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ANTIOXIDENT PROPERTIES OF VARIOUS SOLVENT FROM LEAVES EXTRACTS OF IPOMOEA PES-CAPRAE L.

Botany

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ABSTRACT

The present study was undertaken to find the antioxidant value of Ipomoea pes-caprae is a medicinal plants in Chidhambaram, Cuddalore district, Tamilnadu. Antioxidants have been reported to prevent oxidative damage caused by free radical and can be used in cardiovascular, anti-inflammatory, diabetes and cancer diseases. The amount of total phenols, flavonoids and radical scavenging activity has been studied. Major amount of phenols were determined in methanol extracts of leaves. Moreover, maximum flavonoid content was found to be present in the methanol extract of leaves .However, high radical scavenging activity was observed in methanol extracts of leaves followed by ethyl acetate, chloroform and petroleum ether.

KEYWORDS

Antioxidant, Flavanoid, Medicinal plants, Phenol, Radical scavenger.

INTRODUCTION

"The world is going herbal" is not just a phrase but a phenomenon, which is storming the globe with scientific, rationale and is fast emerging to support better health and life through plants and plant derived products. Mankind depends mainly upon the plant kingdom to meet their needs. In our ancient world, the consumption of plants as a medicine was the only source against various diseases. Fossil records date of human use of plants as medicine at least to the middle Paleolithic age 60,000 years ago(Fabricant and Fransworth, 2001), at the time the use of plants was based on visual observations of some local practioners and their traditional knowledge pass on to generation after generation. At one time, it was thought that the chemically synthesized materials would completely replace the materials of natural origin; but later it was realized that by no means a true copy of a natural substance could be made by synthetic one.

Materials and methods:

Antiaxident activity:

The radical scavenging effects of Ipomoea pes-caprae leaves of crude extracts were evaluated against 1,1-diphenyl-2-picrylhydrazyl, ABTS·+, hydrogen peroxide, superoxide radical, Nitric oxide radical, hydrogen peroxide, hydroxyl radicals scavenging assay, ferric reducing antioxidant power, total phenol, total flavonoids content and total antioxidant activity.

A. Materials and equipments

All the chemicals and solvents used in the study were analytical grade (AR). 1,1-Diphenyl-2-Picryl-Hydrazyl (DPPH), quercetin, gallic acid (TCA), ascorbic acid, monobasic and dibasic phosphate, potassium ferric cyanide, sulphuric acid, sodium phosphate, EDTA, ammonium molybdate, NBT-Nitroblue tetrazolium, BHT, ferric chloride, ammonium acetate, acetyl acetate, potassium phosphate buffer, potassium phosphate saline, phosphoric acid, ferrozine, methanol and were obtain from Hi-media, India.

B.Determination of DPPH radical scavenging method (1-1,Diphenyl -2 Picryl-hydrazyl)

DPPH free radical scavenging activity

The DPPH scavenging activity of different extracts of I.pes-caprae was measured according to the procedure described by Hatano et al. (1988). Radical scavenging activity of plant extracts against stable DPPH radical was determined spectrophotometrically. The colorimetric change (from deep-violet to light yellow), 1 mL of each solution added to 3 mL of 0.004% methanolic DPPH free radical solution. After 30 minutes the absorbance of the preparations were taken at 517 nm by a UV spectrophotometer (Hitachi-U-20) which was compared with the corresponding absorbance of standard ascorbic acid concentrations (62.5, 125, 250 and 500 µg/mL). Methanol was used to zero the spectrophotometer absorbance of the DPPH radical without antioxidants. The control, was measured special care was taken to minimize the loss of free radical activities of DPPH radical stock solution was calculated by the following equation.

$$\text{Radical scavenging activity (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{(\text{Absorbance of control})} \times 100$$

Extracts concentration providing 50% inhibition IC50 calculation.

C. ABTS+ scavenging effects (2,2'-Azino-bis-3-ethylbenzthiazoline- 6-sulphonic acid)

Re et al. (1999) with few modifications. The ABTS.+ radical effects was generated by reaction of 5 mL of aqueous ABTS.+ solution (7 mM) and 88 µL of 140 mM (2.45 mM final concentration) of potassium per sulfate solution. The mixture was held in dark at 29C for 14 h before being used, and then it was diluted with ethanol in order to obtain an absorbance of 0.7≠0.02 units at 734 nm using a UV - Vis spectrophotometer (U-200 Hitachi). Reference substances (ascorbic acid), were allowed to react with 3 mL of the resulting blue-green ABTS+ radical solution in a dark condition. The decrease of absorbance at 734 nm was measured at the end point of 6 min. The standard curve was linear between 0-20 mg of ascorbic acid 100 mL. The results were expressed as an acerbic acid values; the latter is more suitable for food. The activity of petroleum ether, chloroform, ethyl acetate and methanol was estimated at different concentrations. All the tests were performed in triplicate.

$$\text{Radical scavenging activity (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{(\text{Absorbance of control})} \times 100$$

Extracts concentration providing 50% inhibition IC50 calculation.

D.Superoxide radical scavenging assay

Super oxide radical scavenging assay was examined methods of Gow-Chin and Hui-Yin (1995) was adapted and modified in the lab. The reaction mixture consist of 0.75 mg/mL of 1 mL sample, 1 mL 60 µM Phenazine Methosulphate (PMS), in Phosphate Buffer (0.1 M, pH 7.4) and 150 µM 1 mL Nitro Blue Tetrazolium (NBT) in phosphate buffer. Incubation at ambient temperature followed for 5 minutes and the result colour was read spectrophotometrically at 560 nm against a blank (water). Ascorbic acid was taken as standard (1 mg/mL). The effect of ascorbic acid was also determined by replacing plant extracts with 1 mL Ascorbic acid (1 mg/mL) in acetone in the reaction mixture.

$$\text{Radical scavenging activity (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{(\text{Absorbance of control})} \times 100$$

Extracts concentration providing 50% inhibition IC50 calculation.

C. Hydrogen peroxide (H2O2) Scavenging assay

H2O2 Scavenging activity was examined as per the method of Ruch et al. (1989). A solution of H2O2 (40 mM) was prepared in a phosphate buffer (pH 7.4). The concentration of H2O2 was determined spectrophotometrically at 230 nm. Different concentrations (62.5, 125, 250 and 500) µg/mL of leaves extracts of I.pes-caprae were added to 0.6 ml of H2O2 (40 mM) and absorbance of the solution was read at 230 nm after 10 min. against a blank solution containing residue oil in phosphate buffer without H2O2. The percentage scavenging of H2O2 was calculated using the following formula.

$$\text{Radical scavenging activity (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{(\text{Absorbance of control})} \times 100$$

Extracts concentration providing 50% inhibition IC50 calculation.

D. Hydroxyl radical scavenging assay

The scavenging activity of different extracts on hydroxyl radical was measured according to the method of Klein et al. (1991). The various concentrations (62.5, 125, 250 and 500 µg/mL) of extracts were added with 1.0 mL of iron-EDTA solution (0.13% Ferrous ammonium sulfate and 0.26% EDTA), 0.5 ml EDTA solution (0.018%) and 1.0 mL 0.85 % dimethylsulphoxide (DMSO) v/v in 0.1 M phosphate buffer, pH 7.4). The reaction was inhibited by adding 0.5 mL of Ascorbic acid (0.22%) and incubated at 80-90C for 15 min. in a water bath. After incubation, the reaction was terminated by the addition of 0.1 mL of ice-cold TCA (17.5% w/v). Three mL of Nash reagent (75.0 g of ammonium acetate, 3.0 mL of glacial acetic acid and 2 mL of acetyl acetone were mixed and raised to 1 L with distilled water) was added and left at room temperature for 15 min. The reaction mixture without sample was used as control ascorbic acid. The intensity of the colour formed was measured spectroscopically at 412 nm against reagent blank. The percentage hydroxyl radical scavenging activity is calculated by the following formula.

$$\text{Radical scavenging activity (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{(\text{Absorbance of control})} \times 100$$

Extracts concentration providing 50% inhibition IC50 calculation.

E. Ferric reducing antioxidant power (FRAP)

The FRAP assays was determined by the following method of Chu et al. (2000) with modifications. 0.1 M potassium phosphate buffer (pH 6.6) (2.5 mL) and 1 per cent potassium ferricyanide (2.5 mL) w/v were mixed with 1 mL of extracts of different concentration. The reaction mixture was incubated at 50C for 20 min, after which 10% w/v trichloro acetic acid (2.5 mL) was added. 2.5 mL of water and 0.5 mL of 0.1% w/v FeCl3 were then added to 2.5 mL of the reaction mixture. The solution was incubated at ambient temperature for 30 min for colour development. They expressed as mg GAE/g.

F. Total phenolic content

Determination of total phenolic content was carried out following the Folin - Ciocalteu method by Singleton and Rossi (1965). One mL solution containing (1 mg/mL) was added volumetric flask. 1 mL of Folin - Ciocalteu reagent (previously diluted 10-fold with distilled water) and allowed to stand at 22C for 5 min; 7.5% of 0.75 mL of sodium bicarbonate solution was added and mixed thoroughly. The samples were measured spectrophotometrically at 765 nm using spectrometer after 90 min at 22C. The amount of total phenolic was determined as Gallic acid and equivalent and expressed as mg GAE/G dry weight.

G. Total flavonoid content

The flavonoids content was determined by aluminium trichloride method using quercetin as a reference compound (Chang et al., 2002). This method based on the formation of a complex flavonoid-aluminum having the absorptivity maximum at 415 nm, after remained react at room temperature for 30 min. Briefly, 0.5 mL of each extracts (1:10 g/mL) in methanol was separately mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. The amount of total flavonoids was determined as mg quercetin/g.

H. Total antioxidant activity

Total antioxidant activity of different crude extracts of I.pes-caprae was determined according to the method of Prieto et al. (1999). Briefly, 0.3 mL of samples was mixed with 3.0 mL reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Reaction mixture was incubated at 95C for 90 min under water bath. Absorbance of all the sample mixture was measured at 695 nm. Total antioxidant activity is expressed as the number of equivalents of ascorbic acid.

Results:

DPPH(1,1-Diphenyl-2-picrylhydrazylhydrate Radical Scavenging activity) The results of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of various extracts of leaves Ipomoea pes-caprae and the radical scavenging activity of the positive standard (Ascorbic acid) are presented table. Based on the results obtained, methanol extracts of leaves exhibited the highest DPPH activity followed by ethyl acetate, chloroform and petroleum ether. The IC50 values of methanol extracts of I.pes-caprae leaf and L-ascorbic acid were 403, 321, 286 and 219µg/mL respectively.

DPPH activity of different extracts of Ipomoea pes-caprae leaf

S. No	Plant extracts/ solvents	DPPH activity				IC50value (µg/mL)
		Concentrations µg/ml				
		125	250	500	1000	
1	Petroleum ether	24.54 ± 0.56	31.45 ± 0.14	39.18 ± 0.34	43.57 ± 0.09	403
2	Chloroform	28.05 ± 0.50	35.60 ± 0.45	41. 44 ± 0.57	46.34 ± 0.23	321
3	Ethyl acetate	33.56 ± 0.34	42.50 ± 0.50	46.56 ± 0.67	54.23 ± 0.09	286
4	Methanol	37.17 ± 0.34	46.18 ± 0.28	51.04 ± 0.06	62.15 ± 0.50	259
5	Ascorbic acid	41.01 ± 0.50	57.33 ± 0.57	61.10 ± 0.50	83.46 ± 0.67	219

ABTS·+ scavenging effects (2,2'-azino-bis-3-ethylbenzthi-azoline-6-sulphonic acid):

ABTS·+ radical scavenging activity of petroleum ether, chloroform, ethyl acetate, and methanol from leaves of Ipomoea pes-caprae and the positive standard (BHT) were carried out and results are presented in table. Leaves of I.pes-caprae methanol extracts revealed the remarkable antioxidant activity followed by ethyl acetate, chloroform and petroleum ether. The IC50 values methanol extracts of I.pes-caprae leaves and buttylated hydroxyl toluene (BHT) acid were 567,430,371,230 and 202 µg/mL respectively.

ABTS+ Scavenging effects (2, 2-Azino-bis-3-ethyl benzthiazoline-6-sulphonic acid) various extracts of Ipomoea pes-caprae leaf

S. No	Plant extracts/ solvents	ABTS+ Scavenging effects				IC50 value (µg/mL)
		Plant extracts Concentrations µg/ml				
		125	250	500	1000	
1	Petroleum ether	21.23 ± 0.26	27.13 ± 0.26	32.10 ± 0.50	43.14 ± 0.56	567
2	Chloroform	27.05 ± 0.35	32.45 ± 0.55	37.70 ± 0.05	46.90 ± 0.08	430
3	Ethyl acetate	34.20 ± 0.50	39.92 ± 0.90	44.50 ± 0.50	56.23 ± 0.97	371
4	Methanol	39.51 ± 0.87	43.00 ± 0.50	49.32 ± 0.87	61.20 ± 0.09	230
5	BHT	47.24 ± 0.54	61.16 ± 0.04	72.12 ± 0.50	83.73 ± 0.12	202

Hydroxyl peroxide scavenging effects

The results of hydrogen peroxide scavenging activity of petroleum ether, chloroform, ethyl acetate, and methanol of leaves of I.pes-caprae and positive standard are shown in table. Methanol extracts of leaves ofI. Pes-caprae exhibited the highest hydrogen peroxide activity. The value of IC50 of methanol extracts of I.pes-caprae leaves and EDTA were556,490, 423, 346 and 218µg/mL.

Hydrogen peroxide scavenging effects of various extracts of Ipomoea pes-caprae leaf

s.no	Plant extracts/ solvents	Hydrogen peroxide scavenging effects				IC50 value (µg/mL)
		Concentrations µg/ml				
		125	250	500	1000	
1	Petroleum ether	17.50 ± 0.50	23.50 ± 0.35	29.07 ± 0.18	34.67 ± 0.90	556
2	Chloroform	21.32 ± 0.50	27.90 ± 0.50	32.10 ± 0.03	35.20 ± 0.67	490
3	Ethyl acetate	24.56 ± 0.28	31.45 ± 0.97	36.23 ± 0.98	40.69 ± 0.50	423
4	Methanol	31.50 ± 0.50	35.50 ± 0.67	39.50 ± 0.50	49.10 ± 0.30	346

5	EDTA	35.10 ± 0.40	41.50 ± 0.38	49.05 ± 0.16	62.20 ± 0.20	218

Superoxide radical scavenging activity

The results of superoxide radical scavenging activity of petroleum ether, chloroform, ethyl acetate, and Methanol of leaves of I.pes-caprae and positive standard are shown in table. Methanol extracts of I. pes- caprae leaves produced the significant superoxide radical scavenging activity. The IC50 value of methanol extracts of I. pes-caprae leaves and BHT were 568,498,424,300 and 290 µg/mL.

Superoxide radical scavenging activity of various extracts of Ipomoea pes-caprae leaf

S.NO	extracts/ solvents	Superoxide radical scavenging activity				IC50value (µg/mL)
		Concentrations µg/ml				
		125	250	500	1000	
1	Petroleum ether	15.17±0.25	18.19±0.29	26.92±0.37	41.13±0.13	568
2	Chloroform	19.16±0.26	23.10±0.21	29.59±0.30	43.25±0.01	498
3	Ethyl acetate	30.13±0.36	35.41±0.36	41.00±0.25	50.04±0.01**	424
4	Methanol	34.17±0.03	37.19±0.13	48.25±0.14	63.12±0.13	300
5	BHT	38.93±0.03	44.72±0.12	49.95±0.03	76.62±0.06	290

radical scavenging activity

The results of hydroxyl radical scavenging activity various extracts of leaves of I.pes-caprae and Vitamin are shown in table. The highesthydroxyl radical scavenging activity was exhibited with methanol extracts of leaves I.pes caprae. The IC50 values of methanol extracts of I.pes-carae leaves and VitaminC were found to be 439,398,340,310 and 240µg/mL respectively.

Hydroxyl radical scavenging effects of various extracts of Ipomoea pes-caprae leaf

S.No	Plant extracts/solvents	Hydroxyl radical scavenging effects				IC50 value (µg/mL)
		Concentrations µg/ml				
		125	250	500	1000	
1	Petroleum ether	23.17±0.25	25.19±0.29	35.92±0.37	49.13±0.13	439
2	Chloroform	25.16±0.26	31.10±0.21	37.59±0.30	51.25±0.01	398
3	Ethyl acetate	35.13±0.36	42.41±0.36	48.00±0.25	57.04±0.01**	340
4	Methanol	42.17±0.03	43.19±0.13	54.25±0.14	71.12±0.13	310
5	Vitamin C	25.03 ± 0.69	34.14 ± 0.27	42.50 ±0.50	57.94 ± 0.59	240

Total antioxidant activity

The results of total antioxidant activity of petroleum ether, chloroform, ethyl acetate and methanol of leaves of I.pes-caprae and positive standard are shown in table. The highest total antioxidant activity was recorded in methanol extracts of I.pes-caprae followed by ethyl acetate, chloroform and petroleum ether. The IC50 values of methanol extracts of I. pes-caprae leaves and L-ascorbic acid values were found to be 530,414,350,270 and 230 µg/mL respectively.

Total antioxidant activity of various extracts of Ipomoea pes-caprae leaf

S.No	Plant extracts/ solvents	Total antioxidant activity				IC50 value (µg/mL)
		Concentrations µg/ml				
		125	250	500	1000	
1	Petroleum ether	32.17±0.25	35.19±0.29	44.92±0.37	59.13±0.13	530
2	Chloroform	35.16±0.26	40.10±0.21	46.59±0.30	60.25±0.01	414
3	Ethyl acetate	44.13±0.36	50.41±0.36	57.00±0.25	65.04±0.01**	350
4	Methanol	45.17±0.03	51.19±0.13	60.25±0.14	79.12±0.13	270
5	Ascorbic acid	58.17 ±0.72	63.00±0.50	77.75±0.12	88.85 ± 0.50	230

Ferric reducing antioxidant power assay

The results of ferric reducing antioxidant power of petroleum ether, chloroform, ethyl acetate and methanol of leaves of Ipomea pes-caprae and positive standard (Gallic acid) are shown in table. I.pes-caprae methanol extracts produced the highest antioxidant activity. The IC50 value of methanol extracts of leaves and gallic acid were 398,340,287,231and 188 µg/mL respectively.

Ferric Reducing Antioxidant Power Assay in various extracts of Ipomoea pes-caprae leaf

S.No	Plant extracts/solvents	Ferric Reducing Antioxidant Power Assay				IC50value (µg/mL)
		Concentrations µg/ml				
		125	250	500	1000	
1	Petroleum ether	40.17±0.25	42.19±0.29	49.92±0.37	67.13±0.13	398
2	Chloroform	43.16±0.26	49.10±0.21	54.59±0.30	69.25±0.01	340
3	Ethyl acetate	53.13±0.36	60.41±0.36	66.00±0.25	72.04±0.01**	287
4	Methanol	54.17±0.03	59.19±0.13	69.25±0.14	87.12±0.13	231
5	Gallic acid	70.17±0.73	75.51±0.28	89.17±0.48	99.13±0.36	188

Total phenol and flavonoid content

The results of total phenolic and flavonoids content of different extracts of I.pes-caprae are presented in Table respectively. The total phenols (15.82± 0.05) mg gallic acid equivalent (GAE/g) and flavonoids (17.12± 0.09) mg quercetin equivalent (QE/g) were found to be higher in methanol extract of leaves of I.pes-caprae, followed ethyl acetate (total phenols = 13.05± 0.52 mg gallic acid equivalent (GAE/g) and flavonoids = 9.16 ±0.15 mg quercetin equivalent (QE/g), chloroform (total phenols = 2.30± 0.08 mg gallic acid equivalent (GAE/g) and flavonoids = 4.90 ±0.18 mg quercetin equivalent (QE/g) and petroleum ether (total phenols = 0.76± 0.12 mg gallic acid equivalent (GAE/g) and flavonoids = 3.51 ±0.10 mg quercetin equivalent (QE/g).

The total phenol and flavonoid contents of different extracts of Ipomoea pes-caprae

Total phenol GAE mg/ml		
S. No.	Solvents	Leaf
1	Petroleum ether	0.76 ± 0.12a
2	Chloroform	2.30 ± 0.08a
3	Ethyl acetate	9.34± 0.06b
5	Methanol	13.05± 0.52b
6	Gallic Acid	15.82 ± 0.05c

Results expressed mean: ± Standard deviation

Total flavonoids QUE mg/ml		
S. No.	Solvents	Leaf
1	Petroleum ether	3.51 ± 0.10a
2	Chloroform	4.90 ± 0.18a
3	Ethyl acetate	9.16 ± 0.15ab
5	Methanol	12.89 ± 0.04b
6	Quercetin	17.12 ± 0.09c

Results expressed mean: ± Standard deviation

DISCUSSION

The various diseases in the world are due to the production of free radicals. These free radicals exert harmful effects when it reacts with important cellular components like proteins, DNA and cell membrane (Mantena et al., 2008). However, an overload of these radicals had been linked to certain chronic diseases of heart, liver and some form of cancers and diabetes (Prakash et al., 2007). Antioxidants that can neutralize free radicals may therefore be used to protect the human body from diseases and retard rancidity in foods consumed by humans (Leong and Shui, 2002). It is believed that higher intake of antioxidant rich food is associated with decreased risk of degenerative diseases particularly cardiovascular disease and cancer.

In the present study, various solvents like petroleum ether, chloroform, ethyl acetate, and methanol extracts of I.pes-caprae were used to determine the activities of DPPH free radical scavenging, hydrogen peroxide scavenging properties, the superoxide anion radical scavenging, scavenging nitric oxide radical, Ferric Reducing Antioxidant Power, total phenol and flavonoids content.

Maximum antioxidant activities were recorded in methanol extracts of leaves of I.pes-caprae. Chanda et al. (2011) have also reported the antioxidant activities in twelve medicinal plants i.e., the acetone and methanol extracts of Paltophorum ferrugineum and Triumfetta rotundifolia showed maximum DPPH free radical scavenging activity, the methanol and acetone extract of Buchanania lanzan showed the highest hydroxyl radical scavenging activity while, the acetone and methanol extracts of Paltophorum ferrugineum showed the highest superoxide anion radical scavenging activity better than standard and better reducing capacity. The present result is in augment with the previous reports of Ruan et al. (2008), Chatterjee et al. (2012) and Har and Ismail (2012) who studied that Syzygium cumini root extracts, Eugenia jambolana seed extracts and S. polyanthum leaf extracts respectively.

Conclusion

The antioxidant activities of leaves crude extracts were carried out. The results showed that the leaves of I.pes-caprae were found to have considerable antioxidant activities. The highest content of DPPH(1,1-diphenyl-2-picrylhydrazyl hydrate radical scavenging activity), ABTS·+ scavenging (2,2'-Azino-bis-3-ethyl benzthiazoline- 6-sulphonic acid), Hydrogen peroxide scavenging, Superoxide anion radical scavenging, Nitric oxide scavenging, m-hydroxyl radical scavenging, FRAP (Ferric Reducing Antioxidant Power), total phenol and total flavonoids content were observed in methanol extracts of leaves followed by ethyl acetate, chloroform and petroleum ether.

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