



Free radical scavenging activity of *Ipomoea obscura* (L.) Ker-Gawl

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Abstract

Objective: To evaluate the free radical scavenging activity of *Ipomoea obscura* (L.) whole plant belonging to the family Convolvulaceae. **Methods:** Three successive whole plant extracts (petroleum ether, methanol and water) of *Ipomoea obscura* were prepared and the total phenolic content was estimated. The extract were screened for their *in vitro* antioxidant activity using 2,2'-diphenyl-2-picryl hydrazyl (DPPH), 2,2'-azino-bis (3-ethyl-benzo-thiazoline-6-sulfonic acid) diammonium salt (ABTS), hydrogen peroxide, nitric oxide, superoxide and hydroxyl radicals by p-nitroso dimethyl aniline (p-NDA) and deoxyribose assays and the IC₅₀ values were calculated. **Results:** The total phenol content in methanol and water extracts was found to be 18.15 mg/g and 9.12 mg/g, respectively. Among the three extracts tested, the methanol extract showed maximum activity with IC₅₀ values 53.12 ± 0.33, 108.40 ± 2.15, 107.90 ± 1.20 and 424.00 ± 2.90 µg/ml, for ABTS, DPPH, hydrogen peroxide and nitric oxide radical inhibition assays, respectively. The water and petroleum ether extracts showed moderate to low activity compared to methanol extract when tested for ABTS, DPPH, hydrogen peroxide and nitric oxide radical inhibition assays. All the three extracts showed less activity at high concentrations when tested against superoxide and hydroxyl radical inhibition assays. **Conclusion:** The successive methanol extract exhibited significant antioxidant activity and merits further investigations in animal models and isolation of its active constituents.

Key words: *Ipomoea obscura* (L.), free radical scavenging activity, DPPH, ABTS

1. Introduction

Free radicals oxidatively damage lipids and proteins and compromise genomic DNA integrity. They are widely recognized as the root cause of numerous degenerative diseases including cancer. Antioxidants are potent scavengers of free radicals and serve as inhibitors of neoplastic

processes [1]. *Ipomoea obscura* (L.) Ker-Gawl (Family: Convolvulaceae) is a slender, twinning perennial herb found almost throughout India upto an altitude of 3000 ft., in grasslands, hedges and waste lands [2]. In Uganda, it is used for the treatment of diarrhea by traditional healers [3].

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Leaves of this plant are used as an application to aphthous affections after toasting, powdering and boiling with ghee and in admixture with the leaves of *Argyrea mollis* for sores [2]. Chemical investigations of this plant have shown the presence of tropane alkaloids such as Calysteginine B-1, Calysteginine B-2, Calysteginine B-3, Calysteginine B-4 and Calysteginine C-1 and indole alkaloids such as Ipobscurine A, Ipobscurine B, Ipobscurine C and Ipobscurine D [4-6]. Plants belonging to *Ipomoea* species such as *Ipomoea batatas* [L.] have shown significant antioxidant activity [7]. No biological activity, however, is reported for *Ipomoea obscura* (L.) till date. Hence, in the present investigation different extracts of the whole plant, *Ipomoea obscura* (L.), were screened for their *in vitro* antioxidant activity using standard procedures.

2. Materials and Methods

2.1. Plant Material

Whole Plant material was collected in the month of June, 2004 from Masinagudi village, Nilgiris District, Tamilnadu and was authenticated at Medicinal Plants Survey and Collection Unit, Government Arts College, Ootacamund, India. A voucher specimen was deposited for further references at JSS College of Pharmacy Herbarium, Ootacamund, India.

2.2. Chemicals

2, 2-Diphenyl-1-picryl hydrazyl (DPPH) and 2, 2'-Azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt (ABTS) were obtained from Sigma Aldrich Co., St. Louis, USA. Rutin and p-Nitroso dimethyl aniline (p-NDA) were obtained from Acros Organics., New Jersey, USA. Naphthyl ethylene diamine dihydrochloride (NEDD) was from Roch – Light Ltd., Suffolk, UK, Ascorbic acid, nitro blue tetrazolium (NBT) and butylated hydroxy anisole (BHA) were from SD Fine Chemicals Ltd., Mumbai, India, and 2-deoxy-D-ribose was from Hi-Media

Laboratories Pvt. Ltd., Mumbai, India. Sodium nitroprusside was from Ranbaxy Laboratories Ltd., Mohali, India. Sulphanilic acid used was from E-Merck (India) Ltd., Mumbai, India. All chemicals used were of analytical grade.

2.3 Extraction procedure

The fresh whole plant was shade dried, powdered and passed through sieve No.20 and extracted (600 g) successively with 4.5 L each of petroleum ether (60–80° C), methanol and water in a Soxhlet extractor for 18–20 h. The extracts were concentrated to dryness under reduced pressure and controlled temperature (40–50° C) using rotary evaporator. The petroleum ether extract yielded an yellowish green sticky solid, weighing 10.4 g (1.73% w/w), the methanol extract yielded a dark greenish solid residue, weighing 105.3 g (17.55% w/w), and the aqueous extract yielded a dark brown solid residue, weighing 15.5 g (2.58% w/w). All the extracts were preserved in a refrigerator till further use.

2.4. Preparation of Test and Standard Solutions

All the three extracts of *Ipomoea obscura* and the standard antioxidants (ascorbic acid, rutin and butylated hydroxy anisole) were dissolved in distilled dimethyl sulphoxide (DMSO) separately and used for the *in vitro* antioxidant assays using six different methods. As DMSO interferes in the case of hydrogen peroxide method the extracts and the standards were dissolved in distilled methanol and used. The stock solutions were serially diluted with the respective solvents to obtain lower dilutions.

2.5. Total Phenolic Compounds Estimation

Antioxidant compounds generally contain phenolic group(s) and hence, the amount of phenolic compounds in all the three extracts of the whole plant was estimated by using Folin-Ciocalteu reagent [8]. In a series of test tubes, 0.4 ml of the extract in methanol was taken, mixed

with 2 ml of Folin-Ciocalteu reagent and 1.6 ml of sodium carbonate. After shaking, it was kept for 2 h and the absorbance was measured at 750 nm using a Shimadzu-UV-160 spectrophotometer. Using gallic acid monohydrate, a standard curve was prepared. The linearity obtained was in the range of 1-10 µg/ml. Using the standard curve, the total phenolic compounds content was calculated and expressed as gallic acid equivalent in mg/g of extracts.

2.6. Free radical scavenging activity

The three extracts were tested for their *in vitro* antioxidant activity using standard methods. In all these methods, a particular concentration of the extract or standard solution was used which gave a final concentration of 1000 µg/ml to 0.45 µg/ml after all the reagents were added. Absorbance was measured against a blank solution containing the extract or standard, but without the reagents. A control test was performed without the extracts or standards. Percentage scavenging and IC₅₀ values \pm S.E.M (IC₅₀ value is the concentration of the sample required to inhibit 50% of radical) were calculated.

2.6.1. DPPH Radical Scavenging Method

A 10 µl aliquot of the different concentrations of extracts and standards were added to 200 µl of DPPH in methanol solution (100 µM) in a 96-well microtitre plate (Tarson Products (P) Ltd., Kolkata, India). After incubation at 37°C for 20 min, the absorbance of each solution was determined at 490 nm using ELISA reader (Bio Rad Laboratories Inc, California, USA, Model 550) [9].

2.6.2. Scavenging of ABTS Radical Cation

To 0.2 ml of various concentrations of the extracts and standards, 1.0 ml of distilled DMSO and 0.16 ml of ABTS solution were added and incubated for 20 min. Absorbance of these solutions were measured spectrophotometrically at 734 nm [10].

2.6.3. Scavenging of Hydrogen Peroxide

A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffered saline (PBS at pH 7.4). Various concentrations of the extracts and standards in methanol (1 ml) were added to 2 ml of hydrogen peroxide solution in PBS. After 10 min, the absorbance was measured at 230 nm [11].

2.6.4. Nitric Oxide Radical Inhibition Assay

The reaction mixture (6 ml) containing sodium nitroprusside (10 mM, 4 ml), phosphate buffer saline (1 ml) and the extracts and standard solutions (1 ml) were incubated at 25°C for 150 min. After incubation, 0.5 ml of the reaction mixture was removed and 1 ml of sulphanilic acid reagent (0.33% in 20% glacial acetic acid) was mixed and allowed to stand for 5 min for completion of diazotization reaction and then 1 ml of NEDD was added, mixed and allowed to stand for another 30 min in diffused light. The absorbance was measured at 540 nm against the corresponding blank solutions in a 96-well microtitre plate (Tarsons Product (P) Ltd., Kolkata, India) using ELISA reader (Bio Rad Laboratories Inc, California, USA, Model 550) [12].

2.6.5. Scavenging of Hydroxyl Radical by Deoxyribose Method

Various concentrations of the extracts and standards in DMSO (0.2 ml) were added to the reaction mixture containing deoxyribose (3 mM, 0.2 ml), ferric chloride (0.1 mM, 0.2 ml), EDTA (0.1 mM, 0.2 ml), ascorbic acid (0.1 mM, 0.2 ml) and hydrogen peroxide (2 mM, 0.2 ml) in phosphate buffer (pH, 7.4, 20 mM) to give a total volume of 1.2 ml. The solutions were then incubated for 30 min at 37°C. After incubation, ice-cold trichloro acetic acid (0.2 ml, 15% w/v) and thiobarbituric acid (0.2 ml, 1% w/v)

in 0.25 N HCl were added. The reaction mixture was kept in a boiling water bath for 30 min, cooled and the absorbance was measured at 532 nm [13].

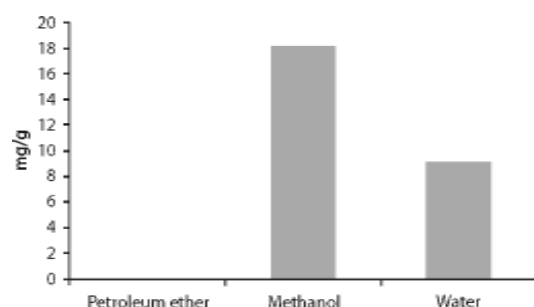


Fig. 1. Total Phenol estimation of successive extracts of *Ipomoea obscura*.

2.6.6. Scavenging of Hydroxyl Radical by p-NDA Method

Various concentrations of the extracts and standards in distilled DMSO (0.5 ml) were added to a solution mixture containing ferric chloride (0.1 mM, 0.5 ml), EDTA (0.1 mM, 0.5 ml), ascorbic acid (0.1 mM, 0.5 ml), hydrogen peroxide (2 mM, 0.5 ml) and p-NDA (0.01 mM, 0.5 ml) in phosphate buffer (pH 7.4, 20 mM), to produce a final volume of 3 ml. Absorbance was measured at 440 nm [14].

2.6.7. Scavenging of Super Oxide Radical by Alkaline DMSO Method

To the reaction mixture containing 0.1 ml of NBT (1 mg /ml solution in DMSO) and 0.3 ml of the extracts and standard in DMSO, 1ml of alkaline DMSO (1 ml DMSO containing, 5 mM NaOH in 0.1 ml water) was added to give a final volume of 1.4 ml and the absorbance was measured at 560 nm [14].

Table 1. Antioxidant activity of *Ipomoea obscura*(L.) Ker-Gawl Whole plant extracts

Extracts/ Standards	IC ₅₀ ⁺ values ± SEM*(µg/ml)						
	DPPH	ABTS	H ₂ O ₂	Nitric oxide	Deoxy- ribose	p-NDA	Super- oxide
Extracts							
Petroleum ether extract	250.00 ± 4.80	203.33 ± 1.66	235.00 ± 1.44	534.40 ± 3.66	>1000	>1000	>1000
Methanol extract	108.40 ± 2.15	53.12 ± 0.33	107.90 ± 1.20	424.00 ± 2.90	>1000	>1000	>1000
Water extract	224.00 ± 5.30	103.60 ± 0.57	215.83 ± 1.50	505.12 ± 3.30	>1000	>1000	>1000
Standards							
Ascorbic acid	5.67 ± 0.26	10.05 ± 0.49	187.33 ± 3.45	-	-	-	>1000
Rutin	7.89 ± 0.51	0.48 ± 0.017	35.26 ± 0.166	88.44 ± 2.56	-	225.63 ± 7.85	-
BHA	-	-	23.45 ± 2.45	-	78.06 ± 1.25	-	>1000

* Average of three independent determinations, three replicates, values are mean ± SEM.

+ IC₅₀ = Concentration of the sample/standard required to inhibit 50% of free radicals.

3. Results and Discussion

The total phenolic compounds of three successive extracts were expressed as gallic acid equivalent in mg/g of extracts (Fig.1). The methanol extract has the highest phenolics content (18.15 mg/g,) followed by water extract (9.12 mg/g). The petroleum ether extract does not contain any phenolic compounds. The antioxidant activity measured are given in Table 1. Among the three extracts tested for *in vitro* antioxidant activity using seven methods, the successive methanol extract exhibited good antioxidant activity in ABTS, DPPH, hydrogen peroxide and nitric oxide radical scavenging methods with IC₅₀ values of 53.12 ± 0.33, 108.40 ± 2.15, 107.90 ± 1.20 and 424.00 ± 2.90 µg/ml, respectively. The successive aqueous extract showed moderate activity in ABTS, DPPH, hydrogen peroxide and nitric oxide radical scavenging methods with IC₅₀ values of 103.60 ± 0.57, 224.00 ± 5.30, 215.83 ± 1.50 and 505.12 ± 3.30 µg/ml, respectively. The petroleum ether extract showed moderate to low activity in ABTS, DPPH, hydrogen peroxide and nitric oxide radical scavenging methods with IC₅₀ values of 203.33 ± 1.66, 250.00 ± 4.80,

235.00 ± 1.44 and 534.40 ± 3.66 µg/ml, respectively. All the three extracts failed to exhibit antioxidant activity in the scavenging of superoxide radical by alkaline DMSO method and hydroxyl radical by deoxyribose and p-NDA method. The IC₅₀ values obtained, however, for all the extracts in all the methods were found to be higher than the standards used, indicating their low activity compared to the standards.

The preliminary phytochemical investigations of the three extracts revealed the presence of steroids, terpenoids, alkaloids, carbohydrate and phenolic compounds such as tannins, flavonoid etc., In conclusion, the successive methanol extract of *Ipomoea obscura* was found to possess significant free radical scavenging activity. The observed antioxidant activity of the extracts of *Ipomoea obscura* may be due to the presence of any of these constituents. The constituents responsible for the antioxidative activity of *Ipomoea obscura*, however, are currently not clear. Further work is, therefore, under progress to identify and isolate the antioxidative constituents and to establish the activity in animal models.

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