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Free radical scavenging activity of water extract of heartwood of *Pterocarpus marsupium*

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Abstract

The antioxidant activities of water extract of heartwood of *Pterocarpus marsupium* were evaluated in different systems viz., radical scavenging activity by DPPH reduction, nitric oxide (NO) radical scavenging in sodium nitroprusside/Griess reagent system. The results indicate that the reducing power of a substance may be an indicator of its potential antioxidant activity, but it is not necessarily a linear correlation between these two activities. The extract was found to have different level of antioxidant properties in the models tested. In scavenging DPPH and nitric oxide (NO), its activity was intense (EC_{50} = 182.74 and 66.028 μg/ml respectively). The free radical scavenging property may be one of the mechanism by which this drug is effective in several free radical mediated disease conditions.

1. Introduction

Pterocarpus marsupium, commonly known as Bija, is a well-known drug in ayurvedic system of medicine. Literature survey revealed that heartwood of *P. marsupium* has antihyperglycemic [1, 2], anticataract activity [3] and was found to be effective on glycogen content of tissue and the key enzyme of carbohydrate metabolism [4]. The antioxidant compound play key role as a heath promoting factor. Scientific evidence suggests that antioxidants reduce the risk for various diseases [5]. In recent years, one of the areas, which attracted a great deal of attention, is antioxidant in the control of degenerative diseases in which oxidative damage has been implicated. In the present study it was planned to evaluate antioxidant activity of water extract of heartwood of *P. marsupium*, in quenching two radicals (implicated in oxidative damage in cellular system) viz., DPPH and nitric oxide.

2. Materials and Methods

1,1-Diphenyl-2-picryl hydrazyl (DPPH), ascorbic acid and linoleic acid were purchased

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from Himedia Ltd., Mumbai. Sodium nitroprusside and sulphanilamide, N-(1-napthyl) ethylenediamine, were purchased from Loba Chemicals, India. All the reagents used for the study were of analytical grade. A Shimadzu model 2401 double beam UV-visible spectrophotometer with a pair of 10 mm matched quartz cells was used to measure absorbance of the resulting solution. Heartwood of *P. marsupium* was procured from a local market.

2.1 Preparation of Water Extract of Pterocarpus marsupium

Heartwood of *P. marsupium* was procured from a local supplier and authenticated in Department of Botany, Nagpur University, Nagpur and voucher specimen (4882/a) was preserved. Wood was separated from bark and dried in air, powdered to 100 mesh and stored in airtight container till further use. The powder (350 g) was extracted with water (3 × 250 ml) by cold extraction and extract was then filtered through Whatman filter paper No. 41 and concentrated under reduced pressure.

2.2 Assay for Antiradical Activity [6]

Antiradical activity was measured by a decrease in absorbance at 516 nm of a methanol solution of colored DPPH. A stock solution of DPPH (4.3 mg in 3.3 ml methanol) was prepared to produce the concentration such that 75 µg of it in 2 ml of methanol gave an initial absorbance of 0.9. This stock solution was used to measure the antiradical activity. Decrease in the absorbance in the presence of the test compounds at different concentrations was noted after 15 min. EC_{50} (i.e the concentration of the test solution required to give a 50 % decrease in absorbance, compared to that of blank solution) was calculated from % inhibition. Ascorbic acid was used as standard.

2.3. Assay for Nitric Oxide Scavenging Activity [7]

The procedure is based on the principle that, sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interferes with oxygen to produce nitric ions that can be established using Griess reagent (1% sulphanilamide, 2 % H_3PO_4 and 0.1 % napthylethylenediamine dihydrochloride). Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric ions.

For the experiment, sodium nitroprusside (5 mM) in phosphate buffered saline was mixed with different concentration of water extract of heartwood of *P. marsupium* and incubated at room temperature for 150 min. the same mixture without the water extract of the sample but with

Table 1. Antioxidant activity of water extract heartwood of *Pterocarpus marsupium* observed with DPPH

Sample	Concentration in µg/ml	% Inhibition*	EC_{50} (µg/ml)
Water extract	100	33.56 ± 0.733	
	200	54.72 ± 1.9052	
	300	74.03 ± 1.949	182.74
	400	84.46 ± 2.040	
	500	85.21 ± 1.782	
	600	88.21 ± 1.649	
Ascorbic acid			23.923

n=5, * Mean±S.D.

Table 2. *In vitro* NO Scavenging activity of water extract of heartwood of *Pterocarpus marsupium*

Sample	Concentration in µg/ml	% Inhibition*	EC_{50} (µg/ml)
Water extract	10	31.96 ± 1.245	
	20	33.33 ± 1.121	
	40	35.29 ± 2.512	
	80	60.58 ± 2.758	66.028
	160	63.72 ± 1.852	
	320	74.11 ± 1.325	
	640	74.92 ± 1.621	
Ascorbic acid			12.312

n=5, * Mean±S.D.

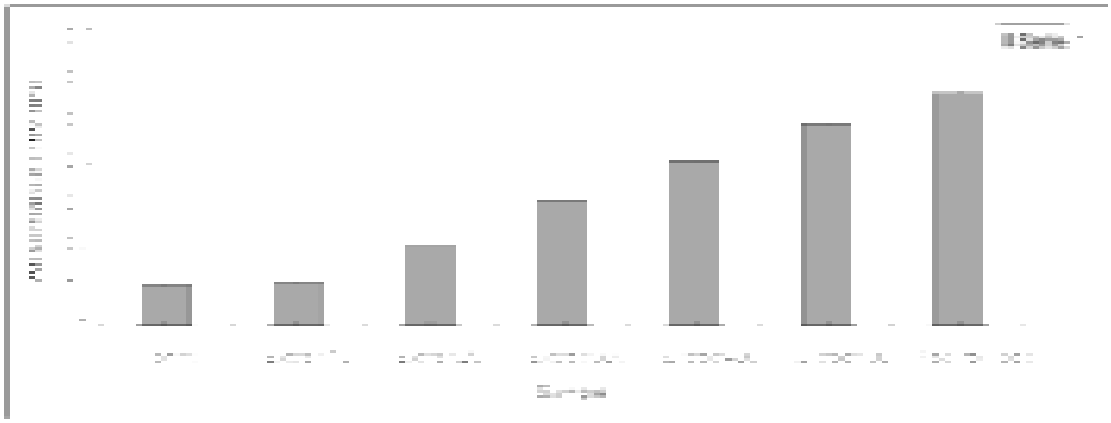

Fig.1. Reducing power of water extract of heartwood of *Pterocarpus marsupium*
(Number following extract indicates the µg of extract added to the emulsion and Ascorbic acid 500 µg).
Values are Mean ± S.D.

equivalent amount of water served as control. After incubation period, 0.5 ml of Griess reagent was added. The absorbance was noted at 546 nm. Ascorbic acid was used as positive control.

2.4. Reducing Power [8]

Extract (100-500 µg) in 1 ml of distilled water was mixed with 2.5 ml of phosphate buffer (0.2mM, pH 6.6) and 2.5 ml potassium ferricynide 1 %, and then the mixture was incubated at 50° for 30 min. Subsequently 2.5 ml of trichloroacetic acid (10 %) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. finally 2.5 ml of upper layer solution was mixed with 2.5 ml distilled water and 0.5 ml $feCl_3$ (0.1 %) and absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

3. Results and Discussion

The antioxidant activities as well as reducing activity of water extract of heartwood of

P. marsupium were evaluated in several *in-vitro* models exhibited different level of antioxidant activity in models studied. The extract showed concentration dependent antiradical activity of inhibiting DPPH radical up to 600 µg/ml, beyond which there was not much improvement in the activity. DPPH radical showed an EC_{50} 182.74 µg/ml (Table I). The extract exhibited a moderate nitric oxide scavenging activity between 100-500 µg/ml in a dose dependent manner with an EC_{50} 66.028 µg/ml (Table II). The reducing power of extract was found to increase as the amount of extract increased (Fig. 1). Even in the presence of 100 µg extract, the reducing power was significantly higher than that of control ($p<0.01$).

4. Conclusion

DPPH is one of the free radicals generally used for testing preliminary radical scavenging activity of a compound of a plant extract. In the present study, water extract showed a good antiradical activity by scavenging DPPH radicals. In addition to this, nitric oxide is also implicated in inflammation, cancer and other pathological condition [9]. The extract showed a moderate nitric oxide scavenging activity.

The reducing capacity of compound may serve as significant indicator of its potential antioxidant activity [10]. The present finding signifies that the water extract of heartwood of *P. marsupium* might be a potential source of natural antioxidant.

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