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A study on the antioxidant activity of *Semecarpus anacardium* L.f. nuts

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Abstract

Objective: To study the antioxidant activity of different extracts of nuts of *Semecarpus anacardium in vitro*. Methods: The *in vitro* antioxdant activity was investigated by two methods: by estimating degree of non-enzymatic haemoglobin glycosylation measured colorimetrically at 520 nm and by assaying DPPH free radical scavenging activity. Results: It was found that petroleum ether and ethanol extracts of nuts of *S. anacardium* showed higher antioxidant activity than other extracts of it. The antioxidant activities of the extracts are close and identical in magnitude and comparable to that of standard antioxidant compounds used. The antioxidant activities of the extracts were concentration dependant. Conclusion: The results of the present study justify the use of the nuts as folk remedies.

Key words: Antioxidant activity, *Semecarpus anacardium* nuts, non-enzymatic haemoglobin glycosylation, DPPH free radical scavenging activity.

1. Introduction

Semecarpus anacardium L.f. (Family: Anacardiaceae, Bhela in Bengali, Bhalia in Oriya), a small tree is distributed throughout the hotter part of India, found widely in North Australia. Various parts of this plant were used in tribal medicine for diseases like herpetic eruption, paralysis and acute rheumatism [1]. The fruits of *S.anacardium* called dhobi-nut is used as an indelible ink to mark laundry [2]. Extract of nuts posses anticancer activity due to its strong cytotoxic effects [3]. The tribes of Simlipal Biosphere Reserve use it for curing headache, hydrocoel etc. It has also lubricant, anticorrosive and antimicrobial properties [3, 4, 5]. The nuts of *S. anacardium* on preliminary chemical analysis are found to contain alkaloid, tannins, saponin, flavonoids, anthraquinones, ascorbic acid [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, polyphenols as

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dietary sources of antioxidant [8]. Hence, the present communication deals with the evaluation of the antioxidant activity of the nuts of *S. anacardium*.

2. Materials and Methods

2.1 Plant material

The nuts of *S. anacardium* were collected during the months of March-April from the hilly area of Simlipal Biospere Reserve of Mayurbhanj district, Orissa and were authenticated by Dr H. J. Chowdhury, Joint Director, Central National Herbarium, Botanical Survey of India, Howrah and West Bengal. The voucher specimen has been preserved in our laboratory for further references (DTA 1). After collection, nuts were washed properly, then shade dried for about 20 days and then pulverized into fine powder by hand grinder.

2.2 Preparation of extracts

The powdered nuts were exhaustively extracted successively with petroleum ether (40-60°C), chloroform, ethyl acetate, ethanol and distilled water using a soxhlet extractor. The extracts were concentrated to dryness in vaccum. The yield of petroleum ether, chloroform, ethyl acetate, ethanol and water extracts were 1.07, 3.60, 4.25, 7.80 and 2.48% w/w, respectively. The extracts were subjected to antioxidant studies.

2.3 *Chemicals*

Haemoglobin was purchased from Nice Chemicals Pvt. Ltd., Cochin. Glucose, phosphates 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.

2.4 Antioxidant studies

2.4.1 Using 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 haemoglobin in the presence of different concentrations of extracts and their absence were measured colorimetrically at 520 nm [9-14].

2.4.2 Using the method of DPPH free radical scavenging activity [15-18]

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 mg/ml and 1 mg/ml.

To 2 ml methanol 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 concentration of extracts and their absence were measured colorimetrically at 517 nm. The degree of free radical scavenging activity was expressed as: % 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.

Table 1: The antioxidant activity of different extracts of *S. anacardium* nuts using non-enzymatic haemoglycosylation method.

Samples	Final concentration of the tested compound (mg/ml)	
	0.5	1.0
PE	29.20 ± 0.87	56.25 ± 1.04
CE	22.75 ± 0.74	43.75 ± 1.19
EA	25.60 ± 0.90	49.50 ± 1.13
EE	29.10 ± 0.94	57.29 ± 0.95
AE	18.0 ± 0.68	34.38 ± 0.79
D-α-tocopherol	11.0 ± 0.52	17.2 ± 0.66
Ascorbic acid	4.8 ± 0.19	7.3 ± 0.31

Percent inhibition of haemoglobin glycosylation was measured at two concentrations of petroleum ether extract (PE), chloroform extract (CE), ethyl acetate extract (EA), ethanol extract (EE) and aqueous extract (AE). The activities were compared with those of D-α-tocopherol and ascorbic acid. Values are mean ± SD (n=3).

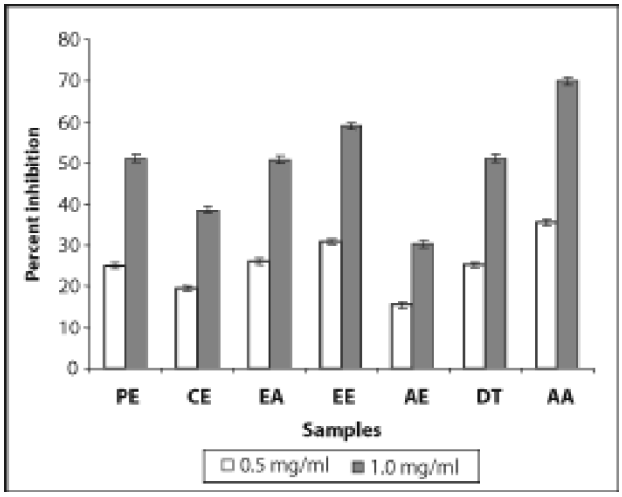

Fig. 1. DPPH free radical scavenging effects of different extracts of *S. anacardium* nuts.

Percent inhibition of DPPH free radicals was measured at two concentrations of petroleum ether extract (PE), chloroform extract (CE), ethyl acetate extract (EA), ethanol extract (EE) and aqueous extract (AE). The activities were compared with those of D-α-tocopherol and ascorbic acid. Values are mean ± SD (n=3).

2.4.3 Statistical analysis

Statistical analysis was performed by the Students '*t*' test. Least square estimation was used to calculate the peaking activity for DPPH screening activity. $P<0.05$ was considered significant.

3. Results and Discussion

Results of antioxidant activity of nuts of *S. anacardium* are summarized in Table 1 and Fig 1. The results obtained from Table 1 indicate that petroleum ether and ethanol extract have remarkable antioxidant activity than the

chloroform, ethyl acetate and aqueous extract. From the assay of DPPH free radical scavenging activity (Fig 1), it is found that ethanol extract has highest and aqueous extract has 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. Data are reported as the mean ± SD of three measurements and differences were statistically significant ($P<0.05$). The antioxidant activities of the extracts were 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, ascorbic acid and tannins, which might be responsible for such activity [12-13].

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