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Research Article

IN-VITRO FREE RADICAL SCAVENGING ACTIVITY OF AERIAL PARTS OF ETHANOLIC EXTRACT OF *ALBIZIA PROCERA (FAMILY: MIMOSOIDEAE)*

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ABSTRACT

Objective: Research study was undertaken to investigate and evaluate the *in-vitro* antioxidant activities of ethanolic extract of *Albizia procera.*

Methods: The ethanolic extract of *Albizia procera* was examined for DPPH (α,α-diphenyl-β-picrylhydrazyl) radical scavenging activity, Superoxide anion, Nitric oxide and Hydroxyl radical scavenging activity with reference standard Rutin, Quercetin and ascorbate respectively through *in-vitro* models.

Results: Ethanolic extract of *Albizia procera* showed significant free radical scavenging activity than that of various standards. The radical scavenging activity was found to be concentration dependent manner.

Conclusion: Ethanolic extract of *Albizia procera* showed strong scavenging activity against free radical compared to various standards. These *in-vitro* assays indicate that this plant extract is a better source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

Keywords: Ethanol extract, *Albizia procera, In-vitro* antioxidant activities, DPPH

INTRODUCTION

There is considerable epidemiological evidence indicating an association between diets rich in fresh fruits and vegetables and a decreased risk of cardiovascular disease and certain forms of cancer. Free-radicals are generated continuously in the body due to metabolism and disease[1]. In order to protect themselves against free radicals, organisms are endowed with endogenous (catalase, superoxide dismutase, glutathione peroxidase/ reductase) and exogenous (C and E vitamins, carotene, uric acid) defenses; yet these defense systems are not sufficient in critical situations (oxidative stress, contamination, UV exposure, etc.) Where the production of free radicals significantly increases[2]. Free radicals can cause lipid peroxidation in foods, which leads to their deterioration[3]. Antioxidants are important in the prevention of human diseases. Antioxidant compounds may function as free radical scavengers, complexes of pro-oxidant metals, reducing agents and quenchers of singlet oxygen formation[4].

Albizia procera is a medium sized deciduous tree, sparingly grown in India. This plant is used traditionally in dropsy, pain, rheumatism, convulsions, delirium, and septicaemia[5]. The bark of the plant is used as an astringent in the treatment of diarrhea. Its decoctions are recommended for ulcers as a useful was solution[6]. They are reported to exhibit various pharmacological activities such as CNS activity, cardiotonic activity, lipid-lowering activity, anti-oxidant activity, hepatoprotective activity, hypoglycemic activity, etc[7]. Even though, traditionally, leaves of *Albizia procera* were extensively used for the treatment of a variety of wounds, and no scientific data in its support are available. Our literature survey revealed that the antioxidant activity of various extracts from the whole plant of *Albizia procera* was not investigated; hence these activities have been investigated in the present study.

MATERIAL AND METHODS

Collection and Identification of Plant materials

The aerial parts of *Albizia procera* were collected from Tuliarai, Thirunelveli District of Tamil Nadu, India. Taxonomic identification was made from Botanical Survey of Medicinal Plants Unit Siddha, Government of India. Palayamkottai. The aerial parts of Albizia *procera,* were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

Preparation of Extracts

The above powdered materials were successively extracted with Ethanol by hot continuous percolation method in Soxhlet apparatus[8] for 24 hrs. The extract was concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

Evaluation of Antioxidant activity by *In-vitro* Techniques

DPPH photometric assay

The effect of extract on DPPH radical was assayed using the method of mensor et al., (2001) [9]. A ethanolic solution of 0.5ml of DPPH (0.4mM) was added to 1 ml of the different concentrations of plant extract and allowed to react at room temperature for 30 minutes. The scavevging activity was measured as the decrease in absorbance of the sample verses DPPH standard solution Choudhury et al (2012) [20]. Ethanol served as the blank and DPPH in ethanol without the extracts served as the positive control. After 30 min, the absorbance was measured at 518 nm and converted into percentage radical scavenging activity as follows.

$$Scavengingactivity(\%) = \frac{A_{518}\,\text{Control} - A_{518}\,\text{Sample}}{A_{518}\,\text{Control}} \times 100$$

Where A_{518} control is the absorbance of DPPH radical+ ethanol; A_{518} sample is the absorbance of DPPH radical+ sample extract/ standard.

Superoxide radical scavenging activity

Superoxide radical (O_2^-) was generated from the photoreduction of riboflavin and the corresponding reduction of nitroblue tetrazolium.Raju et.al(2012)[19].Measurement of superoxide anion scavenging activity was performed based on the method described by Winterbourne et al (1975)[10]. The assay mixture contained sample with 0.1ml of Nitro blue tetrazolium (1.5 mM NBT) solution, 0.2 ml of EDTA (0.1M EDTA), 0.05 ml riboflavin (0.12 mM) and 2.55 ml of phosphate buffer (0.067 M phosphate buffer). The control tubes were also set up where in DMSO was added instead of sample. The reaction mixture was illuminated for 30 min and the absorbance at 560 nm was measured against the control samples. Ascorbate was used as the reference compound. All the tests were performed in

triplicate and the results averaged. The percentage inhibition was calculated by comparing the results of control and test samples.

Hydroxyl radical scavenging activity

This was assayed as described by Elizabeth and Rao (1990)[11]. The assay is based on the quantification of degradation product of 2-deoxy ribose by condensation with TBA. Hydroxyl radical was generated by the Fe^{3+} -Ascorbate –EDTA –H_2O_2system (Fenton reaction). The reaction mixture contained 0.1 ml deoxyribose (2.8mM),0.1 ml EDTA (0.1 mM), 0.1 ml H_2O_2 (1mM), 0.1 ml Ascorbate (0.1mM), 0.1 ml KH_2PO_4-KOH buffer, P^H 7.4 (20mM) and various concentrations of plant extracts in a final volume of 1 ml. The reaction mixture was incubated for 1 hour at 37^0 C. Deoxyribose degradation was measured as TBARS and the percentage inhibition was calculated.

Nitric oxide radical scavenging activity

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which were measured by the method of Garrat (1964)[12].The reaction mixture (3ml) containing 2 ml of sodium nitroprusside (10mM) and 0.5 ml of phosphate buffer saline (1M) was incubated at 25^0C for 150 mins. After incubation, 0.5 ml of the reaction mixture containing nitrite was pipetted and mixed with 1 ml of sulphanilic acid reagent (0.33%) and allowed to stand for 5 min for completing diazotization. Then 1 ml of naphthylethylene diamine dihydrochloride (1% NEDA) was added, mixed and allowed to stand for 30 mins. Sodium nitroprusside in aqueous solution at physiological P^H spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions which can be estimated by the use of Griess Illosvery reaction at 540 nm.

RESULTS AND DISCUSSION

Antioxidant compounds may function as free radical scavengers, initiator of the complexes of pro-oxidant metals, reducing agents and quenchers of singlet oxygen formation[13]. Therefore, the importance of search for natural antioxidants has increased in the recent years so many researchers focused the same[14].

DPPH scavenging activity

DPPH is a stable free radical at room temperature often used to evaluate the antioxidant activity of several natural compounds. The reduction capacity of DPPH radicals was determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants. The percentage of DPPH radical scavenging activity of ethanolic extract of *Albizia procera* presented in Table 1. The ethanolic extract of *Albizia procera* exhibited a maximum DPPH scavenging activity of 63.88% at 1000 µg/ml whereas for Rutin (standard) was found to be 69.33% at 1000 µg/ml. The IC_{50} of the ethanolic extract of *Albizia procera* and Rutin were found to be 460µg/ml and 495µg/ml respectively.

Table 1: DPPH scavenging potential of Ethanol extract of *Albizia procera*

S. No.	Concentration (µg/ml)	% of activity(±SEM)*	
		Ethanol extract of *Albizia procera*	**Standard (Ascorbate)**
1	125	16.77±0.01	19.44±0.30
2	250	38.45±0.04	36.03±0.07
3	500	55.88±0.90	51.33±0.05
4	1000	63.88±0.09	69.33±0.07
		IC_{50}=460 µg/ml	**IC_{50}=495µg/ml**

*All values are expressed as mean ± SEM for three determinations

Superoxide anion scavenging activity

Superoxide is a highly reactive molecule that reacts with various substances produced through metabolic processes. Superoxide dismutase enzymes present in aerobic and anaerobic organisms catalyses the breakdown of superoxide radical[15].

Percentage scavenging of superoxide anion examined at different concentrations of ethanolic extract of *Albizia procera* (125, 250, 500, 1000 µg/ml) was depicted in table 2. The percentage scavenging of superoxide radical surged with the enhanced concentration of plant extracts. The maximum scavenging activity of plant extract and Quercetin at 1000 µg/ml was found to be 67.65% and 62.28% respectively. Superoxide scavenging ability of plant extract might primarily be due to the presence of flavanoids[16]. The IC_{50} value of plant extract and Quercetin was recorded as 260µg/ml and 285µg/ml respectively.

Table 2: Superoxide anion radical scavenging activity of Ethanol extract of *Albizia procera*

S. No.	Concentration (µg/ml)	% of activity(±SEM)*	
		Ethanol extract of *Albizia procera*	**Standard (Quercetin)**
1	125	33.20±0.02	35.44 ± 0.48
2	250	49.73±0.29	47.88 ± 0.49
3	500	60.29±0.70	61.39 ± 0.18
4	1000	67.65±0.05	62.28 ± 0.09
		IC_{50}=260 µg/ml	**IC_{50}=285µg/ml**

*All values are expressed as mean ± SEM for three determinations

Hydroxyl radical scavenging activity

The highly reactive hydroxyl radicals can cause oxidative damage to DNA, lipids and proteins[17]. The percentage of Hydroxyl radical scavenging activity of ethanolic extract of *Albizia procera* presented in Table 3. The ethanolic extract of *Albizia procera* was exhibited a maximum Hydroxyl radical scavenging activity of 64.53 % at 1000 µg/ml whereas for ascorbate (standard) was found to be 55.23 % at 1000 µg/ml. The IC_{50} values of the ethanolic extract of *Albizia procera* and ascorbate were found to be 380µg/ml and 410µg/ml respectively.

Nitric oxide scavenging activity

Nitric oxide is a diffusible free radical which is an important effector molecule in diverse biological systems[18]. So it is worthful to investigate the NO scavenging potential of the plant extract.The reduction of nitric oxide radical by the ethanolic extract of *Albizia procera* and ascorbate was illustrated in Table 4. The maximum scavenging activity of ethanolic extract of *Albizia procera* and ascorbate at 1000 µg/ml were found to be 63.49 % and 55.23% respectively. The IC_{50} values of plant extract and ascorbate were recorded as 565µg/ml and 410µg/ml respectively.

Table 3: Hydroxyl radical scavenging activity of Ethanol extract of *Albizia procera*

S. No.	Concentration (µg/ml)	% of activity(±SEM)*	
		Ethanol extract of *Albizia procera*	**Standard (Ascorbate)**
1	125	13.01±0.01	26.87 ± 0.08
2	250	36.09±0.02	30.30 ± 0.05
3	500	63.58±0.31	60.64 ± 0.02
4	1000	64.53±0.02	55.23 ± 0.01
		IC_{50}=380 µg/ml	**IC_{50}=410µg/ml**

*All values are expressed as mean ± SEM for three determinations

Table 4: Nitric oxide radical scavenging activity of Ethanol extract of *Albizia procera*

S. No.	Concentration (µg/ml)	% of activity(±SEM)*	
		Ethanol extract of *Albizia procera*	**Standard (Ascorbate)**
1	125	25.24±0.05	26.87 ± 0.08
2	250	29.08±0.01	30.30 ± 0.05
3	500	47.80±0.34	60.64 ± 0.02
4	1000	63.49±0.41	55.23 ± 0.01
		IC_{50}= 565g/ml	**IC_{50}=410µg/ml**

*All values are expressed as mean ± SEM for three determinations

CONCLUSION

From the results obtained in the present study, it is concluded that the ethanolic extract of *Albizia procera,* which contains large amounts of phenolic compounds, exhibits high antioxidant and free radical scavenging activities. These *In-vitro* assays indicate that this plant extract is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses. Furthermore, the *In- vivo* antioxidant activity of this extract needs to be assessed prior to clinical use.

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