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NOTE

Evaluation of the Antioxidant Activity of Aerial Parts of *Cynodon dactylon*

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The *in vitro* antioxidant activity of aerial parts of *Cynodon dactylon* has been investigated by estimating degree of non-enzymatic haemoglobin glycosylation measured colorimetrically at 520 nm. The ethyl acetate extract of aerial parts of *C. dactylon* showed higher antioxidant activity than other extracts of it. The antioxidant activity of the extracts is close and identical in magnitude and comparable to that of standard antioxidant compounds used.

Key Words: Antioxidant activity, *Cynodon dactylon*, Non-enzymatic haemoglobin glycosylation.

Cynodon dactylon Pers (Bengali: Durba, Hindi: Dhub, English: Bermuda grass; family: Graminae) is a creeping grass, found in warm climates all over the world between 45º South and North altitude. It grows in open areas where there are frequent disturbances such as grazing, flooding and fire. The juice of the plant is astringent and is applied externally to fresh cuts and wounds. It is also useful in treatment of catarrhal opthalmia, dropsy, hysteria, epilepsy, insanity, chronic diarrhoea and dysentery. The plant is folk remedy for anasarca, calculus, cancer, carbancles, cough, hypertension, snakebites, stones, gout and rheumatic affection[1-4]. The aerial parts of *C. dactylon* on preliminary chemical analysis are found to contain flavonoids[5,6]. Recently, a great deal of interest has been directed towards the bioactivity of flavonoids as dietary sources of antioxidant[7]. Hence, the present communication deals with the evaluation of the antioxidant activity of aerial parts of *C. dactylon* 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 *C. dactylon* were collected from Panua, in the district of Bankura, West Bengal in the month of December and were authenticated by Dr H.J. Chowdhury, Joint Director, Central National Herbarium, Botanical Survey of India, Howrah and West Bengal. A voucher specimen has been preserved in our laboratory for future reference (DM1). Shade-dried, powdered, sieved (40 mesh size) plant materials were exhaustively extracted successively with petroleum ether (40-60 ºC), chloroform, ethyl acetate, ethanol and distilled water using a soxhlet extractor. The extracts were concentrated to dryness in vacuum. The yield of petroleum ether, chloroform, ethyl acetate, ethanol and water extracts were 0.9, 1.1, 1.5, 2.6 and 0.5 % 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[8-12].

Results of antioxidant activity of aerial parts of *C. dactylon* extracts are summarized in Table-1. The results obtained indicate that chloroform and ethyl acetate extract have better antioxidant activity than petroleum ether, ethanol and aqueous extract. The activities were compared with D-α-tocopherol and ascorbic acid that were used as standard antioxidant compounds.

The detailed chemical nature of the active principle(s) responsible for antioxidant activity is not known. However, preliminary phytochemical screening has confirmed the presence of flavonoids, which might be responsible for this activity[11].

TABLE-1
ANTIOXIDANT ACTIVITY OF DIFFERENT EXTRACTS OF *C. dactylon*

Samples	Final concentration of the tested compound (mg/mL)	
	0.5	1.0
Petroleum ether extract	15.6 ± 0.40	37.5 ± 0.60
Chloroform extract	20.5 ± 0.35	47.9 ± 0.65
Ethyl acetate extract	26.7 ± 0.43	54.2 ± 0.63
Ethanol extract	18.0 ± 0.30	34.4 ± 0.52
Aqueous extract	13.3 ± 0.28	27.1 ± 0.42
D-α-tocopherol	11.0 ± 0.30	20.0 ± 0.34
Ascorbic acid	6.0 ± 0.19	10.0 ± 0.21

Per cent inhibition of haemoglobin glycosylation was measured at two concentrations of petroleum ether 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 ± SEM of three replicates.

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