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Research Article

ANTIOXIDANT POTENTIAL, TOTAL PHENOLIC AND FLAVONOIDS CONTENT OF AERIAL PARTS OF ETHANOLIC EXTRACT OF *Albizia procera (FAMILY: MIMOSOIDEAE)*

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ABSTRACT

The aim of this work was to estimate the total phenolic and flavonoids content, and to evaluate *in-vitro* antioxidant activity of ethanolic extract of *Albizia procera*. The ethanolic extract of *Albizia procera* was showed significant free radical scavenging activity than that of various standards. The greater amount of phenols and flavonoids were found in ethanolic extract of *Albizia procera* than that of other extracts. The radical scavenging activity was found to be concentration dependent manner. Further studies on isolation of constituents from the extract and their biological activities are under investigation.

Keywords: *Albizia procera,* antioxidant activities, phenolics, flavonoids.

INTRODUCTION

The role of free radicals and tissue damage in diseases such as atherosclerosis, heart failure, neurodegenerative disorders, aging, cancer, diabetes mellitus, hypertension and several other diseases are becoming increasingly recognized[1]. Reactive oxygen species (ROS), as well as reactive nitrogen species (RNS), are products of normal cellular metabolism and they are well recognized for playing a dual role as both deleterious and beneficial species, since they can be either harmful or beneficial to living systems[2].Antioxidant supplements or foods rich in medicinal plants may be used to help the human body in reducing oxidative damage by free radicals and active oxygen[1].

Therefore currently, the research interest is focused on the potential role of antioxidants in the treatment and prevention of above diseases. The most commonly used antioxidants at present are butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), propyl gallate (PG) and *tert*-butylhydroquinone (TBHQ). However, they are suspected of being responsible for liver damage and carcinogenesis in laboratory animals[3].Therefore, the development and utilization of more effective antioxidants of natural origin are desired.

Albizia procera is a medium sized deciduous tree, sparingly grown in India. This plant is used traditionally in dropsy, pain, rheumatism, convulsions, delirium, and septicemia[4]. 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[5]. 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[6]. Even through, traditionally, leaves of *Albizia procera* were extensively used for the treatment of variety of wounds[7], and no scientific data in its support is available. Our literature survey revealed that the antioxidant activity of various extracts from 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:

Iron chelating activity[9]

The method of Benzie and strain (1996)[9] was adopted for the assay. The principle is based on the formation of *O*-Phenanthroline-Fe^{2+} complex and its disruption in the presence of chelating agents. The reaction mixture containing 1 ml of 0.05% O-Phenanthroline in methanol, 2 ml ferric chloride (200µM) and 2 ml of various concentrations ranging from 10 to 1000µg was incubated at room temperature for 10 min and the absorbance of the same was measured at 510 nm. EDTA was used as a classical metal chelator. The experiment was performed in triplicates.

Total antioxidant activity (Phosphomolybdic acid method) [10]

The antioxidant activity of the sample was evaluated by the transformation of Mo (VI) to Mo (V) to form phosphomolybdenum complex (Prieto et al., 1999)[10]. An aliquot of 0.4 ml of sample solution was combined in a vial with 4 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The vials were capped and incubated in a water bath at 95^0C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The antioxidant activity was expresses relative to that of ascorbic acid.

FRAP assay[11]

A modified method of Benzie and Strain (1996)[11] was adopted for the FRAP assay. The stock solutions included 300 mM acetate buffer, pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-S-triazine) solution in 40 mMHCl and 20 mM$Fecl_3.6H_2O$. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ and 2.5 ml $Fecl_3.6H_2O$. The temperature of the solution was raised to 37^0 C before using. Plant extracts (0.15 ml) were allowed to react with 2.85 ml of FRAP solution for 30 min in the dark condition. Readings of the colored product (Ferrous tripyridyltriazine complex) were taken at 593 nm. The standard curve was linear between 200 and 1000 µM $Feso_4$. Results are expressed in µM (Fe (II) /g dry mass and compared with that of ascorbic acid.

Estimation of total phenol[12]

0.5 ml of Folins phenol reagent and 2 ml of sodium carbonate (20%). The reaction mixture was kept in boiling water bath for 1 min. the absorbance was measured at 650 nm in a spectrophotometer.

Estimation of total flavonoids[13]

0.5 ml of extract and 4 ml of the vanillin reagent (1% vanillin in 70% conc. H_2SO_4) was added and kept in a boiling water bath for 15 mins.

The absorbance was read at 360 nm. A standard was run by using catechol (110 µg/ml).

RESULTS AND DISCUSSION

Free radical is a molecule with an unpaired electron and is involved in bacterial and parasitic infections, lung damage, inflammation, reperfusion injury, cardiovascular disorders, atherosclerosis, aging and neoplastic diseases[14]. They are also involved in autoimmune disorders like rheumatoid arthritis etc[15].

Iron chelating activity

Iron binding capacity of the ethanolic extract of *Albizia procera* and the metal chelator EDTA at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values were presented in table 1 Maximum chelating of metal ions at 1000µg/ml for plant extract and EDTA was found to be 63.45 ± 0.97% and 63.78±0.03% respectively. The IC_{50} value of plant extract and EDTA was recorded as 420µg/ml and 390µg/ml respectively.

Table 1: Iron chelating activity of Ethanol extract of *Albizia procera*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Ethanol extract of *Albizia procera*	Standard (EDTA)
1	125	14.61 ± 0.24	18.02 ±0.07
2	250	39.58 ± 0.24	47.77±0.07
3	500	53.97 ± 0.38	56.89±0.05
4	1000	63.45 ± 0.97	63.78±0.03
		IC_{50}=420µg/ml	**IC_{50}=390µg/ml**

Total antioxidant activity (Phosphomolybdic acid method)

The percentage of total antioxidant activity of ethanolic extract of *Albizia procera* presented in Table 2. The ethanolic extract of *Albizia procera* exhibited a maximum total antioxidant activity of 59.36±0.01% at 1000 µg/ml whereas for ascorbate (standard) was found to be 55.23 ± 0.01% at 1000 µg/ml. The IC_{50} of the ethanolic extract of *Albizia procera* and ascorbate were found to be 237µg/ml and 410µg/ml respectively.

Table 2: Total antioxidant capacity of Ethanol extract of *Albizia procera*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Ethanol extract of *Albizia procera*	Standard (Ascorbate)
1	125	23.56±0.05	26.87 ± 0.08
2	250	51.63±0.01	30.30 ± 0.05
3	500	56.36±0.03	60.64 ± 0.02
4	1000	59.36±0.01	55.23 ± 0.01
		IC_{50}=237 µg/ml	**IC_{50}=410µg/ml**

FRAP assay

The reducing ability of the ethanolic extract of *Albizia procera* and ascorbate at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values were presented in Table 3. The maximum reducing ability at 1000µg/ml for ethanolic extract and ascorbate were found to be 63.59±0.04% and 55.23 ± 0.01% respectively. The IC_{50} values of methanolic extract and ascorbate were recorded as 253µg/ml and 410µg/ml respectively.

Table 3: FRAP assay of Ethanol extract of *Albizia procera*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Ethanol extract of *Albizia procera*	Standard (Ascorbate)
1	125	31.26±0.06	26.87 ± 0.08
2	250	49.63±0.01	30.30 ± 0.05
3	500	58.47±0.08	60.64 ± 0.02
4	1000	63.59±0.04	55.23 ± 0.01
		IC_{50}=253 µg/ml	**IC_{50}=410µg/ml**

Total phenol

Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups[16]. The total phenolic content of ethanolic extract of *Albizia procera* was presented in Table 4. Ethanolic extract of *Albizia procera* had found higher content of phenolic components.

Table 4: The total Phenolic content of ethanolic extract of *Albizia procera*

S.No	Extracts	Total phenol content (mg/g of Catechol) (±SEM)*
1	Ethanolic extract of *Albizia procera*	3.980±0.006

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

Total flavonoids

The total amount of flavonoids content of ethanolic extract of *Albizia procera* was summarized in Table 5. Flavonoids present in food of plant origin are also potential antioxidants[17]. The higher content of flavonoids was found ethanolic extract of *Albizia procera*.

Table 5: The total flavonoids content of ethanolic extract of *Albizia procera*

S.No	Extracts	Total flavonoids content (mg/g) (±SEM)*
1.	Ethanolic extract of *Albizia procera*	2.651 ± 0.077

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

CONCLUSION

The results of the present study was clearly indicated that the ethanolic extract of *Albizia procera* can be used as easily accessible source of natural antioxidants and as a possible food supplement in pharmaceutical industry. *In vitro* study indicates that these plant extracts is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses. Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the plant extract.

REFERENCES

1. Flora SJ. Role of free radicals and antioxidants in health and disease. Cell Mol Biol 2007; 53: 1-2.
2. Valko M, Izakovic M, Mazur M, Rhodes CJ and Telser J. Role of oxygen radicals in DNA damage and cancer incidence. Mol Cell Biochem 2004; 266: 37-56.
3. Anagnostopoulou MA, Kefalas P, Papageorgiou VP and Boskou D. Radical scavenging activity of various extracts and fractions of sweet orange peel (Citrus sinensis). Food Chem2006; 94:19-25.
4. Asolker LV, Kakkar KK, Chakre OJ. Supplement to glossary of Indian medicinal plants. Part-I (A-K). National Institute of Science Communication, New Delhi: 2000. P. 116-7.
5. Kirthikar KR, Basu DB. Indian Medicinal Plants. Vol. 4.2n ed. Dehradun: Oriental Enterprises; 2000.p. 1255-7.
6. Rajanarayana K, Reddy MS, Chaluvadi MR, Krishna DR. Bioflavonoids classification, pharmacological, biochemical effects and therapeutic potential. Indian J Pharmacol 2001; 33:2-16.
7. Chopda MZ, Mahajan RT. Wound Healing Plants of Jalgaon District of Maharashtra state, India. Ethnobotanical Leaflets 2009; 13:1-32.
8. Harborne J.B. (1984) Phytochemical methods 11 Edn. In Chapman &, Hall. New York: 4-5.
9. Benzie IEF and Strain JJ (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem.239,70-76.

10. Prieto, P., Pineda, M., Aguilar, M (1999). Spectrophotometricquantitation of antioxidant capacity through the formationof a Phosphomolybdenum Complex: Specific applicationto the determination of vitamin E. Anal. Biochem, 269,337-341.
11. Benzie IEF and Strain JJ (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem.239,70-76.
12. Mallick CP and Singh MB. Plant enzymology and Histoenzymology (eds), Kalyani publishers, New Delhi, 1980; 286.
13. Cameron GR, Milton RF and Allen JW. Measurement of flavonoids in plant samples. Lancet, 1943; 179.
14. Roy, H. and Burdon, (1994). *Free Radical Damage and Its Control,* Elsevier Science b. V. Netherlands, p.125
15. Rao, M.S. and Raman, M.V., (2004). *Biochemical Systematics and Ecology*, 32, pp.447-448.
16. Hatano T, Edamatsu R, Mori A, et al. Effect of interaction of tannins with co-existing substances. VI. Effects of tannins and related polyphenols on superoxide anion radical and on DPPH radical. Chemical and Pharmaceutical Bulletin 1989; 37: 2016–2021.
17. Salah N, Miller NJ, Paganga G, Tijburg L, Bolwell GP and Rice Evans C, Polyphenolic flavonoids as scavengers of aqueous phase radicals and as chain-breaking antioxidants, Arch Biochem Biophys, 1995; 322(2): 339-346.