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Antioxidant and Antiinflammatory Activities of Different Solvent Extracts and Isolated Compounds of *Ipomoea pes-caprae* (L) Sweet of Sunderban Mangrove Eco-complex

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In the present study, we carried out for the first time the antioxidant and antiinflammmatory activities of the different solvent extracts of the plant *Ipomoea pes-caprae* (L) sweet grown up in the marine enviornment of the Sunderban Mangrove eco-complex. It was observed that chloroform and ethyl acetate solvent extracts obtained from the methanolic extract of the plant had significant antioxidant and antiinflammatory activities. To trace the molecules responsible for these bioactivities, the chemical investigation of the plant yielded four pure compounds, 7-hydroxy-6-methoxycoumarin; 5,7,4'-trimethoxykaempferol; 3,7,8,3'4'-pentahydroxy flavone; *trans*-[3-(4'-hydroxyphenyl)-2-propenoic acid] which were identified by spectral methods. These compounds when tested individually, showed appreciable radical scavenging activities.

Key Words: *Ipomoea pes-caprae*, Antioxidant activity, Antiinflammatory activity.

INTRODUCTION

Ipomoea pes-caprae (L) sweet belonging to the family Convolvulaceae are widely distributed in tropical and subtropical countries. Some of the species belonging to the genus *Ipomoea* are frequently used in folk medicine for the treatment of several diseases[1,2]. It had been employed as a herbal remedy in countries like Brazil, Mexico, Thailand, India to cure inflammations, colonic, diuretic disorders, gonorrhoea, dolorous processes[3,4]. *I. pes-caprae* is known as Rinonina in Mexico which is used as infusions for kidney ailments and as decoctions to treat functional digestive disorders, colic, dysentery, internal-external pain and inflammatory conditions[5]. Pre-clinical and clinical investigations have confirmed some pharmacological properties[6-9] of this plant, but no systematic pharmacological and chemical work was done on this species, particularly grown up in the struggling marine environment of the Sunderban mangrove eco-complex where it is used for the prevention of rheumatoid arthritis[10]. This prompted the present investigator to study the antioxidant and antiinflammatory activities of the plant to establish the scientific basis of such application and to identify the chemical entities responsible for such activities.

EXPERIMENTAL

The UV spectra were recorded in UV-visible spectrophotometer (Ultrospec 2000, Pharmacia Biotech). IR spectra were obtained in KBr on a Perkin-Elmer 599 B spectrophotometer. The NMR spectra were recorded in $CDCl_3/MeOD_4$ using a Bruker AV-300 spectrometer (300 MHz for ^1H NMR and 75 MHz for ^{13}C and DEPT). Electron-impact mass spectrometry (EIMS), FAB and HRMS data were recorded using Shimadzu-QP5050A, JEOL JMS600 and Qtof Micro YA263, respectively.

The aerial part of *Ipomoea pes-caprae* was collected from Sagar Island (21º56'N, 88º16'E) of the Sunderban mangrove eco-complex (West Bengal, India) during December 2008. The voucher specimen (CU1/046) was authenticated by Botanical Survey of India (BSI), Howrah, West Bengal, India and preserved in our laboratory.

Extraction, isolation and characterization: The air-dried and powdered aerial part of *I. pes-caprae* (750 g) was soaked in methanol (5.5 L) for 48 h at room temperature. The methanol extract of the plant was filtered and concentrated under reduced pressure in a rotary evaporator (Hanshin, Rotavapour) to get the crude oily extract (84.83 g). The crude extract was then fractionated successively with *n*-hexane,

chloroform, ethyl acetate and water. The resulting fractionated extracts were concentrated and dried to get *n*-hexane extract (17.03 g), chloroform extract (3.63 g), ethyl acetate extract (11.46 g) and water extract (22.67g). The chloroform extract was subjected to column chromatography over silica gel (60-120 mesh), eluted with different solvents of increasing polarity and four pure compounds **I-IV** were isolated. The structures of the compounds, were settled as 7-hydroxy-6-methoxycoumarin (**I**), 5,7,4'-trimethoxykaempferol (**II**), 3,7,8,3,'4'-pentahydroxyflavone (**III**) and *trans*-[3-(4'-hydroxyphenyl)-2-propenoic acid] (**IV**) by the exhaustive application of UV, IR, ^{1}H NMR, ^{13}C NMR, DEPT and mass spectral studies and comparing the data with those described in the literature[11-14].

Compound I: Scopoletin (7-hydroxy-6-methoxycoumarin) 8.6 mg; white crystalline; $C_{10}H_8O_4$ UV λ_{max} (MeOH): 204 (4.138) nm, 228 (3.824) nm, 252 (3.394) nm, 297 (3.384) nm, 344 (3.728) nm; IR (KBr, ν_{max}, cm^{-1}): 3339, 1704, 1565, 1509; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.59 (1H, d, *J* = 9.5 Hz, H-4), 6.88 (1H, s, H-8), 6.82 (1H, s, H-5), 6.26 (1H, d, *J* = 9.5 Hz H-3), 3.95 (3H, s, -OMe); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 161.38 (C=O), 150.3 (C-8a), 149.7 (C-7), 144.01 (C-5a), 143.23(C-3), 113.4(C-4), 111.5(C-6), 107.52 (C-8), 103.21(C-5), 56.43 (-OCH_3); mass: m/z 193 $[M + H]^+$, 177, 164, 149, 135, 121, 105, 71.

Compound II: 5,7,4'-Trimethoxykaempferol; $C_{18}H_{16}O_6$; 6.8 mg; light yellow amorphous solid UV λ_{max} (MeOH): 257 (3.278), 296 (3.041) nm, 356 (3.204); IR (KBr, ν_{max}, cm^{-1}): 3463, 1732, 1609, 1511, 1460; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.17 (2H , dd, *J* = 7 and 2 Hz, H-2',H-6'), 7.03 (2H, dd, *J* = 9 and 2 Hz, H-3',H-5'), 6.55 (1H, d, *J* = 2.2 Hz H-8),6.35 (1H, d, *J* = 2.2 Hz H-6), 3.98 (3H, s, -OMe), 3.92 (3H, s, -OMe), 3.89 (3H, s, -OMe); mass: m/z 328 $[M + H]^+$, 299, 282, 164, 135, 79.

Compound III: 3,7,8,3'4'-Pentahydroxy flavone $C_{15}H_{10}O_7$; 6.6 mg; light yellow amorphous solid UV λ_{max} (MeOH): 203 (4.307), 215 (4.041) nm, 323 (4.149); IR (KBr, n_{max}, cm^{-1}): 3163, 1702, 1606, 1509, 1457; ^{1}H NMR (300 MHz, $MeOD_4$) δ: 7.81 (1H , d, *J* = 9.5 Hz, H-5), 7.41 (1H, d, *J* = 8.5 Hz, H-5'), 6.75 (1H, dd, *J* = 8.5 and 2.2 Hz H-6'),6.67 (1H, d, *J* = 2.2 Hz H-2'), 6.14 (1H, d, *J* = 9.5 Hz, H-6); mass: m/z 302 $[M^+ - 1]$, 185.

Compound IV: *Trans*-[3-(4'-hydroxyphenyl)-2-propenoic acid]; $C_9H_8O_3$; 6.3 mg; white amorphous solid; UV λ_{max} (MeOH) 222 (3.633) nm, 288 (3.748) nm; IR (KBr, ν_{max}, cm^{-1}) 3437, 2931, 1664, 1596, 1511, 1434 and 981; ^{1}H NMR (300 MHz, $MeOD_4$) δ 7.49 (1H , d, *J* = 15.9 Hz, H-3), 7.34 (2H, d, *J* = 8.6 Hz H-2',H-6') 6.70 (2H, d, *J* = 8.5 Hz H-3', H-5'), 6.18 (1H, d, *J* = 15.9 Hz H-2); mass: m/z 164 $[M + H]^+$, 147, 119, 118, 91, 65.

Antioxidant activity

Total phenolic content: The total phenolic content of crude methanolic extract and four solvent extracts (hexane, chloroform, ethyl acetate and aqueous) was determined according to standard protocol[15]. Samples were introduced into test tubes; 1.0 mL of Folin-Ciocalteu reagent and 0.8 mL of sodium carbonate (7.5 %) were added. The tubes were mixed and allowed to stand for 0.5 h. Absorption at 765 nm was measured (Ultrospec 2000 UV-visible spectrophotometer, Pharmacia Biotech, USA). The total phenolic content was expressed as gallic acid equivalents (GAE) in microgram per mg of extract.

DPPH Radical scavenging activity: The DPPH radical scavenging activity of the crude extract, four solvent extracts and isolated four compounds was determined according to standard protocol[15]. The free radical scavenging activity of different extracts and butylated hydroxyl toluene (BHT) as positive control was determined using the stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl). Aliquots (20-100 mL) of the tested sample were placed in test tubes and 3.9 mL of freshly prepared DPPH solution (25 mg/L) in methanol was added in each test tube and mixed. 0.5 h later, the absorbance was measured at 517 nm. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenged}(\%) = \left\{\frac{(A_c - A_t}{A_c}\right\} \times 100$$

where A_c is the absorbance of the control reaction and A_t is the absorbance in presence of the sample of the extracts. The antioxidant activity of the extract was expressed as IC_{50}. Each value was determined from regression equation.

Antiinflammatory activity

Animal: Adult male inbred albino rats (Wister Strain) weighing between 150-175 g and male mice weighing between 20-22 g were used for anti-inflammatory and acute toxicity study, respectively. They were fed on commercial diet (Hindustan Lever) and water ad libitum. All the animals were acclimatized for a week before use. The room temperature was maintained at 25 ± 1 ºC. The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC Reg. 507).

Acute toxicity: Methanolic extract and four solvent extracts (hexane, chloroform, ethylacetate and aqueous) were fed and injected to the mice orally and intraperitoneally to determine lethality or morbidity within 3 days. The dose was given in progressive manner[16].

Study of antiinflammatory activity using carrageenan induced paw edema in rat: Acute inflammation was induced by carrageenan according to the model of Winter *et al.*[17] with slight modification[18]. For this purpose, 0.1 mL of 1 % suspension of carrageenan in normal saline was injected into the subplantar tissues of right hind paw in rats. The right paw volume was plethysmometrically (Techno, India) measured at 0 and 3 h after carrageenan injection. The test extracts were administered at dose of 250 and 500-mg/kg body weight, orally, 1 h before experiment. Diclofenac sodium (10 mg/kg, p.o.) was used as standard, while 0.9 % NaCl (2.5 mL/kg, p.o.) was given to control animals. The antiinflammatory activity was expressed as the average percent inhibition of edema in each group, which was calculated according to the general formula :

$$\text{Inhibition}(\%) = 100 - \left(100 \times \frac{V_t}{V_c}\right)$$

where, V_t and V_c represent the increase in paw volumes of rats treated with drug and control, respectively.

RESULTS AND DISCUSSION

The total phenolic content and the DPPH radical scavenging activities of the different solvent extracts of the plant *Ipomoea pes-caprae* are reported in Table-1. The antioxidant status of the chloroform (379.8 µg GAE/ mg extract and IC_{50} 1390 µg/ mL) and ethylacetate extracts (973.16 µg GAE/mg extract and IC_{50} 340 µg/mL) of the plant was found to be promising, hence these were subjected to antiinflammatory activity assay, the results of which are recorded in Table-2. The active ethyl acetate extract was found to have the highest antiinflammatory activity (42.59 and 60.20 % inhibition at 250 and 500 mg/kg doses) followed by chloroform extract (37.04 and 57.41 % inhibition at 250 and 500 mg/kg doses) (Table-2). The potency of the crude extract and four solvent extracts was observed more in higher doses. It may be mentioned that the antiinflammatory activity of the chloroform and ethylacetate extract was better than that reported by Pongprayoon *et al.*[6,7] (54.68 % inhibition). This may be due to ecological influence on the plant. The methanolic extract and other four solvent extracts of the plant did not show any signs of toxicity up to 2 g/kg body weight per oral and i.p. dose in mice. Hence, these were quite safe for use. Individual assay of the isolated compounds (Fig. 1) showed that compound **IV** possesses highest radical scavenging activity (795.72 µg/mL) followed by compound **III** (1311.02 µg/mL), compound **II** (2148.06 µg/mL) and compound **I** (3027.99 µg/mL) as is evident from the bar diagram (Fig. 2). Several compounds had been reported from hexane, petroleum ether and ethylacetate extract of *I. pes-caprae*[6,19-24] but there was no report regarding the isolation of these four compounds from *I. pes-caprae*. All these compounds isolated from other sources were shown to possess antiinflammatory activity[11,25-27], further strengthening our observation.

TABLE-1
TOTAL PHENOLIC CONTENT AND DPPH RADICAL SCAVENGING ACTIVITY OF *Ipomoea-pes-caprae*

Sample	Total phenolic content (µg GAE/ mg extract)	DPPH radical scavenging activity (IC_{50} in µg/mL)
Methanolic extract	0.189 ± 0.001	1520 ± 44.01
Hexane extract	94.67 ± 0.87	5130 ± 69.01
Chloroform extract	379.80 ± 6.67	1390 ± 8.12
Ethylacetate extract	973.16 ± 1.46	340 ± 3.14
Aqueous extract	40.98 ± 0.58	5580 ± 130.12

Values are in mean ± SEM (n = 3).

TABLE-2
ANTIINFLAMMATORY ACTIVITY OF *Ipomoea pes-caprae*

Drug/extracts	Doses (mg/kg)	Paw volume increased in 3 h (cc)	Edema (%) inhibition relative to control at 3 h
Control	2.5	1.08 ± 0.092	–
Methanolic extract	250	0.76 ± 0.074**	29.62
	500	0.48 ± 0.065***	55.55
Chloroform extract	250	0.68 ± 0.041***	37.04
	500	0.46 ± 0.029***	57.41
Ethyl acetate extract	250	0.62 ± 0.083***	42.59
	500	0.43 ± 0.051***	60.20
Diclofenac sodium	10	0.38 ± 0.052***	64.81

Values are in mean ± SEM (n = 6), $^{**}p < 0.01$-0.005, $^{***}p < 0.002$-0.001, "n" indicate number of animals in each group. Statistical analysis-student's paired "*t*" test. All values were compared with control.

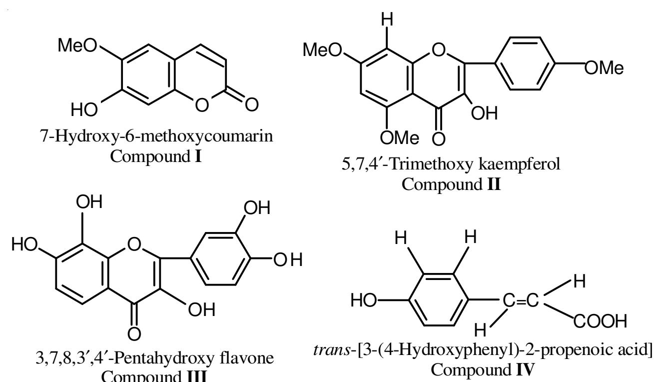

Fig. 1. Compounds (**I-IV**) isolated from *Ipomoea pes-caprae*

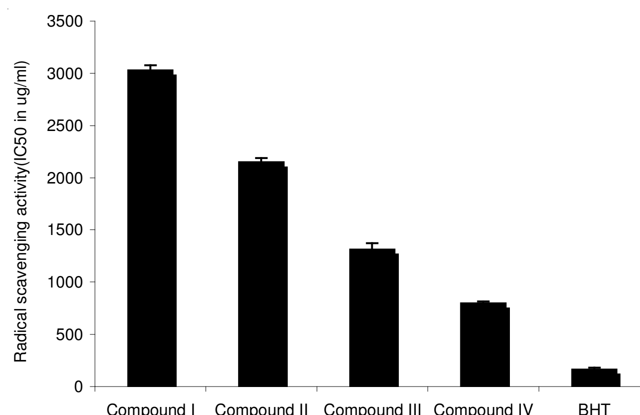

Fig. 2. DPPH radical scavenging activity of compounds (**I-IV**) isolated from *Ipomoea pes-caprae* and BHT

Conclusion

Hence, the folkloric use of *Ipomoea pes-caprae* in the Sunderban area is scientifically justified.

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