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Original Research Article

PHYTOCHEMICAL AND *IN-VITRO* ANTIOXIDANT SCREENING OF *Ipomea hederacea*

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Abstract: In present study, phytochemical and *in-vitro* antioxidant potential of *Ipomea hederacea*, a widely used medicinal plant, were investigated. Methanolic and aqueous extract of leaves and stem of *Ipomea hederacea* showed inhibition of scavenging of 1,1-diphenyl-2picrylhydrazyl (DPPH) radical and inhibition of reactive oxygen species (ROS) *in vitro.*

Key-words: *Ipomea hederacea*, DPPH, ROS, Anti-oxidant

Introduction: *Ipomea hederacea* Jacq. (kaladana or ivy leaf morning-glory), a member of the family Convolvulaceae, is used primarily for its seeds and recognized for its medicinal properties, especially in Asian countries[1-4]. This medicinal herb contains various valuable chemical constituents such as ecdysteriods, steroidal glycosides, aromatic acids, triterpenes, amino acids, organic acids, mineral elements and vitamins. A number of pharmacological properties such as diuretic[5], anthelmintic[6], blood purifier[7], deobstruent[8], laxative[9], carminative[10] and anti-inflammatory[11] actions have been ascribed to this plant, besides its use to treat abdominal diseases, fevers, headache and bronchitis.

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Ipomoea hederacea is a medicinal herb currently gaining popularity among researchers due to its potential health benefits. It is an affordable source of protein, carbohydrates, minerals and vitamins and health-promoting fatty acids[12]. However *in vivo* toxicological studies should be performed before inclusion of its seeds in foods. The survey of the literature revealed the presence of alkaloids and tri-terpenoids. The plant may be exploited as a source for seed gums and may serve as a renewable reservoir of industrial gums. It has also revealed a broad spectrum of pharmacological activities. More advanced techniques should be used to further explore high-value bioactive constituents responsible for tagged bioactivities.)

Therefore, present study deals with evaluation of some of these parameters that may act as a

beneficial role in standardization and authentication of plant *Ipomoea hederacea*. In addition, the study also includes *in-vitro* anti-oxidant activity of stem leaves of the plant.

Material and Methods: The plant *Ipomoea hederacea* (L.) Jacquin were procure from campus of P.B.R.I. Bhopal, Madhya Pradesh and were authenticated by Dr. Zeaul Hasan, Department of Botany, Safia Science College, Bhopal, M.P., India. A Voucher Specimen (299/Bot/Safia/2011) of the plant has been deposited for future reference in Pharmacognosy Department, P.S.I.T. College, Kanpur.

Preparations of Extract of *Ipomoea hederacea:* The leaves with stem were shade dried and powdered. The leaves with stem (500 gm) were extracted with petroleum ether in soxhlet apparatus by simultaneous extraction for 48 hrs. The extract was concentrated in vacuum. On concentration it yielded petroleum ether stem extract (5.2 gm). The extracts were studied for preliminary phytochemical analysis. The marc of petroleum ether leaves with stem were extracted with chloroform on concentration, it yielded (7.2 gm).

The marc of chloroform were extracted with acetone on concentration, it yielded (3.9 gm). After that the marc of acetone extracted with methanol on concentration it yielded (22.4 gm). At the last stage the marc of methanol extracted with water on concentration it yielded (50.3 gm).

The *in-vitro* anti-oxidant activity was studied in rats in methanolic extract of *Ipomoea hederacea* leaves with stem. The percentage yield were found to be for petroleum ether leaves with stem extract (1.04%) and the percentage yield were found to be for chloroform extract (1.44%), for acetone it was (0.78%), for methanol extract it was (4.48%), for water it was (10.06%).

Determination of Total Phenolic Content: 1 mg/ml of extracts sample were prepared and then 0.1 ml of sample, 1.9 ml distilled water and 1.0 ml of Folin Ciocalteau reagent were added in a tube, and then 1.0 ml of 20% Na_2CO_3 was added. The reaction mixture was incubated at room temperature in dark for 30 min and the absorbance of the blue colour sample was recorded at 765 nm on UV-Visible spectrophotometer. The blank consist of all reagents and solvents but no sample.

The sample was tested in triplicate and a calibration curve for gallic acid was obtained. The results were compared to gallic acid calibration curve and the total phenolic content of extracts was expressed as µg of gallic acid equivalents (GAE) per mg of dry extract. Drug used Gallic acid from (Merck, India).

Determination of Total Flavonoids Content: 1 mg/ml of extracts sample were prepared and then extract or standard solutions (0.25ml) were mixed with 1.25 ml distilled water and 75 µ L 5% $NaNO_2$. After 6 min. 75 µ L of 10% $AlCl_3$ was added. After 5 min, 0.5 ml of 1 M NaOH was added to the mixture. Immediately, the absorbance of the mixture was determined at 510 nm on UV-Visible spectrophotometer. The blank consist of all reagents and solvents but no sample.

The sample was tested in triplicate and a calibration curve for rutin was obtained. Total flavonoids content was expressed as µg quercetin equivalents (QE) per mg of dry extract. Drug used Quercetin from (Sigma Aldrich, US).

***In-vitro* Antioxidant Activity**

DPPH (2, 2- Diphenyl-1-Picrylhydrazyl) Radical Scavenging Method: Free radical scavenging activity of the petroleum ether, acetone, chloroform, methanolic and aqueous extract of stem and leaves of *Ipomoea hederacea* were determined using a stable 2, 2-Diphenyl-1-picrylhydrazyl (DPPH). DPPH is a free radical of violet colour. The antioxidants in the sample scavenge the free radical and turn it into yellow colour. The change of colour from violet to yellow is proportional to the radical scavenging activity.

Antioxidants reacts with DPPH, which is stable free radical and is reduced to DPPHH and as

consequence the absorbance's decreased from the DPPH radical to the DPPH-H form. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in term of hydrogen donating ability.

Preparation of Standard Ascorbic acid solutions: Different solutions of the ascorbic acid were prepared in 90% methanol solution to obtain different concentrations (10 - 100µg/ml). 200 µM solution of DPPH in methanol will be prepared and 1.5 ml of this solution will be added to 1.5 ml of methanol ascorbic acid solution at different concentrations and incubated for 30 min at room temperature in dark conditions. After 30 minutes, the absorbance of each solution of ascorbic acid was taken against methanol (as blank) at 517 nm.

Preparation of Test solutions: Different solutions of the leaves stem extracts were prepared in 90% methanol solution to obtain different concentrations (10 - 100µg/ml). 200 µM solution of DPPH in methanol will be prepared and 1.5 ml of this solution will be added to 1.5 ml of methanol extract solution at different concentrations and incubated for 30 min at room temperature in dark conditions. After 30 minutes, the absorbance of each solution of leaves, stem and root extracts was taken against methanol (as blank) at 517 nm.

Preparation of Control Solution: For control, 1.5 ml of methanol was mixed with 200 µM DPPH solution and incubated for 30 min at room temperature in dark condition. Absorbance of the control was taken after 30 min against methanol (as blank) at 517 nm.

Percentage antioxidant activity of plant extract and Ascorbic acid was calculated by using formula:

$$I\% = \frac{Ac - At}{Ac} \times 100$$

Where,

I % = Percentage inhibition

Ac = Absorbance of control (methanol and 200 µM DPPH solution)

At= Absorbance of ascorbic acid / plant extract with 200 µM DPPH solution after 30 min.

Assay of Reduction Power: Principle: This method is based on the principle of increase in the absorbance of the reaction mixture. Increase in the absorbance indicates increase in the antioxidant activity. Substances, which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm. Increase in absorbance of the reaction mixture indicates the reducing power of the samples.

Potassium ferricyanide + Ferric chloride

↓

Preparation of Standard Ascorbic acid Solutions

Different concentrations of the ascorbic acid were prepared in distilled water to give solutions of concentration (10 - 100µg/ml). 1ml of each concentration of ascorbic acid solutions were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% Potassium ferricyanide solution. The reaction mixtures were incubated for 20 min at 50°C. Afterwards 2.5ml of 10% trichloroacetic acid solution was added and centrifuged at 560 X g for 10 min. After separation, 2.5 ml of upper layer of each solution were mixed with 2.5 ml of distilled water and 1ml of 0.1% Ferric chloride (freshly prepared solution). Absorbance was recorded for each solution of ascorbic acid against (0.2 M, pH 6.6) phosphate buffer (used as blank) at 700 nm.

Preparation of Test Solutions: Different solutions of extract were prepared in distilled water to give various concentrations (10 - 100µg/ml). 1ml of each solution of plant part extract was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% Potassium ferricyanide solution. The reaction mixtures were incubated for 20 min at 50°C. Afterwards 2.5ml of 10% trichloroacetic acid

solution was added and centrifuged at 560 X g for 10 min. After separation, 2.5 ml of upper layer of each solution were mixed with 2.5 ml of distilled water and 1ml of 0.1% Ferric chloride (freshly prepared solution). Absorbance was recorded for each solution of plant part extract against (0.2 M, pH 6.6) phosphate buffer (used as blank) at 700nm.

The absorbance v/s concentration curve for ascorbic acid and extract was plotted. The 'Y' & 'R^2' values obtained in both curve and the cases were comparatively studied to determine the reducing power of extract.

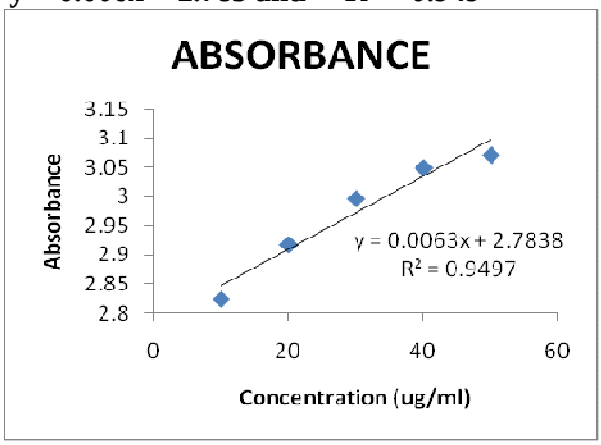
Results and Discussion

Total Phenolic Content: All determination was performed in triplicate. The folin-Ciocalteu reagent is sensitive to reducing compounds including polyphenols. They produce a blue color upon reaction. This blue colour was measured spectrophotometrically.

Line of regression from Gallic acid was used for estimation of unknown phenol content.

From standard curve of gallic acid line of regression was found to be

y = 0.006x + 2.783 and R^2 = 0.949



Graph No. -1

S. No.	Conc. (mg/ml)	Absorbance	Total Phenolic content in mg/g equivalent of Gallic acid
1	1 mg/ml	3.428	107.5

Table 1: Total Phenolic Content of Aqueous Extract Standard curve of Gallic acid

S. No.	Conc (µg/ml)	Absorbance
1	10	2.824
2	20	2.918
3	30	2.997
4	40	3.05
5	50	3.072

Table 2: Total Flavonoid Content of Aqueous Extract

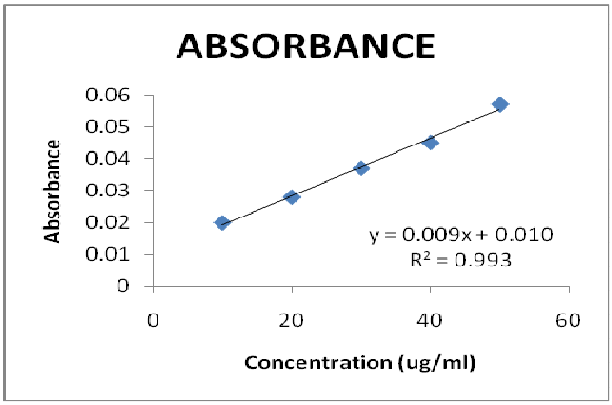
S. No.	Conc (µg/ml)	Absorbance
1	10	0.02
2	20	0.028
3	30	0.037
4	40	0.045
5	50	0.057

Procedure: Total flavonoids were measured by a colorimetric assay according to Dewanto et al. An aliquot of diluted sample or standard solution of rutin was added to a 75 µl of $NaNO_2$ solution, and mixed for 6 min, before adding 0.15 mL $AlCl_3$ (100 g/L). After 5 min, 0.5 mL of NaOH was added. The final volume was adjusted to 2.5 ml with distilled water and thoroughly mixed. Absorbance of the mixture was determined at 510 nm against the same mixture, without the sample, as a blank. Total flavonoid content was expressed as mg rutin/g dry weight (mg rutin/g DW), through the calibration curve of Rutin. Samples were analyzed.

Line of regression from rutin was used for estimation of unknown flavonoid content. From standard curve of rutin, line of regression was found to be

$y = 0.009x + 0.010$ and $R^2 = 0.993$

Thus the goodness of fit was found to be good for selected standard curve. By putting the absorbance of test sample (y = absorbance) in line of regression of above mentioned rutin.



Graph no.3

S. No.	Conc. (mg/ml)	Absorbance	Total Phenolic content in mg/g equivalent of Gallic acid
1	1 mg/ml	0.103	10.33

DPPH Assay: The percentage (%) inhibition curves for DPPH of ascorbic acid and extract were plotted, from which IC_{50} values of percentage inhibition of DPPH by ascorbic acid and extract were calculated using regression equation.

Table 3: DPPH Assay

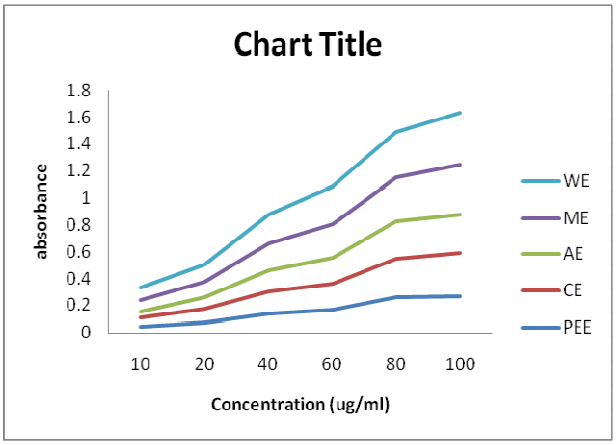
Conc (µg/ml)	% Inhibition				
	Pet Ether	Acetone	Chloroform	Methanolic	Aqueous
10	12.54613	17.89668	30.4428	42.06642	44.28044
20	16.42066	20.84871	32.65683	44.09594	45.57196
30	19.37269	22.87823	37.26937	45.75646	47.97048
40	20.4797	25.46125	38.92989	47.23247	50
50	23.24723	28.22878	42.25092	48.15498	52.21402
60	25.27675	30.4428	45.01845	49.44649	53.69004
70	27.30627	35.42435	46.86347	50.92251	55.35055
80	29.15129	37.45387	48.52399	52.58303	57.19557
90	31.91882	39.6679	50.73801	53.69004	59.40959
100	36.53137	44.28044	53.13653	54.79705	61.43911

S. No.	Extract	IC_{50} (µg/ml)
1	Pet Ether	163.05
2	Acetone	123.86
3	Chloroform	84.84
4	Methanolic	63.33
5	Aqueous	41

Table 4: Reducing Power Assay

Conc (µg/ml)	Absorbance				
	Pet Ether	Acetone	Chloroform	Methanolic	Aqueous
10	0.043	0.046	0.064	0.084	0.092
20	0.076	0.088	0.097	0.116	0.129
40	0.136	0.157	0.168	0.202	0.213
60	0.166	0.195	0.191	0.257	0.278
80	0.257	0.278	0.293	0.322	0.342
100	0.269	0.294	0.318	0.363	0.388

The absorbance v/s concentration curve for ascorbic acid and extract was plotted. The 'Y' & 'R^2' values obtained in both curve and the cases were comparatively studied to determine the reducing power of extract.



References

1. Souza, M.M.; Oliveira, A.M.; Filho, V.C.; Berti, C.; Yunes, R.A.; Krogh, R. Estudos preliminares da propriedade antinociceptiva de Ipomoea pes-caprae em modelos farmacologicos especificos. Alcance 1998, 5, 53–57.
2. Ahmad, M.; Mehjabeen; Zia-Ul-Haq, M.; Jahan, N. Determination of LD50 and ED50 by dose response relationship and assessment of toxicological and non-toxicological behaviour of Ipomoea hederacea. J. Pharm. Res. 2011, 4, 1176–1178.
3. Zia-Ul-Haq, M.; Ahmad, S.; Calani, L.; Mazzeo, T.; Del Rio, D. ; Pellegrini, N.; De Feo, V. Compositional study and antioxidant potential of Ipomoea hederacea Jacq. and Lepidium sativum L. seeds. Molecules 2012, 17, 10306–10321.
4. Ruckmani, D.S.; Chitra, M.; Jayamathi, P. Hepatoprotectivity and an antioxidant study of Ipomoea hederacea on experimentally induced hepatotoxic rats. Rec. Res. Sci. Tech. 2010, 2, 17–19.
5. Zia-Ul-Haq, M.; Ahmad, M.; Mehjabeen; Jehan, N.; Ahmad, S.; Qayum, M.; Inamullah, K.M. Antimicrobial screening of selected flora of Pakistan. Arch. Biol. Sci. 2011, 63, 691–695.
6. Singh, B.P.; Singh, S. Chemical investigation of seed of Ipomoea hederacea and its biological activity. J. Chem. Pharm. Res. 2012, 4, 1441–1448.
7. Nam, K.A.; Lee, S.K. Evaluation of cytotoxic potential of natural products in cultured human cancer cells. Nat. Prod. Sci. 2000, 6, 183–188.
8. Meira, M.; da Silva, E.P.; David, J.M.; David, J.P. Review of the genus Ipomoea: Traditional uses, chemistry and biological activities. Braz. J. Pharmacog. 2012, 22, 682–713.
9. Zaman, M.K.; Ali, M.; Siddiqui, A.W.; Rafiullah, M.R.M. New di- and triterpenic compounds from Ipomoea hederacea seeds. J. Saudi Chem. Soc. 2005, 9, 161–170.
10. The Wealth of India, Raw Materials; Council of Scientific and Industrial Research: New Delhi, India, 1992; pp. 249–251.
11. Kritikar, K.R.; Basu, B.D. Indian Medicinal Plants (Illustrated), 3rd ed.; Sri Satguru Publication: Delhi, India, 2000; pp. 2368–3269.
12. Nadkarni, K.M. Indian Plants and Drugs; Asiatic Publishing House: New Delhi, India, 1985; pp. 202–203.