Evaluation of the Antioxidant Activity of the Roots and Rhizomes of *Cyperus rotundus* L.

D. K. PAL* AND S. DUTTA

Division of Pharmaceutical Chemistry, Seemanta Institute of Pharmaceutical Sciences, Jharpokharia, Mayurbhanj-757 086, India.

The *in vitro* antioxidant activity of the roots and rhizomes of *Cyperus rotundus* L. has been investigated by estimating degree of non-enzymatic haemoglobin glycosylation, measured colorimetrically at 520 nm. The ethanol extract of the roots and rhizomes of *C. rotundus* showed higher activity, than other extracts of it. The antioxidant activity of the extracts are close and identical in magnitude, and comparable to that of standard antioxidant compounds used.

***For correspondence**

E-mail: drdilip2003@yahoo.co.in

Cyperus rotundus L., (Muthaghas (Bengali), Motha (Hindi), *Nutgrass* (English); family: Cyperaceae) is a perennial sedge distributed throughout India. The roots and rhizomes of this plant are used in different diseases like chronic diarrhoea, inflammation, skin rashes, and excess bleeding. It has also antiestrogenic, antimicrobial, anthelmintic, antihistaminic, antiemetic, antipyretic, and antidiabetic activities[1-5]. The roots and rhizomes of *C. rotundus* on preliminary chemical analysis, is found to contain β-sitosterol, cyperene, cyperol, flavonoids, sesquiterpenoids, ascorbic acid, and polyphenols[6,7]. It is of timely interest, to search for new antioxidants from plant sources. Recently, a great deal of interest has been directed towards the bioactivity of flavonoids, ascorbic acid, and polyphenols, as dietary sources of antioxidant[8]. Hence, the present communication deals with the evaluation of the antioxidant activity of the roots and rhizomes of *C. rotundus* L.

Evaluation of the antioxidant activity of any drug sample or herbal extract can be carried out, either by *in vitro* or *in vivo* models. Various procedures are available in each model to determine the antioxidant capacity. Here, the evaluation is carried out by *in vitro* non-enzymatic glycosylation of haemoglobin method. Since non-enzymatic glycosylation of haemoglobin is an oxidation reaction, an antioxidant is expected to inhibit the reaction. The degree of haemoglycosylation *in vitro*, in the presence of different concentration of extracts, can be measured colorimetrically.

Haemoglobin was purchased from Nice Chemicals Pvt. Ltd., Cochin. Glucose and D-α-tocopherol were procured from Merck, Mumbai. Ascorbic acid and gentamycin were obtained from Biokem International Pvt. Ltd., Bangalore and Nicholas Piramal India Ltd. Pithampur, respectively. All other reagents and solvents used, were of analytical grade.

The roots and rhizomes of *C. rotundus* L. were collected from Panua, in the district of Bankura, West Bengal in the month of June, and were authenticated at the Central National Herbarium, Botanical Survey of India, Howrah, West Bengal. A voucher specimen has been preserved in our laboratory for future reference (DPS 1). Shade-dried, powdered, sieved (40 mesh size) plant materials were exhaustively extracted successively with petroleum ether (40-60 C), chloroform, ethanol, and distilled water, using a soxhlet extractor. The extracts were concentrated to dryness in vacuum. The yield of petroleum ether, chloroform, ethanol, and water extracts, were 1.5%, 2.4%, 12.3% and 9.2%, respectively. The ethanol extract was subjected to silica gel preparative TLC, where two compounds were isolated using chloroform : ethanol (9:1) as solvent system.

Compound A (mp: 210°, R_f value: 0.59, λ_{max}: 224.2 nm) having characteristic IR (Perkin Elmer, IR-297) peaks at 3350 cm^{-1}, 2930 cm^{-1}, 2850 cm^{-1}, 1715 cm^{-1}, 1690 cm^{-1}, 1640 cm^{-1}, 1600 cm^{-1}, 1450 cm^{-1}, 1380 cm^{-1} and 1300 cm^{-1} and compound B (mp: 255°, R_f: 0.64, λ_{max}: 220 nm) having characteristic IR peaks at 2930 cm^{-1}, 2850 cm^{-1}, 1700 cm^{-1}, 1600 cm^{-1}, 1450 cm^{-1}, 1380 cm^{-1} and 1300 cm^{-1} suggest the structural similarities with the polyphenolic type of compounds[9].

The antioxidant activity of different extracts were investigated by estimating degree of non-enzymatic haemoglobin glycosylation, measured colorimetrically. Haemoglobin, 60 mg/100 ml in 0.01 M phosphate buffer (pH 7.4) was incubated in presence of 2 g/100 ml concentration of glucose for 72 h, in order to find out the best condition for haemoglobin glycosylation. The assay was performed by adding 1 ml of glucose solution, 1 ml of haemoglobin solution, and 1 ml of gentamycin (20 mg/ 100 ml), in 0.01 M phosphate buffer (pH 7.4). The mixture was incubated in dark at room temperature for 72 h. The degree of glycosylation of hemoglobin in the presence of different concentration of extracts and their absence, were measured colorimetrically at 520 nm[10-13].

Results of antioxidant activity of *C. rotundus* extracts are summarized in Table 1. The result obtained, indicates that ethanol extract has more antioxidant activity than the petroleum ether, chloroform, and aqueous extract. Ethanol extract showed 35.3%, aqueous extract 14.1%, chloroform extract 12%, petroleum ether extract 7.9% and inhibition of haemoglobin glycosylation with a concentration of 1.0 mg/ml of each. The antioxidant activity of the extracts are concentration dependent. The detailed chemical nature of the active principles responsible for antioxidant activity is not known. However, preliminary phytochemical screening has confirmed the presence of flavonoids (1.25%), ascorbic acid (0.01%) and polyphenols (1.62%) which might be responsible for this activity. D-α- tocopherol (vitamin E) and ascorbic acid (vitamin C) were used as standard antioxidant compounds.

TABLE 1: THE ANTIOXIDANT ACTIVITY OF DIFFERENT EXTRACTS OF *C. rotundus* L.

Sample	Final concentration of the tested compound (mg/ml)	
	0.5	1.0
PE	2.9±0.16	7.9±0.24
CE	6.1±0.30	12.0±0.32
EE	23.3±0.25	35.3±0.29
AE	9.2±0.17	14.1±0.21
D-α-tocopherol	11.4±0.15	16.3±0.14
Ascorbic acid	5.3±0.11	9.4±0.13

Percent inhibition of haemoglobin glycosylation was measured at two concentrations of petroleum ether extract (PE), chloroform extract (CE), ethanol extract (EE) and aqueous extract (AE). The activities were compared with those of D-α-tocopherol and ascorbic acid, Values are mean±SEM of three observations.

ACKNOWLEDGEMENTS

The authors are grateful to the Principal, S. I. P. S. and Mr. P. C. Basa, President, S. I. P. S., Orissa for providing necessary facilities. The authors wish to express their sincere thanks to Dr H. J. Chowdhury, Joint Director, Central National Herbarium, Botanical Survey of India, West Bengal for his generous help in taxonomical identification.

REFERENCES

1. Kirtikar, K.R. and Basu, B.D., In; Mhaskar, K.S., Blatter, E. and Caius, J.F., Eds., Indian Medicinal Plants, 3rd Edn, Vol.VIII, Sri Satguru Publications, Delhi, 2002, 3632.
2. Weenan, H., Nkunya, M.H., Bray, D.H., Mwabumbi, L., Kinabo, L.S., Kilimali, V.A. and Winjnberg, T.B., **Planta Med.**, 1990, 66, 371.
3. Indira, M., Sirsi, M., Radonir, S. and Dev, S., **J. Sci. Ind. Res.,** 1956, 15C, 202.
4. Zhu, M., Luk, H.H., Fung, H.S. and Luk, C.T., **Phytother. Res.**, 1997, 11, 391.
5. Bawden, K., Quant, J., Ali F., Raman, A. and Houghton, P.J., **J. Pharm. Pharmacol.**, 2003, 55 (suppl), 531.
6. Thebtaranonth, C., Thebtaranonth, Y., Wanauppathamkut, S. and Yuthavong, Y., **Phytochemistry**, 1995, 40, 125.
7. Sonwa, M.M. and Konig, W.A., **Phytochemistry**, 2001, 58(5), 799.
8. Harborne, J.B., In; The Flavonoids: Advances in Research since 1986, Chapman & Hall, London, 1994, 619.
9. Dyer, J.R., In; Applications of Absorption Spectroscopy of Organic Compounds, 9th Edn., Prentice-Hall of India Pvt Ltd, New Delhi, 1994, 22.
10. Acharya, S. and Maning, J.M., **J. BioI. Chem.,** 1980, 255, 7216.
11. Fluckiger, R. and Winterhalter, K.H., In; Biochemical and Chemical Aspects of Haemoglobin Abnormalities, Academic Press, New York, 1978, 208
12. Asgary, S., Naderi, G.H., Sarrafizadegan, N., Ghassemi, N., Boshtam, M., Rafie, M. and Arefian, A., **Pharmaceutica Acts Helvetiae,** 1999, 73, 223.
13. Yadav, S.B., Tripathy, V., Singh, R.K. and Pandey, H.P., **Indian J. Pharm. Sci**., 2000, 6, 477.

Accepted 20 March 2006
Revised 4 June 2005
Received 18 February 2005
Indian J. Pharm. Sci., 2006, 68 (2): 256-258