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Antioxidant and Radical Scavenging Effect of Ipomoea pes-caprae Linn. R.Br

Umamaheshwari .G, T.Ramanathan*, and R. Shanmugapriya

C.A.S. in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai 608502, Tamil Nadu, India.

Telephone: 04144-243223 Extn: 213, Mobile: + 91 98941 75200: Fax: 04144-243555.
***Corres.author: drtrcasmb@gmail.com**

Abstract: Numerous diseases are associated with oxidative stress caused by free radicals. Current research is directed towards finding naturally-occurring antioxidants of the plant origin. The aim of the present study was to evaluate the *in vitro* antioxidant activities of *Ipomoea pes-caprae* extract. 80% ethanol extract of *Ipomoea pes-caprae* was studied *in vitro* for total antioxidant activity, for scavenging of hydroxyl radicals, nitric oxide and phenolic contents. Subsequent quantification showed the presence of 0.84% (*m/m*) phenolics (calculated as gallic acid) and 0.23% (*m/m*) flavonoids calculated as catechin equivalents per 100 g of fresh mass. The presence of phenolic compounds prompted us to evaluate its antioxidant activity. The present study provides evidence that the ethanol extract of *Ipomoea pes-caprae* is a potential source of natural antioxidants.
Keywords: antioxidants, ethanol extract, free radical scavenging, *Ipomoea pes-caprae.*

INTRODUCTION:

Ipomoea pes-caprae (Convolvulacea) is a valuable medicinal plant, distributed in the tropics and subtropics regions and uses in folk and tribal medicines. *Ipomoea pes-caprae* (L.) R. Br.-bayhops is a pan tropical, trailing vine that routinely colonizes on sand dunes. It grows just above the high tide line along coastal beaches, forming large mats that assist in stabilizing sands. This is an evergreen perennial with a large, thick root that can be 10 ft long and 2 inch in diameter. The entire plant is glabrous and somewhat fleshy. The stem runs along the ground rooting at the nodes with only the flowers being erect.[1,2] The plant contains active component likes naphthalenone, (-)-mellein, eugenol, 4-vinyl-guaiacol. lipophilic glycosides, 2-methylpropanoic, (2S)-methylbutyric, n-hexanoic, n-decanoic, and n-dodecanoic acids. (Manigaunha). *Ipomoea pes-caprae* (Convolvulacea) plant is highly reputed in folk and tribal medicines. Known by different names like Railroad vine, coast morning glory, goat's-foot morning glory, salsa-da-praia etc, grows on sand dunes and beaches above the high tide line in tropical and subtropical regions throughout the world. *Ipomoea pes-caprae* has the potential in scavenging free radicals and can be a vital source of antioxidant phytochemicals [3]and good antinociceptive property due to the

presences of compounds, such as glochidone, betulinic acid, alpha- and beta-amyrin acetate, isoquercitrin in the writhing test and formalin test in mice, and to treat dolorous processes. [4]. Leaves are used in rheumatism, and as stomachic and tonic. The extract of the leaves have the astringent, diuretic and laxative properties. It has biological activity like antioxidant, analgesic and anti-inflammatory, antispasmodic, anticancer, antinociceptive, antihistaminic, insulogenic and hypoglycemic [5]. It is also used in inhibition of platelet aggregation, diarrhea, vomiting, and piles [6]. The antioxidant compounds in a typical diet are mostly derived from plant sources and polyphenolic components of higher plants act as antioxidant or other mechanisms contributing to anti-carcinogenic action [7]. The antioxidants from natural sources are given preference than synthetic sources and the DPPH method has been widely applied for estimating antioxidant activities recently [8]. Research also indicates that the DPPH test is particularly suitable for the evaluation of antioxidant activity of crude extracts [9]. Besides, evaluation of the antioxidant polyphenols from less known ethno-medicinal plant from Asia are urgently needed [10].

MATERIALS AND METHODS

The present study was carried out to evaluate the Antioxidant properties and radical scavenging effect from the plant of *Ipomoea pes-caprae*. Hence it is an imperative need to do the following steps

PREPARATION OF PLANT EXTRACTS

The plant sample was collected from Parangipettai, Cuddalore District, Tamilnadu. The dried leaves (1.0 kg) were powdered by electrical blender. The soxhlet apparatus which contain the 80% of ethyl alcohol used for the 20 gm of plant sample followed by the standard procedure [11]. The plant material was loaded in the inner tube of the soxhlet apparatus and then fitted into a round bottomed flash containing ethanol separately. The solvents were boiled gently (40°C) over a heating mantle using the adjustable rheostat. The extraction was continued for 8 h and the solvent was removed at the reduced pressure with the help of rotary vacuum evaporator to yield a viscous dark green residue (12.5g) of ethyl alcohol of leaf extracts.

ESTIMATION OF TOTAL PHENOLIC CONTENT

Total phenolic content was determined using Folin-Ciocalteu (FC) reagent according to the method of Singleton and Rossi with a slight modification. Briefly, the plant extract (0.1 ml) was mixed with 0.75 ml of FC reagent (previously diluted 1000-fold with distilled water) and incubated for 5 min at 22°C, then 0.06% Na_2CO_3 solution was added. After incubation at 22°C for 90 min, the absorbance was measured at 725 nm. All tests were performed six times. The phenolic content was evaluated from a gallic acid standard curve.

ESTIMATION OF TOTAL FLAVONOID

The total flavonoid content was determined with aluminum chloride ($AlCl_3$) according to a known method (12) using quercetin as a standard. The *Ipomoea pes-caprae* extract (0.1 ml) was added to 0.3 ml distilled water followed by $NaNO_2$ (0.03 ml, 5%). After 5 min at 25°C, $AlCl_3$ (0.03 ml, 10%) was added. After a further 5 min, the reaction mixture was treated with 0.2 ml 1 mM NaOH. Finally, the reaction mixture was diluted to 1 ml with water and the absorbance was measured at 510 nm. All tests were performed six times. The flavonoid content was calculated from a quercetin standard curve.

DPPH FREE RADICAL SCAVENGING ACTIVITY

The free-radical scavenging activity of *Ipomoea pes-caprae* extract was measured by the decrease in absorbance of ethanol solution of DPPH (13). A stock solution of DPPH (33 mg L^{-1}) was prepared in methanol and 5 mL of this stock solution was added to 1 mL of the *Ipomoea pes-caprae* extract solution at different concentrations (200, 400, 600, 800 and 1000 mg mL^{-1}).After 30min,absorbance was measured at 517nm and compared with the standards, *i.e.*, ascorbic acid, BHA and a-tocopherol (10–50mg mL^{-1}). Scavenging activity was expressed as the percentage inhibition.

HYDROXYL RADICAL SCAVENGING ACTIVITY

Ethanol extract at different concentrations was placed in a test tube and evaporated to dryness. One mL of iron-EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5 mL of 0.018% EDTA, 1 mL of DMSO -0.85%, *V/V*, in 0.1 mol L–1 phosphate buffer, pH 7.4 and 0.5 mL of 0.22% ascorbic acid were added to each tube (14). The tubes were capped tightly and heated in a water bath at 80–90 °C for 15 min. The reaction was

terminated by adding 1 mL of ice-cold TCA (17.5% *m/V*). Three ml of Nash reagent (75.0 g ammonium acetate, 3 mL glacial acetic acid and 2 mL acetyl acetone were mixed and water was added to a total volume of 1 L) was added to each tube; the tubes were left at room temperature for 15 min for colour development. The intensity of the yellow colour formed was measured at 412 nm against a blank of the reagent. Percentage inhibition was determined by comparing the results of the test and standard compounds.

NITRIC OXIDE SCAVENGING ACTIVITY

Nitric oxide scavenging activity was measured spectrophotometrically (15). Sodium nitroprusside (5 mM L^{-1}) in phosphate buffered saline pH 7.4, was mixed with different concentrations of the extract (200–1000 mg mL–1) prepared in methanol and incubated at 25 °C for 30 min. A control without the test compound, but with an equivalent amount of methanol, was taken. After 30 min, 1.5 mL of the incubated solution was removed and diluted with 1.5 mL of Griess reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% N-1- naphthylethylenediamine dihydrochloride). Absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with N-1- naphthylethylene diamine dihydrochloride was measured at 546 nm and the percentage scavenging activity was measured with reference to the standard.

STATISTICAL ANALYSIS

Values were represented as mean ± SD of three parallel measurements and data were analyzed using the t-test.

RESULTS AND DISCUSSION

From the results on the total phenolic content, it was found that there were 0.84% of gallic acid equivalents of phenolic compounds while the total flavonoid content was 0.23% of catechin equivalent of fresh mass of *Ipomoea pes-caprae* extract. The results of antioxidant and free radical scavenging activity are given in Table 1. The free radical scavenging activity was evaluated by using various *in vitro* assays. DPPH radical was used as a substrate to evaluate the free radical scavenging activity of *Ipomoea pes-caprae* extract. The scavenging effect of *Ipomoea pes-caprae* extract on the DPPH radical was

56.12±2.4 % ($p < 0.005$), at a concentration of 1000 mg mL^{-1} compared to the scavenging effects of ascorbic acid, BHA and a-tocopherol at 50 mg mL^{-1} of 58.11±

1.2, 51.12± 1.1 and 37.12± 0.2 ($p < 0.05$) respectively. Hydroxyl radicals are the major active oxygen species that cause lipid oxidation and enormous biological damage (L. W. Aurand). The percentage of hydroxyl radical scavenging increased with the increasing concentration of *Ipomoea pes-caprae* extract. Nitric oxide (NO) is a potent pleiotropic mediator of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity [16]. The percentage inhibition of nitric oxide generation by *Ipomoea pes-caprae* at 1000 mg mL^{-1} concentration was found to be 29.3± 3.3 % ($p < 0.005$). On the other hand, ascorbic acid at 50 mg mL^{-1} concentration showed 14.2± 0.8 % ($p < 0.05$) inhibition of nitric oxide.

CONCLUSION

Free radical scavenging effect of *Ipomoea pes-caprae* increases with increasing concentration and maximum antioxidant activity was observed at 1000 mg mL^{-1}. Antioxidant activity may be due to phenolic compounds in *Ipomoea pes-caprae* but further work should be done on the isolation and identification of other antioxidant components of *Ipomoea pes-caprae.*

Table 1.Antioxidant profile of *Ipomoea pes-caprae* extract

Sample	Concentration	DPPH radical scavenging (%)	Hydroxyl radical scavenging (%)		Nitrite radical scavenging (%)	
Ipomoea pes-caprae	1000	56.12±2.4	38.2	1.4	29.3	3.3
Ascorbic acid	50	58.11±1.2	-	32.1	14.2	0.8
BHA	50	51.12±1.1	50.4	1.3	-	1.4
Tocopherol	50	37.12±0.2	-	-	-	-

A Mean ± SD, $n = 3$

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