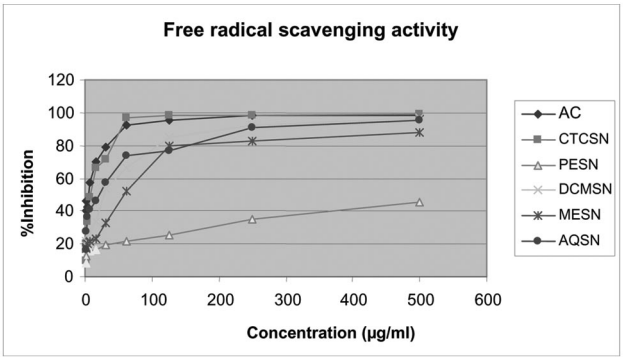

Figure 1. DPPH free radical scavenging activity of different extractives of *O. mungos.*

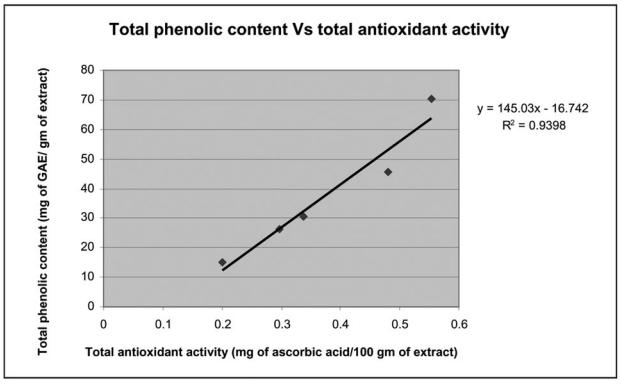

Figure 2. Correlation between the total phenolic content and total antioxidant capacity.

Results and Discussion

The methanolic crude extract of *O. mungos* as well as different Kupchan partitionates derived from it were subjected to assay for total phenolic content, free radical scavenging activity and preliminary cytotoxicity. The total phenolic content in crude MeOH extract and its dichloromethane, carbon tetrachloride, aqueous and pet-ether soluble sub-fractions were found to be demonstrated 30.45, 70.20, 45.50, 40.20 and 14.95 mg GAE/ gm of sample, respectively. The result indicated the presence of highest amount of phenolic compounds in the dichloromethane soluble Kupchan fraction.

In the analysis of free radical scavenging activity, the IC_{50} values of the extractives were found in the range of 8.50±0.85 to 125.65±0.61 µg/ml. The free radical scavenging activity of dichloromethane soluble fraction (IC_{50} = 8.50±0.85 µg/ml) was significant when compared to that of synthetic antioxidant, butylated hydroxytoluene (IC_{50} = 27.5 µg/ml) and ascorbic acid ($IC_{50=}$ 5.8 µg/ml). The prominent free radical scavenging activity may be related to its high phenolic content (70.20±0.05 mg GAE/ gm of sample) or due to synergistic antioxidant activity of various components present in the extractive. A positive correlation was seen between total phenolic content and total antioxidant activity of *O. mungos* having correlation coefficient (R^2) values of 0.9398.

In the brine shrimp lethality bioassay, different mortality rate of the nauplii by the test samples and the control group suggested that the methanolic crude extract of the whole plant and its Kupchan fractions have promising toxic activities. The lowest LC_{50} value (0.474 µg/ml) obtained with the dichloromethane soluble fraction and the highest (2.204 µg/ml) LC_{50} was revealed by the aqueous soluble fraction, whereas Vincristine sulphate showed the LC_{50} value of 0.451 µg/ml.

It is clearly evident from the above findings that the whole plant of *O. mungos* has high antioxidant potential and significant cytotoxic activities. Therefore, the plant is a good candidate for further studies to isolate the bioactive principles.

Acknowledgements

One of us (Farhana Islam) acknowledges the Ministry of Science, Information and Communication Technology (MOSICT), Government of the Peoples Republic of Bangladesh for a fellowship to her to carry out the work. We are also grateful to the Centre for Biomedical Research, University of Dhaka, Dhaka-1000, Bangladesh for providing laboratory facilities.

References

Ayyanar M. and Ignacimuthu, S., 2009. Herbal medicines for wound healing among tribal people in southern India: ethnobotanical and scientific evidences. *Intl. J. Appl. Res. Nat. Prod.* **2**, 29-42.

Brand-Williams, Cuvelier, W. and Berset, C., 1995. Use of free radical method to evaluate antioxidant activity. *Lebensm. Wiss. Technol*. **28**, 25-30.

Harbertson J. and Spayd S., 2006. Measuring phenolics in the winery. *Am. J. Enol. Vitic*. **57**, 280- 288.

Meyer B.N., Ferringni N.R., Puam, J.E., Lacobsen L.B. and Nichols D.E., 1982. *Drug Info Journal*, **31**, 516-554.

Peter J. H. and Ibironke M.O., 1993. Flowering plants used against snakebite, Review article, *J. Ethnopharmacol*. **39**, 1-29.

Prieto, P., Pineda, M. and Aguilar, M., 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal. Biochem.* **269**, 337-341

Vanwagenen, B.C., Larsen, R., Cardellina, J.H. II, Ran dazzo, D., Lidert, Z.C. and Swithenbank, C., 1993. Ulosantoin, a potent insecticide from the sponge *Ulosa ruetzleri*. *J. Org. Chem*. **58**, 335-337.