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Research Article

ANTIOXIDANT AND ANTIHEMOLYTIC ACTIVITY OF AN ENDANGERED PLANT SPECIES, *HILDEGARDIA POPULIFOLIA* (ROXB.) SCHOTT & ENDL.

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ABSTRACT

Objective: The present study was aimed at to estimate total content of ascorbic acid, saponins and condensed tannins and to evaluate the antioxidant activity and antihemolytic activity of methanolic extracts of leaf and stem bark parts of *Hildegardia populifolia.*
Methods: Antioxidant activity was carried out by studying nitric oxide scavenging activity, inhibition of β – carotene bleaching activity and antihemolytic activity also studied.
Results: Higher amount of ascorbic acid (93.47 g AA/100 g extract), saponins (18.81 g DE/100g extract) and condensed tannins (0.70 mg LE/g dry sample) was observed in methanolic leaf extract than that of the stem bark extract. Nitric oxide scavenging and β – carotene bleaching activity were relatively higher in stem bark extract (IC_{50} -122.60 and 56.67% respectively). Leaf extract has higher percentage of antihemolytic activity than that of the stem bark extract.
Conclusion: The results of the study confirmed that the studied plant might indeed be potential sources of natural antioxidant and antihemolytic agents.

Keywords: Antioxidant, antihemolytic activity, *Hildegardia populifolia.*

INTRODUCTION

Hildegardia populifolia, an endangered indegenous medium sized tree species, belongs to the family, Sterculiaceae is distributed in dry deciduous forests of Tamil Nadu and Andra Pradesh. The plant extract is used to cure malaria and dog bite [1]. Leaf and stem bark extracts are reported to have antimicrobial [2], antioxidant [3] and antiinflammatory activities [4]. The fiber extracted from the bark is used for domestic purposes. It contained rich varieties of phytochemical constituents like alkaloids, flavonoids, phenols, tannins, terpenoids, steroids, etc [5].

Medicinal plants have many therapeutic potential in which recently there has been an upsurge of interest in identifying the plants that are having antioxidant ability. The main characteristic of an antioxidant is its ability to trap free radicals. Plant derived antioxidants such as polyphenols, carotenoids, flavonoids, phenolics, ascorbic acid (vitamins C) and E have multiple biological effects, including antioxidant activity. Phytochemicals present in plant foods exert health beneficial effects, as they combat oxidative stress in the body by maintaining a balance between oxidants and antioxidants [6]. Many other plant species have been investigated in the search for novel antioxidants but generally there is still a demand to find more information concerning the antioxidant potential of plant species as they are safe and bioactive also. As there is no work in the species, *H. populifolia* on antioxidant and antihemolytic activitities, the present study was carried out in this line.

MATERIALS AND METHODS

Collection of plant material

Leaves and stem bark parts of the species, *Hildegardia populifolia* was collected from Forest Genetics Division, Bhavanisagar, Erode district.

Preparation of plant extract

The shade dried leaves and stem bark of the study species were made into fine powder of 40 mesh size using the pulvarizer separately. Hundred gram of the powder was filled in the filter paper and successively extracted by using 500 mL methanol in soxhlet extractor for 8 to 10 hours [7]. Then the extract was filtered through Whatman No. 1 filter paper to remove all undissolved matter, including cellular materials and other constituents that are insoluble in the extraction solvent.

Determination of ascorbic acid (vitamin C)

It was determined according to the method of Klein and Perry [8]. The dried methanolic extract (100 mg) was extracted with 10 mL of 1% metaphosphoric acid for 45 min at room temperature and filtered through Whatman No. 4 filter paper. The filtrate (1 mL) was mixed with 9 mL of 2,6-dichlorophenolindophenol and the absorbance was measured within 30 min at 515 nm against a blank. Content of ascorbic acid was calculated on the basis of the calibration curve of authentic L-ascorbic acid (0.020 – 0.12 mg/mL). The assays were carried out in triplicate; the results were mean values ± standard deviations and expressed as mg of ascorbic acid/g of extract.

Determination of total saponins

Total saponin content was determined based on vanillin-sulphuric acid colorimetric reaction with some modifications [9]. 50 µL of sample was added with 250 µL of distilled water and about 250 µL of vanillin reagent (800 mg of vanillin in 10 mL of 99.5% ethanol). Then 2.5 mL of 72% sulphuric acid was added. The content was mixed well and kept in a water bath at 60ºC for 10 minutes. Then it was cooled in ice cold water and the absorbance was read in a spectrophotometer at 544 nm. The values were expressed as diosgenin equivalents (DE) derived from a standard curve.

Determination of condensed tannin

200 mg of the plant sample was taken in a test tube and to this 10 mL of 70% acetone was added. The contents were placed over night in a shaker. Then they were centrifuged at 5000 rpm for 5 min and the supernatant was collected. 0.5 mL of the supernatant was pipetted out into a test tube, 3.0 mL of the butanol-HCl reagent (95:5 v/v) and 0.1 mL of ferric reagent (2% ferric ammonium sulfate in 2N HCl) were added sequentially. The contents were vortexed and the mouth of each test tube was covered with a glass marble, and then kept in a heating block adjusted to 97 to 100°C for 60 min. After cooling the test tubes, the absorbance was recorded at 550 nm. Suitable blank was subtracted, which is usually the absorbance of

unheated mixture [10]. Condensed tannins (% in dry matter) as leucocyanidin equivalent (LE) were calculated by the formula:

Condensed tannins = (Absorbance at 550 nm × 78.26 × Dilution factor) / (% dry matter).

Nitric oxide scavenging activity

Nitric oxide scavenging activity was determined according to the method suggested by Sreejayan and Rao [11]. Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacted with oxygen to produce nitrite ions, which can be estimated using the Griess reagent. Scavengers of nitric-oxide act against oxygen, leading to reduced production of nitrite ions. In brief, 3.0 mL of 10 mM sodium nitroprusside in phosphate buffered saline was mixed with different concentrations of the extract and incubated at 25°C for 150 min. 0.5 mL of the incubated solution was removed and diluted with 0.5 mL of Griess reagent (1% sulphanilamide, 2% orthophosphoric acid and 0.1% N-1-naphthylethylenediamine dihydrochloride). The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with N-1-naphthylethylenediamine dihydrochloride were measured at 546 nm and percentage scavenging activity was measured with reference to standards. IC_{50}, an inhibitory concentration was estimated from the per cent inhibition plot. Quercetin and BHT were used as standards.

Inhibition of β – carotene bleaching

The antioxidant capacity of the methanolic leaf and stem bark extracts of *H. populifolia* was evaluated using β – carotene-linoleate model system [12]. 1.0 mg of β – carotene was dissolved in 10 mL of chloroform and mixed with 20 mg of linoleic acid and 200 mg of Tween – 40 emulsifier mixture. Chloroform was completely removed at 45°C under vacuum using a rotary vacuum evaporator. 50 mL of oxygenated distilled water was added slowly to the semi-solid residue with vigorous agitation, to form an emulsion. A 5.0 mL aliquot of the emulsion was dispensed into tubes containing 100 µg/mL of the sample extract. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm using a spectrophotometer. Subsequent absorbance readings were recorded at 15 min intervals by keeping the sample tubes in a water bath at 50°C until the visual colour of β – carotene in the control sample disappeared (about 120 min). A blank, devoid of β – carotene was prepared for background subtraction. Quercetin and BHT were used as standards. All determinations were performed in triplicate and averaged.

The antioxidant activity (AA) was measured in terms of reduction in β – carotene bleaching activity using the following formula:

$$AA\ (\%) = [1- (A_s^0 – A_s^{120}) / (A_c^0 – A_c^{120})] \times 100$$

Where,

A_s^0 is the absorbance of sample at 0 min, A_s^{120} is the absorbance of sample at 120 min, A_c^0 is the absorbance of control sample at 0 min and A_c^{120} is the absorbance of control sample at 120 min.

Antihemolytic activity

The preparation of erythrocyte membrane ghost and the subsequent determination of the antioxidant activity of the extracts on the chemically induced lipid peroxidation were performed according to the method set forth by Naim *et al*. [13]. The erythrocytes from cow blood were separated by centrifugation (2000 rpm for 10 min) and washed with saline phosphate buffer (0.9 g of sodium chloride dissolved in 100 mL of 0.2 M phosphate buffer of pH 7.4) until the supernatant becomes colourless. The erythrocytes were then diluted with saline phosphate buffer to give 4% (v/v) suspension. 500 µg of extract/ mL of saline phosphate buffer was added to 2.0 mL of erythrocyte suspension and the volume was made up to 5.0 mL with saline phosphate buffer. This mixture was pre-incubated for 5 min and then 0.5 mL of H_2O_2 solution of appropriate concentration in saline buffer was added. The concentration of H_2O_2 in the reaction mixture was adjusted so as to bring about 90% hemolysis of blood cells after 240 min. After the incubation time, the reaction mixture was centrifuged at 1500 rpm for 10 min and the extend of hemolysis was determined by measuring the absorbance at 540 nm corresponding to haemoglobin liberation. Natural and synthetic standards at the same concentration as sample extract were used for comparison.

The per cent hemolysis inhibition was calculated using the formula:

Inhibition percentage = [A control –A sample / A control] × 100.

RESULTS AND DISCUSSION

Ascorbic acid

Total content of ascorbic acid determined in leaf and stem bark parts of *Hildegardia populifolia* is presented in Table 1 and the data revealed that study plant contained considerable amount of ascorbic acid in both leaf and stem bark parts. However, higher amount of ascorbic acid was found in methanolic leaf extract (93.47 g AA/100 g extract) than that of the methanolic stem bark extract (83.66 g AA/100 g extract). Ascorbic acid is needed for the growth and repair of tissues in all parts of human body and it is also one of the antioxidants found in plants [14].

Table1: Total contents of ascorbic acid, saponins and ondensed tannins in methanolic leaf and stem bark parts of *Hildegardia populifolia.*

S. No.	Parts	Ascorbic acid (g AA/100 g extract)	Total saponins (g DE/100g extract)	Condensed tannins (mg LE/g dry sample)*
1	Leaf	93.47±2.37^a	18.81±1.05^a	0.70±0.09^a
2	Stem bark	83.66±3.51^b	15.21± 0.68^b	0.28±0.05^b

AA – Ascorbic acid; DE – Diosgenin equivalent
LE - Leucocyanidin equivalent; *70% acetone
Values are expressed as mean±SD (n=6).
Values within the same column not sharing common superscript letters (a,b) differ significantly at p<0.05 by DMRT

Table2: Antioxidant and antihemolytic activity of methanolic leaf and stem bark extracts of *Hildegardia populifolia.*

S. No.	Extract	Antioxidant activity		Antihemolytic activity (%)
		Nitric oxide scavenging activity (IC_{50} µg/mL)	β-carotene activity (%)	
1	Leaf	130.51±1.34^d	44.55±1.34^c	71.90±2.41^a
2	Stem bark	122.60±2.19^c	56.67±2.08^b	52.63±1.46^b
3	Quercetin	50.82±1.25^b	57.7±1.37^a	70.18±1.73^{ab}
4	BHT	38.47±1.09^a	42.0±1.42^d	7.65±0.34^c

Values are expressed as mean±SD (n=6).
Values within the same column not sharing common superscript letters (a-d) differ significantly at p<0.05 by DMRT

Saponins

The results of the study report that the species, *H. populifolia* contained sizeable amount of saponins in these parts. Similar to the findings for ascorbic acid, the methanolic leaf extract (18.81 g DE/100g extract) has more amount of saponins than that of stem bark extract (15.21 g DE/100g extract) (Table 1). The saponins content make the leaves an important source of detergents, surface active agents used in industrial applications and also possesses beneficial health effects [15].

Condensed tannins

The highest content of condensed tannins (Proanthocyanidins) was found in leaf (0.70 mg LE/g) (Table 1). Condensed tannin was exists as oligomers and polymers of three-ring flavanols [16]. Proanthocyanidins has various biological activity and it has greater effects on arteriosclerosis [17] and free radical scavenging ability [18].

Antioxidant activity

Nitric oxide scavenging activity

The calculated IC_{50} values obtained from the plots of concentration-dependent inhibition of NO were presented in Table 2. The extracts registered excellent to moderate antioxidant activity with their IC_{50} values ranged between 38.57 and 130.51µg/mL respectively. The highest measurable activity was found in the stem bark extract (122.60 µg/mL) followed by leaf (130.51µg/mL). NO is a free radical which is an effective inhibitor of several physiological processes such as smooth muscle relaxation and neuronal signaling. Further, it is a diffusible free radical that plays many roles as an effectors molecule in diverse biological systems including neuronal messenger, vasodilatation and antimicrobial and antitumor activities [19].

Inhibition of β – carotene bleaching

H. populifolia leaf and stem bark extracts were evaluated for their β-carotene/linoleic acid bleaching assay the results of the study report that at the concentration of 100 µg/mL in the final reaction mixture, both the parts were inhibited peroxidation of linoleic acid and subsequent bleaching of β-carotene to various degrees (Table 2). Apparently, the most effective in this line were the *H. populifolia* stem bark (56.70%) followed by leaf 44.55%. Furthermore, these values were comparably higher than that of the synthetic antioxidants used. In this assay, oxidation of linoleic acid, an unsaturated fatty acid occurs due to the production of reactive oxygen species formed from halogenated water. The reactive oxygen species will initiate β-carotene oxidation leading to discolouration [20].

Antihemolytic activity

The protective effect of *H. populifolia* leaf and stem bark extracts against H_2O_2 mediated hemolysis was investigated. In general, both extracts performed adequate antihemolytic activity in terms of percentage inhibiting activity which was ranging from 52.37 to 71.00% (Table 2). It seems that the inhibition of hemolysis of leaf extract was observed to be higher than those of the standard antioxidants tested. However, leaf extracts of *H. populifolia* was performed well than the bark extract in offering protection against erythrocyte hemolysis. The oxidation of erythrocytes serves as good model for the oxidative damage of biological membranes [21]. When red blood cells were treated with extracts along with H_2O_2, marked reduction in hemolysis was found. This may be due to the radical scavenging activity of the bioactive compounds present in the extracts.

From the results of the study it is concluded that the traditional medicinal tree, *H. populifolia* possessed considerable level of antioxidant and antihemolytic properties. Therefore, this species can be used as a potential source of antioxidant drug. However, *in vitro* studies are suggested to confirm the property.

REFERENCES

1. Varaprasad B, Katikala PK, Naidu KC, Penumajji S. Antifungal activity of selected plant extracts against pytopathogenic fungi *Aspergillus niger*. *Indian Journal of Science and Technology* 2009; 2(4): 87-90.
2. Saradha M, Paulsamy S. Antibacterial activity of leaf and stem bark extracts of the endangered tree species, *Hildegardia populifolia* (Roxb.) Schott and Endl. (Sterculiaceae). *Journal of Research in Antimicrobials* 2012a; 1: 023-027.
3. Saradha M, Paulsamy S. *In vitro* antioxidant activity and polyphenol estimation of methanolic extract of endangered medicinal tree species, *Hildegardia populifolia* (Roxb.) Schott & Endl. *International Journal of Phytomedicine* 2012b; 4: 362-368.
4. Saradha M, Paulsamy S. Antinociceptive and antiinflammatory activities of stem bark of an endangered medicinal plant, *Hildegardia populifolia* (Roxb.) Schott and Endl. *International Journal of Pharma and Bio Sciences* 2013; 4(3): 30-36.
5. Saradha M. Development of propagation strategies and evaluation of medicinal properties for the endangered plant species, *Hildegardia populifolia* (Roxb.) Schott & Endl. (Sterculiaceae). Ph.D thesis submitted to Bharathiar University, Coimbatore. Tamilnadu, 2013.
6. Scalbert A, Manach C, Morand C, Remesy C, and Jimenez L. Dietary polyphenols and the prevention of diseases. *Critical Reviews in Food Science and Nutrition* 2005; 45(4): 287–306.
7. Gafner F, Msonthi JD, Hostettmann K. Molluscicidal saponins from *Talinum tenuissimum* Dinter. *Helvet. Chim. Acta*. 1985; 68: 555-558.
8. Klein BP, and Perry AK. Ascorbic acid and Vitamin A activity in selected vegetables from different geographical areas of the United States. *J. Food Sci* 1982*;* 47: 941-945.
9. Makkar HPS, Siddhuraju P, and Becker K. Plant Secondary Metabolites. 1st Edn. Humana Press, Totowa, New Jersey, USA; 2007. p. 130.
10. Porter LJ, Hrstich LN, and Chan BG. The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. *Phytochemistry* 1986; 25: 223–230.
11. Sreejayan N, and Rao MNA. Nitric oxide scavenging by curcuminoids. *Journal of Pharmacyand Pharmacology* 1997; 49: 105–107.
12. Taga MS, Miller EE, and Pratt DE. Chia seeds as a source of natural lipid antioxidants. *Journal of the American Oil Chemists' Society* 1984; 61: 928-931.
13. Naim M, Gestener B, Bondi A, and Birk Y. Antioxidant and antihemolytic activities of soyabean isoflavones. *Journal of Agricultural and Food Chemistry.* 1976; 24: 1174-1177.
14. Roman L, Stanila A, and Stanila S. Bioactive compounds and antioxidant activity of *Rosa canina* L. biotypes from spontaneous flora of Transylvania. *Chemistry Central Journal* 2013; 7: 1-10.
15. Shi J, Arunasalam K, Yeung D, Kakuda Y, Mittal G, and Jiang Y. Saponins from Edible Legumes: Chemistry, Processing, and Health Benefits. *Journal of Medicinal Food* 2004; **7**(1): 67-78.
16. Hernes PJ, and Hedges JI. Determination of condensed tannin monomers in environmental samples by capillary gas chromatography of acid depolymerization extracts. *Anal. Chem* 2000; 72: 5115-5124.
17. Zivkovic J, Mujic I, Zekovic Z, Nikolic G, Vidovic S, Mujic A. Extraction and analysis of condensed tannins in *Castanea sativa* Mill. *Journal of Central Europeon Agriculture* 2009; 10(3): 283-288.
18. Ricardo-da-Silva JM, Darmon N, Fernandez Y, Mitjavila S. Oxygen free radical scavenger capacity in aqueous models of different procyanidins from grape seeds. *Journal of Agricultural and Food Chemistry* 1991; 39: 1549-1552.
19. Hagerman AE, Riedl KM, Jones GA, Sovik KN, Ritchard NT, Hartzfeld PW, and Riechel TL. High molecular weight plant polyphenolics (tannins) as biological antioxidants. *J. Agric. Food Chem* 1998; 46: 1887-1892.
20. Gutierrez RMP, Luna HH, and Garrido SH. Antioxidant activity of *Tagates erecta* essential oil. *J. Chil. Chem. Soc* 2006; 51: 883-886.
21. Niki E. Antioxidants in relation to lipid peroxidation. *Chemical Physical Lipids* 1982; 44: 227-253.