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Screening of the Antioxidant Activity of *Hydrilla verticillata* Plant

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The *in vitro* antioxidant activity of *Hydrilla verticillata* plant has been investigated by two methods: by estimating the degree of non-enzymatic haemoglobin measured colorimetrically at 520 nm and by assaying DPPH free radical scavenging activity. From the analysis, it is found that the chloroform extract has the highest and petroleum ether extract has the lowest antioxidant activity. The antioxidant activities of these extracts are closely identical in magnitude and comparable to those of the standard antioxidant compounds used.

Key Words : Antioxidant activity, *H. verticillata* plant, Non-enzymatic haemoglycosylation, DPPH free radical scavenging activity.

INTRODUCTION

Hydrilla verticillata (Oriya: *Chingudia dala*, English: Tape grass; Family: Hydrocharitacea) is a perennial sedge distributed throughout India, Malaysia, Srilanka, China and the United States up to altitude 2005 ft. It is widely found in water including lakes, ponds, rivers, streams and marshes and can survive in few inches of water or in depth up to 20 ft. Therapeutically, *H. verticillata* plant may be used for the following purposes[1, 2]: more complete nutrition, improved digestion and gastrointestinal function, improved circulation, improved clearance of waste products for metabolism and detoxification, improved neurological health[3], improved cardiovascular function and health, increased blood sugar control, strengthened immunity, slowed ageing, etc. It has also antibacterial properties[4]. *H. verticillata* plant, on preliminary chemical analysis, is found to contain saponin, β-carotene, vitamins, minerals, antioxidants and detoxifying agents. 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 β-carotene, vitamins, micro- and macronutrients, minerals as dietary sources of antioxidants[5–9]. Hence the present communication deals with the evaluation of the antioxidant activity of *H. verticillata* plant.

Evaluation of the antioxidant activity of any drug sample or herbal extract can be carried out 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 *in vitro* by (i) non-enzymatic glycosylation of haemoglobin, and (ii) assaying DPPH free radicals scavenging activity.

EXPERIMENTAL

Haemoglobin was purchased form Nice Chemicals Pvt. Ltd., Cochin. Glucose phosphate buffer and D-α-tocopherol were procured from Merck, Mumbai. Ascorbic acid and gentamycin were obtained from Biochem International Pvt. Ltd., Bangalore and Nicholas Piramal India Ltd., Pithampore respectively. DPPH was procured from Sigma-Aldrich, Mumbai. All other reagents and solvents used were of analytical grade.

Preparation of extracts: Fresh *H. verticillata* plants were collected from Subarnarekha river in the Mayurbhanj district of Orissa in the month of August-September and were authenticated by Dr. H.J. Chowdhury, Joint Director, Central National Herbarium, Botanical Survey of India, Howrah, West Bengal. A voucher specimen has been preserved in our laboratory for future reference (DNS1). Shade-dried, powdered, sieved (40 mesh size) plant materials were exhaustively extracted successively with petroleum ether (40–60°C), benzene, chloroform, ethyl acetate, ethanol and distilled water using a Soxhlet extractor. The extracts were concentrated to dryness in vaccum. The yield of petroleum ether, benzene, chloroform, ethyl acetate, ethanol and aqueous extracts were 0.4, 1.2, 0.9, 2.5, 2.4 and 7.2% w/w respectively. The extracts were subjected to antioxidant studies.

Antioxidant Studies

(i) Non-enzymatic haemoglycosylation method: The antioxidant activities of different extracts were investigated by estimating the 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 haemoglobin in the presence of different concentrations of extracts and their absence were measured colorimetrically at 520 nm[10–13].

(ii) The method of DPPH free radical scavenging activity[14–17]: 7.886 mg of DPPH was accurately weighed and dissolved in 100 mL methanol to obtain 200 μM solution of DPPH. All the sample solutions were prepared against two concentrations, *i.e.*, 0.5 and 1 mg/mL.

To a 2 mL methanolic solution of DPPH, 2 mL of sample solution was added. The mixture was incubated in dark at room temperature for 15 min. The degree of free radical scavenging activity in the presence of different concentrations of extracts and their absence were measured colorimetricaly at 517 nm. The degree of free radical scavenging activity was expressed as:

$$\% \text{ Inhibition} = [A_{control} - A_{sample} / A_{control}] \times 100$$

where $A_{control}$ = absorbance of DPPH alone,

A_{sample} = absorbance of DPPH with different concentrations of extracts.

RESULTS AND DISCUSSION

Results of antioxidant activity of *H. verticillata* plant extracts are summarized in Table-1 and Fig. 1. The results obtained from Table-1 indicate that chloroform and ethyl acetate extract have better antioxidant activity than petroleum ether, benzene, ethanol and aqueous extract. From the assay of DPPH free radical scavenging activity (Fig. 1), it is found that chloroform extract has the highest and petroleum ether extract has the lowest antioxidant activity. The activities were compared with D-α-tocopherol (vitamin E) and ascorbic acid (vitamin C) which were used as standard antioxidant compounds.

TABLE-1
THE ANTIOXIDANT ACTIVITY OF DIFFERENT EXTRACTS OF *H. VERTICILLATA* PLANT

Samples	Final concentration of the tested compound (mg/mL)	
	0.5	1.0
Petroleum ether extract	2.3 ± 0.11	4.9 ± 0.15
Benzene extract	13.6 ± 0.34	16.1 ± 0.39
Chloroform extract	20.5 ± 0.38	43.2 ± 0.69
Ethyl acetate extract	22.7 ± 0.43	32.1 ± 0.53
Ethanol extract	6.8 ± 0.18	13.5 ± 0.35
Aqueous extract	3.6 ± 0.10	6.1 ± 0.19
D-α-tocopherol	11.0 ± 0.30	15.8 ± 0.37
Ascorbic acid	4.8 ± 0.11	6.9 ± 0.14

Per cent inhibition of haemoglobin was measured at two concentrations of petroleum ether extract, benzene extract, chloroform extract, ethyl acetate extract, ethanol extract and aqueous extract. The activities were compared with those of D-α-tocopherol and ascorbic acid. Values are mean ± S.E.M. of three replicates.

The detailed chemical nature of the active principles responsible for antioxidant activity is not known. However, preliminary phytochemical screening has confirmed the presence of β-carotene, which might be responsible for such activity[18, 19].

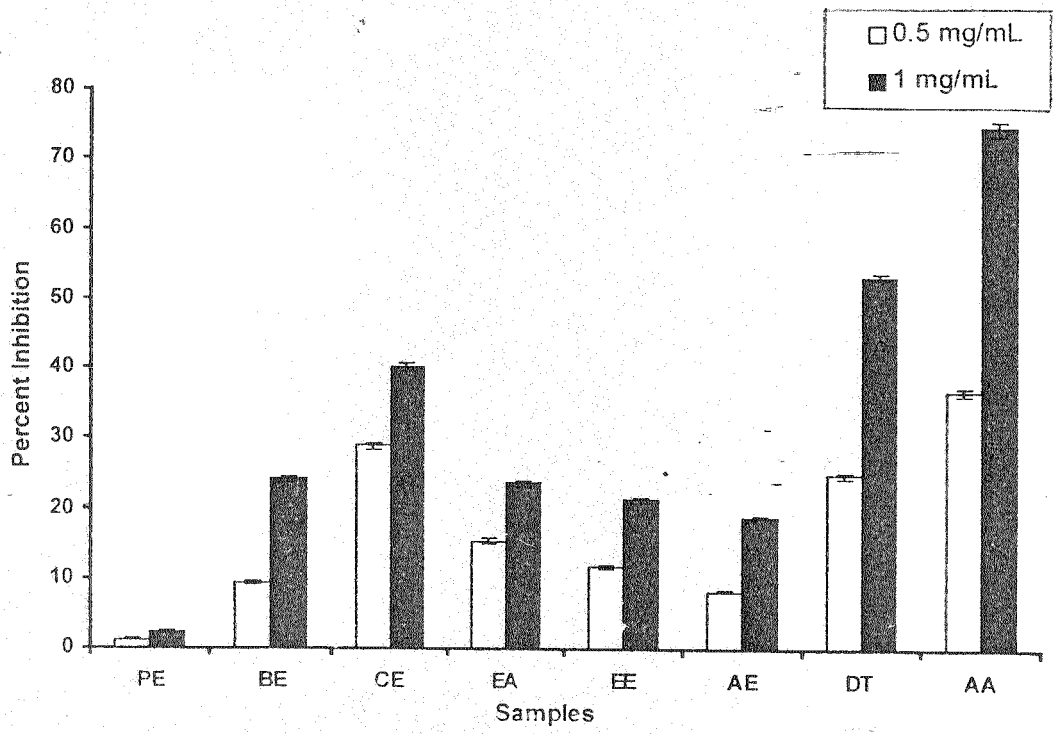

Fig. 1. DPPH free radical scavenging effects of different extracts of *H. verticillata* plant. Percent inhibition of DPPH free radicals was measured at two concentrations of petroleum ether extract (PE), benzene extract (BE), chloroform extract (CE), ethyl acetate extract (EA), ethanol extract (EE) and aqueous extract (AE). The activities were compared with those of D-α-tocopherol (DT) and ascorbic acid (AA). Values are mean ± S.E.M. of three replicates.

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