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NOTE

Evaluation of Antioxidant Activity of Aerial Parts of *Hygrophila difformis*

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The *in vitro* antioxidant activity of aerial parts of *Hygrophilla difformis* has been investigated by estimating degree of non-enzymatic haemoglobin glycosylation measured colorimetrically at 520 nm. The benzene and chloroform extract of aerial parts of *H. difformis* showed higher antioxidant activity than other extracts of it. The antioxidant activity of the extracts is close and comparable to that of the standard antioxidant compounds used.

Key Words: Antioxidant activity, *Hygrophilla difformis*, Non-enzymatic haemoglobin glycosylation.

Hygrophilla difformis Blume (commonly known as water Wisteria, family: Acanthaceae) is a tropical aquarium plants used as environmental ornaments. It is very fast growing plant that requires high light and nutrients to get good green growth. Its rapid growth helps prevention of algae. It grows to a height of 20-50 cm with a width of 15-25 cm. It has slender lacey leaves and upright growth. It is found in marshy habitats on the Indian subcontinent in Bangladsh, Bhutan, India and Nepal. The plant is used as anticoagulant by tribal people[1]. The aerial parts of *H. difformis* on preliminary chemical analysis are found to contain hygrophiloside[2]. Recently, a great deal of interest has been directed towards the bioactivity of natural plants as sources of antioxidant[3-5]. Hence, the present communication deals with the evaluation of the antioxidant activity of aerial parts of *H. difformis* Pers.

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, phosphate buffer and D-α-tocopherol were procured from Merck, Mumbai. Ascorbic acid and gentamycin were obtained from Biokem International Pvt. Ltd., Bangalore

and Nicholas Piramol India Ltd., Pithampur, respectively. All other reagents and solvents used were of analytical grade.

Preparation of extracts: Aerial parts of *H. differmis* were collected from fields of Midnapur District, West Bengal in the month of December and authenticated by Mondal, Additional Director, Central National Herbarium, Botanical Survey of India, Howrah and West Bengal. A voucher specimen has been preserved in our laboratory for future reference (DPKS1). Shade-dried, powdered, sieved (40 mesh size) plant materials were exhaustively extracted successively with petroleum ether (40-60 ºC), benzene, chloroform, ethanol and distilled water using a soxhlet extractor. The extracts were concentrated to dryness in vacuum. The yields of petroleum ether, benzene, chloroform, ethanol and water extracts were 4.26, 3.25, 1.85, 9.17 and 2.58 % w/w, respectively. The extracts were subjected to antioxidant studies.

Antioxidant studies: Non-enzymatic haemoglycosylation method: The antioxidant activities 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[6-10].

Results of antioxidant activity of aerial parts of *H. differmis* extracts are summarized in Table-1 and Fig. 1. The results indicate that the benzene and chloroform extract of aerial parts of *H. differmis* have better antioxidant activity than petroleum ether, ethanol and aqueous extract. The activities were compared with D-α-tocopherol (vitamin E) and ascorbic acid (vitamin C) that were used as standard antioxidant compounds.

TABLE-1
ANTIOXIDANT ACTIVITY OF DIFFERENT EXTRACTS OF *H. differmis*

Samples	Final concentration of the tested compound (mg/mL)	
	0.5	1.0
Petroleum ether extract	28.2 ± 0.41	56.0 ± 0.65
Benzene extarct	34.2 ± 0.45	67.1 ± 0.70
Chloroform extract	35.5 ± 0.47	70.6 ± 0.75
Ethanol extract	18.2 ± 0.25	35.4 ± 0.45
Aqueous extract	11.2 ± 0.30	20.5 ± 0.34
D-α-tocopherol	11.5 ± 0.25	19.8 ± 0.30
Ascorbic acid	6.50 ± 0.19	10.4 ± 0.21

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

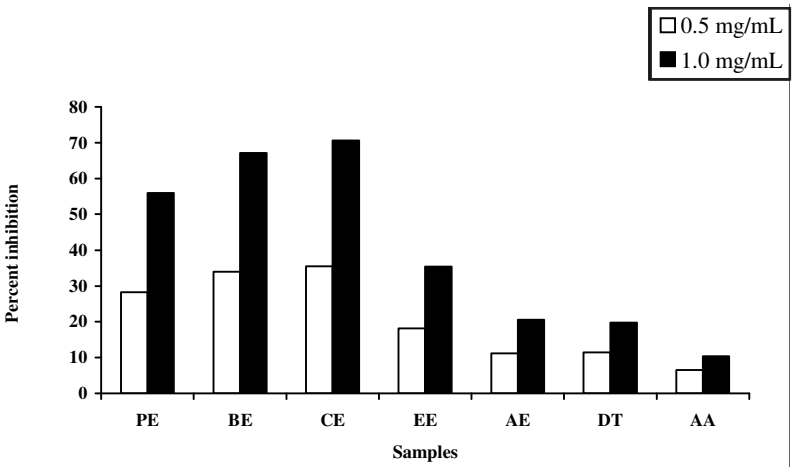

Fig. 1. The antioxidant activity of different extracts of *H. difformis*. Per cent inhibition of the glycosylation of haemoglobin was measured at two concentrations of the pe troleum ether extract (PE), benzene extract (BE), chloroform extract (CE), ethanol extract (EE), aqueous extract (AE), D-α- tocopherol (DT) and ascorbic acid (AA)

The detailed chemical nature of the active principle (s) responsible for antioxidant activity and their mode of action are under investigation.

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