Pharmacologia 3 (9): 377-386, 2012
ISSN 2044-4648 / DOI: 10.5567/pharmacologia.2012.377.386
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Antioxidant and Cytotoxic Activities of Few Selected *Ipomoea* Species

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Abstract: Aim of study: Numerous species of the genus *Ipomoea* are widely used in traditional medicine as powerful cathartics and are reported to have anti-inflammatory, anti-hypertensive, anti-diabetic and anticancer activities. The objective of the present investigation was to evaluate *Ipomoea pes-caprae*, *Ipomoea batatas* and *Ipomoea nil* for their antioxidant and cytotoxic activity. **Materials and methods:** Successive petroleum ether and methanolic extracts of different plant parts viz., leaves, stem, roots and whole plant of *Ipomoea pes-caprae*, *Ipomoea batatas* and *Ipomoea nil* were evaluated for their free radical scavenging activity by different *in vitro* assays including DPPH radical scavenging assay, Nitric oxide radical inhibition activity, ABTS radical scavenging assay, Alkaline DMSO assay, Haemolysis prevention, Hydrogen peroxide scavenging assay, Hydroxyl radical scavenging assay and Lipid peroxidation assay. Methanolic extracts of these *Ipomoea* plants were also investigated for their total phenolic and flavonoid content. Free phenolics isolated from the most active methanolic extract were also evaluated for antioxidant activity. Polarity based successive fractionation was performed for the methanolic extract using chloroform and ethyl acetate. Cytotoxicity of methanolic extracts, petroleum ether extracts and isolated free phenolics was evaluated by MTT and SRB assay using HEp-2 cell line. **Results:** All methanolic extracts of selected *Ipomoea* plants showed the presence of Glycosides, alkaloids, flavonoids, carbohydrates and tannins. Methanolic extracts showed better antioxidant activity when compared to petroleum ether extracts with IC_{50} value in the range of 25-475 and 5-50 μg mL^{-1} for DPPH and ABTS methods, respectively. Methanolic extract of *Ipomoea pes-caprae* leaves, *Ipomoea nil* leaves and *Ipomoea pes-caprae* roots showed comparatively better antioxidant activity. These extracts also showed comparatively higher phenolic and flavonoid content. Free phenolics isolated from leaves of *Ipomoea pes-caprae* showed better activity than crude methanolic extract when investigated by nitric oxide, ABTS and Hydrogen peroxide methods but when tested by DPPH and alkaline DMSO method, crude extracts showed better activity. All methanolic extracts and isolated free phenolics showed good cytotoxicity against HEp-2 cell line with CTC_{50} value in the range of 50-100 μg mL^{-1}. **Conclusion:** The successive methanolic extract of selected *Ipomoea* plants showed potent ability to scavenge different types of free radicals generated by various *in vitro* assays. The total phenol and flavonoid content estimation of these methanolic extracts revealed their presence in good quantities. Among different methanolic extracts tested for antioxidant activity, the leaf extract of *Ipomoea pes-caprae* was the most potent extract. The same extract contained highest amount of total phenolics and flavonoids which suggests that the antioxidant nature of the extract is due to its high phenolic and flavonoid content. Among the fractions of the above extract, prepared according to their polarity, the ethyl acetate fraction showed best activity. Ethyl acetate fraction is believed to possess phenolics and flavonoids. Thus, this study reconfirms that the potent antioxidant activity of *Ipomoea pes-caprae* leaf is due to its high phenol and flavonoid contents. All methanolic extracts and free phenolics showed good cytotoxicity against Hep-2 cell line with CTC_{50} value in the range of 50-100 μg mL^{-1}. So it can be concluded that *Ipomoea pes-caprae*, *Ipomoea batatas* and *Ipomoea nil* are having good antioxidant and cytotoxic activity and their antioxidant activity is mainly due the phenolics and flavonoids. Further studies are needed to prove the anticancer activity of these plants.

Key words: *Ipomoea*, Convolvulaceae, antioxidant activity, cytotoxicity, phenolics, flavonoids

INTRODUCTION

Generation of oxygen radicals such as superoxide radicals (O_2^-), hydroxyl radicals and non-free radical species such as hydrogen peroxide and singlet oxygen ($^1O_2$) are associated with cellular and metabolic injury, aging and number of diseases including cancer, cardiovascular diseases, neurogenerative diseases and inflammation (Salim, 1996; Mimi-Oka *et al*., 1999). On an average, every cell in the body comes under the attack of

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free radicals once every ten seconds. Although, the body possesses defense mechanisms as enzymes and antioxidant nutrients, continuous exposure to chemicals and contaminants may lead to an increase in the amount of free radicals in the body beyond its capacity to control them and cause irreversible oxidative damage (Halliwell, 1994). The free radical damage can be counteracted by the consumption of antioxidants. Antioxidants act by donating an unpaired electron to free radicals and thereby inhibiting the oxidation of macromolecules and cellular structures (Dekkers *et al*., 1996). Vitamin C, vitamin E, carotenoids, glutathione are some of the basic antioxidants in the body. Plants produce lot of antioxidants to protect themselves against oxidative stress caused by sunbeams and oxygen. Vitamin C, vitamin E, flavonoids, tannins and other phenolic compounds present in many plants are potential antioxidants.

Convolvulaceae is a large family characterized by widespread occurrence of flavonols, kaempferol, quercetin and their O-methylated derivatives (Mann *et al*., 1999). Genus *Ipomoea* belonging to this family is widely distributed in tropical and subtropical countries. These plants are used in traditional medicine as powerful cathartics, diuretic, aphrodisiac and in the treatment of skin diseases, ulcers, bronchitis, inflammation, diabetes, fever and general weakness (Singh, 1988). Their biological activities mainly include anticancer, antioxidant, anti-diabetic, anti-inflammatory etc. Three plants were selected for evaluation of their antioxidant and anticancer activity.

- *Ipomoea batatas* (Linn) Lam. is a creeping herb with tuberous roots (Singh, 1988). Chemical constituents which have been reported include vitamin A, vitamin C, carbohydrates, vitamin B_6 (Dini *et al*., 2009), polyhydroxyalkaloids (Schimming *et al*., 2005), saponins, caffeoylquinic acid derivatives (Yoshimoto *et al*., 2002), resin glycosides (Yin *et al*., 2008)
- *Ipomoea pes-caprae* R.Br. (syn. *Ipomoea biloba* Forssk.) is a long trailing sand binding perennial herb with a thick root. Chemical constituents include steroids, terpenoids, alkaloids, flavonoids (De-Souza *et al*., 2000), quinic acid esters, resin glycosides
- *Ipomoea nil* Roth (syn. *Ipomoea hederacea* Auct. Non (Linn) Jacq.) is a twinning hairy slender. Its chemical constituents include alkaloids, flavonoids and anthocyanins (Saito *et al*., 2005)

The objective of the present study was to investigate the antioxidant and cytotoxic activities of these three plants and to evaluate the nature of the compound responsible for antioxidant activity.

MATERIALS AND METHODS

Plant materials: *Ipomoea pes-caprae*, *Ipomoea batatas* and *Ipomoea nil* were collected from sea-shore of Malpe beach, field areas near Manipal and area near Malpe beach, Udupi, Karnataka, India, respectively in October and November 2008. These plants were identified and authenticated by Mr. V. Aravinda Hebbar, The head, Department of Botany, M.G.M. College, Udupi, Karnataka, India. Voucher specimens of these plants were deposited at herbarium of this institute.

Preparation of plant extracts: Leaves, stem, roots and whole plant of *Ipomoea pes-caprae*, *Ipomoea batatas* and *Ipomoea nil* were separated and shade dried. The dried parts were chopped, coarsely powdered and extracted successively with petroleum ether and methanol by Soxhlet extraction. The extracts were concentrated under reduced pressure and controlled temperature using rotary evaporator and evaporated to dryness. The percentage yield of each extract was calculated and dry extracts were stored in refrigerator at 4°C for further studies. The extracts were abbreviated to indicate plant name, part and the solvent with which it was extracted. IP indicates *Ipomoea pes-caprae*, IB indicates *Ipomoea batatas*, in indicates *Ipomoea nil* where as L indicates Leaves, S indicates Stem, R indicates Roots and W indicates Whole plant. This is followed by P or M which indicates Petroleum ether or Methanol, respectively.

Preparation of test and standard solutions: Extracts and standard antioxidants (ascorbic acid, rutin) were dissolved in distilled dimethyl sulphoxide (DMSO) separately and used for different *in vitro* antioxidant assays except the hydrogen peroxide method where methanol was used to dissolve the extracts in order to avoid the interference noted with DMSO. The stock solutions were two-fold serially diluted with the respective solvents to obtain the lower dilutions.

Chemicals: 1, 1-diphenyl-2-picryl hydrazyl (DPPH), 2, 2'-azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt (ABTS), rutin, Nitro blue Tetrazolium (NBT), 3-(4,5-dimethyl thiazole-2-yl) 2,5 diphenyl tetrazolium bromide (MTT), sulphorhodamine-B (SRB) and Thiobarbituric acid (TBA) were obtained from Sigma Aldrich Co., St. Louis, USA. p-nitroso dimethyl aniline (p-NDA) was obtained from National chemicals, Vadodara, India. Naphthyl ethylene diamine dihydrochloride (NEDD) was obtained from S.D. Fine Chemicals Ltd., Mumbai, India. Ascorbic acid, hydrogen peroxide, dimethyl sulphoxide (DMSO) and chloroform were obtained from

Nice chemicals Pvt. Ltd., Cochin, India. Folin-ciocalteau phenol reagent, potassium persulphate, methanol, petroleum ether and glacial acetic acid were obtained from Merck (India) Ltd., Mumbai, India. All chemicals used were of analytical grade.

Cell-lines and culture medium: HEp-2 (Human laryngeal epithelial carcinoma) cell culture was procured from National Centre for Cell Sciences (NCCS), Pune, India. The cells were cultured in MEM medium supplemented with 10% inactivated FBS, Penicillin (100 IU mL^{-1}), streptomycin (100 μg mL^{-1}) and Amphotericin-B (5 μg mL^{-1}) in a humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were trypsinized with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 flat bottles and the cytotoxicity studies were carried out in 96 well microtitre plates.

Preliminary phytochemical screening: All methanolic extracts were tested for the presence of carbohydrates, alkaloids, steroids, saponins, flavonoids and tannins by preliminary phytochemical tests (Khandelwal, 2006).

***In vitro* antioxidant assays:** In all methods, a particular concentration of the extract or standard solution was used which gave a final concentration of 1000 to 1.95 μg mL^{-1} after all the reagents were added. Absorbance was measured against blank solutions that contain extract or standard but without the reagents. A control was performed without adding extracts or standards. Percentage scavenging and IC_{50} values±SDM (Standard Deviation from Mean) were calculated by the following formula.

$$\text{Percentage scavenging} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

DPPH method: The hydrogen atom or electron donating ability of the extracts and pure compounds were measured from the bleaching of purple coloured methanol solution of DPPH using the method of Hwang *et al*. (2001). The degree of discolouration is directly proportional to the antioxidant activity of the compound. A 10 μL aliquot of the extract was added to 200 μL of DPPH in methanol (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 well was measured at 540 nm using ELISA reader (Biotek ELx 800 MS, USA).

Nitric oxide radical inhibition assay: Sodium nitroprusside in aqueous solution spontaneously generates nitric oxide at physiological pH which interacts with oxygen to produce nitrite ions which can be estimated by the use of Griess Illosvoy reaction (Garrat, 1964). It was modified by using NEDD (0.1%, w/v) instead of 1-naphthylamine (5%). The reaction mixture (6 mL) containing sodium nitroprusside (10 mM, 4 mL), phosphate buffer saline (1 mL) and extract or standard solutions (1 mL) were incubated at 25°C for 150 min. After incubation, 0.5 mL of the reaction mixture containing nitrite was transferred and 1 mL of sulphanilic acid (0.33% w/v in 20% glacial acetic acid) and 1 mL of NEDD (0.1% w/v in 50% glacial acetic acid) were added and allowed to stand for 30 min in diffused light. The absorbance was measured at 540 nm against the corresponding blank solutions.

ABTS radical scavenging activity: ABTS radical cation (ABTS+) was generated by reaction of ABTS stock solution (7 mM concentration) with 2.45 mM potassium persulphate and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. To 0.2 mL of the extract or standards, 1 mL of distilled DMSO and 0.16 mL of ABTS solution were added. After 20 min of incubation, the absorbance was measured at 690 nm (Re *et al*., 1999).

Scavenging of Superoxide radical by alkaline DMSO method: Superoxide was generated according to alkaline DMSO method. The reduction of Nitro Blue Tetrazolium (NBT) by superoxide was determined in presence and absence of the extracts using the method of Kunchandy and Rao (1990). To the reaction mixture containing 1 mL of alkaline DMSO, 0.3 mL of extract, 0.1 mL of NBT (0.1 mg) was added. The absorbance was measured at 560 nm.

Haemolysate method: Nitrite-induced conversion of haemoglobin to methaemoglobin: In the presence of sodium nitrite, haemoglobin is converted to methaemoglobin which is toxic to the cells. If the extract has ability to scavenge the radicals generated by sodium nitrite, it will prevent the conversion of haemoglobin to methaemoglobin which can be measured at 630 nm as this wavelength is specific for methaemoglobin. The absorbance measured is directly proportional to the amount of methaemoglobin formed (Unnikrishnan and Rao, 1992). The tubes were prepared by mixing 0.9 mL of Haemolysate solution and 0.5 mL of extract and the tubes were incubated for 15 min. 0.1 mL of 10 mM sodium nitrite was added and incubated for 5 min and absorbance was measured at 631 nm.

Prevention of haemolysis: The oxidation of Red Blood Cells (RBC) by molecular oxygen is performed with ferrous

sulphate and hydrogen peroxide as a free-radical initiator. The RBC's are oxidized at a constant rate by a free-radical chain mechanism resulting in haemolysis. The extent of haemolysis is proportional to the concentration of free radical. Because of this α-tocopherol levels in the membrane would be maintained which will result in the prevention of haemolysis (Miki *et al*., 1987). Red Blood Cells (RBC) were obtained from KMC blood bank, Manipal, Karnataka, India. Heparinized blood was centrifuged at 3000 rpm for 10 min. After removal of plasma and buffy coat, the RBC's were washed thrice with phosphate buffer saline (pH-7.4) containing NaCl (150 mM), NaH_2PO_4 (1.9 mM) and Na_2HPO_4 (8.1 mM) and resuspended in PBS four times its volume. RBC (10% v/v) were incubated with extract or standard in presence of $FeSO_4$-H_2O_2 in shaker incubator for 15 min at 37°C. After which an aliquot was centrifuged and haemolysis was measured at 540 nm as haemoglobin released from cells in supernatant (Beutler, 1975).

Lipid peroxidation (LPO) assay: Hundred μL of test samples were added to 1 mL liposome mixture, control was maintained without test sample. Lipid peroxidation was induced by adding 10 μL $FeCl_3$ (400 mM) and 10 μL L-ascorbic acid (200 mM). After incubation for 1 h at 37°C, the reaction was stopped by adding 2 mL of 0.25 N HCl containing 15% trichloroacetic acid and 0.375% thiobarbituric acid. The reaction mixture was boiled for 15 min, centrifuged and absorbance of the supernatant was measured at 540 nm (Cook and Samman, 1996).

p-NDA method: Hydroxyl radical scavenging was measured by inhibition of p-nitrosodimethylaniline (p-NDA) bleaching. Hydroxyl radical generated through Fenton reaction can bleach p-NDA specifically. To a reaction 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), 0.5 mL of extract or standard in distilled DMSO were added. Absorbance was measured at 450 nm (Kunchandy and Rao, 1989).

Scavenging of hydrogen peroxide: A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffered saline (pH 7.4). One mL of extract or standard in methanol was added to 2 mL of hydrogen peroxide solution. After 10 min, the absorbance was measured at 230 nm (Jayaprakasha *et al*., 2004).

Total phenol estimation: In test tubes, 0.1 mL of extract or gallic acid in methanol, 0.2 mL of Folin-Ciocalteau phenol's reagent, 1 mL of 15% sodium carbonate and 2 mL of distilled water were added, incubated for 2 h and the absorbance was measured at 765 nm using spectrophotometer (Shimadzu Corporation, Japan). The total phenolic content was expressed as % w/w of the extracts equivalent to Gallic acid (Ainsworth and Gillespie, 2007).

Total flavonol estimation: The flavonol content was measured using aluminium chloride colorimetric assay. 0.25 mL of extract or rutin was mixed with 0.75 mL methanol, 0.05 mL of 1 M potassium acetate. After 5 min, 0.05 mL of 10% aluminium chloride and 1.4 mL of distilled water was added. After 30 min, the absorbance of the reaction mixture was measured at 415 nm. The total flavonol content was expressed as % w/w of the extracts equivalent to rutin (Chang *et al*., 2002).

Estimation of total antioxidant capacity: The assay is based on the reduction of Mo (VI)-Mo (V) and subsequent formation of a green phosphate/Mo (V) complex at acidic pH (Huong *et al*., 1998; Salawu *et al*., 2006). The sample and standard tubes were prepared by mixing 100 μL of extract or standard and 1 mL of TAC reagent. Control tube contained methanol instead of extract or standard. All the tubes were heated at 95°C for 90 min and absorbance was measured at 695 nm. Total antioxidant capacity of an extract was expressed as rutin equivalent in mg g^{-1} or % w/w of dry extract.

Polarity based successive fractionation: IPL-M was used for this study because of its better antioxidant activity and higher phenolic and flavonoid content. Around 3 g of crude methanolic extract was dissolved in water and shaken vigorously in a separating funnel first with chloroform (100×3) and then with ethyl acetate (100×3) for 20 min each. All the chloroform and ethyl acetate fractions were collected and evaporated under reduced pressure. After complete evaporation of the solvent, the extract was weighed and antioxidant activity was checked by DPPH method as described in earlier.

Isolation of free phenolics: Phenolics can be responsible for antioxidant activity of the plants because it provides protection to the plants from oxidative stress. Because of the very high phenolic content of these plants, free phenolics were isolated. 10 g of *Ipomoea pes-caprae* leaves were defatted by Soxhlet extraction with petroleum ether. Defatted powder was extracted with 70% ethanol (250 mL×4) for a period of one hour each. Powders were filtered and filtrate was centrifuged. Supernatant was concentrated. pH of the concentrate was adjusted to 2-3

by using 4 M HCl. These concentrates were shaken with ethyl acetate (200 mL×2) for 1 h each. Ethyl acetate fraction was separated, evaporated to dryness and weighed.

In vitro cytotoxicity assays

Determination of mitochondrial synthesis by MTT assay: This assay is based on the assumption that dead cells or their products do not reduce tetrazolium. The principle involved is the cleavage of tetrazolium salt 3-(4, 5 dimethyl thiazole-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into a purple coloured product formazan by mitochondrial enzyme succinate dehydrogenase. The number of living cells is proportional to extent of formazan produced by the cells. The monolayer cell culture was trypsinized and the cell count was adjusted 1.0×10^5 cells mL^{-1} using medium. To each well of the 96 well microtitre plate, 100 µL of the diluted cell suspension was added and the plates were incubated at 37° C for 24 h in 5% CO_2 incubator. After 24 h, the supernatant was flicked off and 100 µL of different drug concentrations (prepared in MEM) were added. The plates were incubated at 37°C for 72 h in 5% CO_2 incubator. After 72 h, drug solutions were flicked off and 50 µL of MTT in PBS (2 mg mL^{-1}) was added. The plates were incubated for 3 h at 37°C in 5% CO_2 incubator. The supernatant was flicked off and 50 µL of isopropanol was added and gently shaken to solubilize the formazan. The plates were incubated for 10 min and the absorbance was measured at 540 nm.

Determination of total cell protein content by sulphorhodamine B (SRB) assay: SRB is a bright pink aminoxanthine dye with two sulfonic groups. Under mild acidic conditions, SRB binds to basic amino acids in TCA fixed cells to provide a sensitive index of cellular protein content (Eisenbrand *et al.*, 2002). The procedure is same as that of MTT assay (2.13.1.) after 72 h, 25 µL of 50% trichloro acetic acid was added and the plates were incubated at 4°C for 1 h. The plates were flicked and washed with distilled water and the plates were air-dried. 50 µL of SRB (0.4% in 1% acetic acid) was added and incubated for 30 min. The unbound dye was removed by washing with 1% acetic acid. The plates were air-dried and 100 µL of 10 mM Tris base was added to solubilize the dye. The absorbance of each well was measure at 540 nm.

RESULTS AND DISCUSSION

Preliminary phytochemical studies: All methanolic extracts showed the presence of carbohydrates, flavonoids, glycosides, alkaloids and tannins. All the extracts showed the presence of saponins except IPL, IBL and INL.

In vitro antioxidant studies

DPPH, nitric oxide and ABTS assay: Methanolic extracts of these plants showed potent antioxidant activity by DPPH and ABTS radical scavenging assay which was comparable to the standard ascorbic acid. IPL-M was the most potent extract to scavenge DPPH and ABTS free radicals with the IC_{50} values 19.33±0.545 µg mL^{-1} and 3.22±0.373 µg mL^{-1}, respectively (Fig. 1). All other methanolic extracts showed good activity with IC_{50} values in the range of 25-475 and 5-50 µg mL^{-1} for DPPH and ABTS assays, respectively (Fig. 2). Among the extracts evaluated, only IPR-M showed moderate activity against nitric oxide radical with IC_{50} value of 806.47±1.055 µg mL^{-1} where as other methanolic and petroleum ether extracts were not active against nitric oxide radical. All petroleum ether extracts showed moderate antioxidant activity by DPPH and ABTS radical scavenging methods. Among the petroleum ether extracts tested, IPR-P showed maximum DPPH scavenging activity with IC_{50} value 79.78±4.197 µg mL^{-1} where as IPL-P was showing maximum ABTS radical scavenging activity with IC_{50} value of 46.07±4.40 µg mL^{-1} (Fig. 3-4).

The results of preliminary antioxidant studies of petroleum ether and methanolic extracts of *Ipomoea*

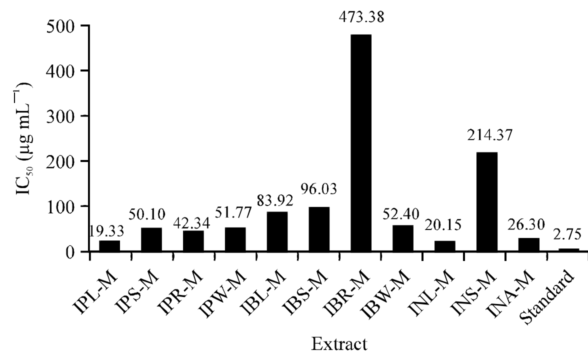

Fig. 1: IC_{50} values of methanolic extracts of selected *Ipomoea* plants by DPPH method

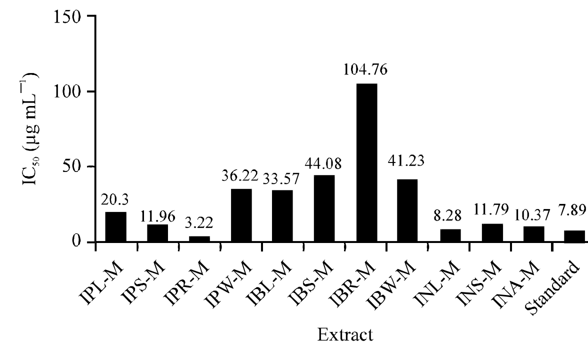

Fig. 2: IC_{50} values of methanolic extracts of selected *Ipomoea* plants by ABTS method

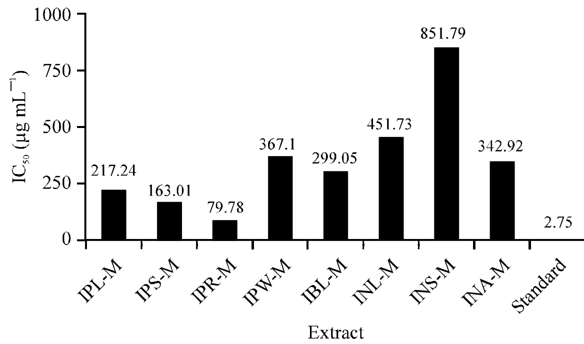

Fig. 3: IC_{50} values of petroleum ether extracts of selected *Ipomoea* plants by DPPH method

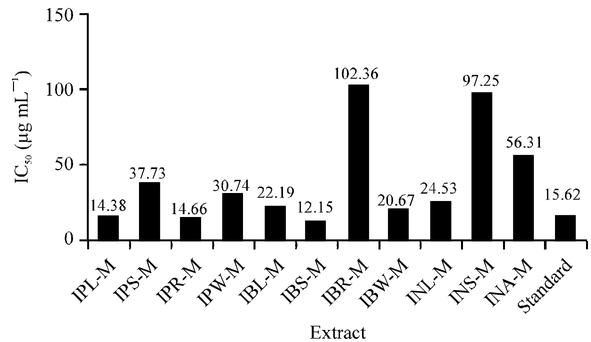

Fig. 5: IC_{50} values of methanolic extracts of selected *Ipomoea* plants by Alkaline DMSO method

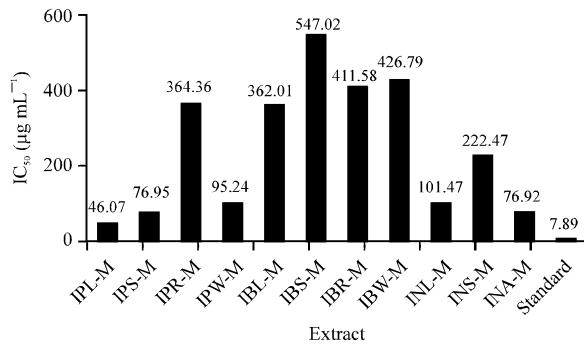

Fig. 4: IC_{50} values of petroleum extracts of selected *Ipomoea* plants by ABTS method

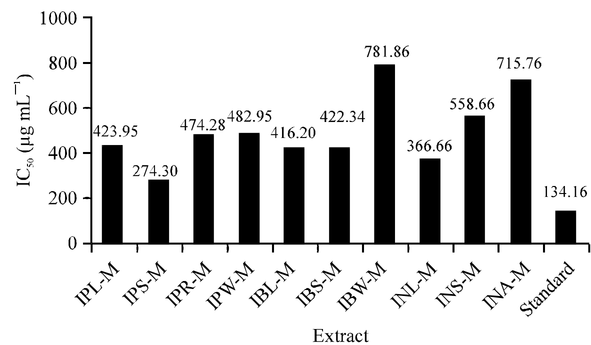

Fig. 6: IC_{50} values of methanolic extracts of selected *Ipomoea* plants by Haemolysate method

species revealed that, petroleum ether extracts failed to show promising antioxidant properties. However, the methanolic extracts showed potent antioxidant activity. Hence, in our further investigations, antioxidant activity of only methanolic extracts was studied.

Alkaline DMSO assay: The methanolic extracts showed potent antioxidant activity by effectively inhibiting superoxide radical by alkaline DMSO method. The IC_{50} values of IPL-M, IPR-M and IBS-M were better than the standard antioxidant (ascorbic acid) with IC_{50} value of 12.15±0.121, 14.38±0.809 and 14.66±2.521 µg mL^{-1}, respectively. All other extracts showed good activity with IC_{50} value ranging between 20.67 to 56.31 µg mL^{-1}, except IBR-M and INS-M which showed IC_{50} value around 100 µg mL^{-1}. The IC_{50} value of standard (ascorbic acid) was 15.62±1.276 µg mL^{-1} (Fig. 5).

Haemolysate method: All selected methanolic extracts moderately prevented nitrite induced conversion of haemoglobin to methaemoglobin. Among the extracts tested, IPS-M and INL-M showed better activity with IC_{50} value of 274.3±2.025 and 366.66±4.33 µg mL^{-1}, respectively. IC_{50} value of all other extracts ranged between 416.2-781.85 µg mL^{-1}. However, the standard antioxidant, ascorbic acid showed good activity with IC_{50} value 134.16±0.069 µg mL^{-1} (Fig. 6).

Prevention of haemolysis: Prevention of haemolysis of RBC's was estimated using oxidative stress induced damage of RBC by Fe^{2+}-H_2O_2 model. Among the extracts tested, leaf extract of *Ipomoea nil* was most active with IC_{50} value of 8.53±0.212 µg mL^{-1}. IBS-M, IPR-M and IPL-M also showed good activity with IC_{50} value of 10.55±0.228, 13.83±1.521 and 29.53±2.327 µg mL^{-1}, respectively. All other methanolic extracts were not active in preventing haemolysis. Standard (Ascorbic acid) showed very good activity with IC_{50} value of 6.84±0.534 µg mL^{-1} (Fig. 7).

Lipid peroxidation assay, p-NDA assay and hydrogen peroxide scavenging assay: The methanolic extracts of selected species of *Ipomoea* plants were screened for their ability to inhibit generation of reactive oxygen species (by hydrogen peroxide method) and hydroxyl radicals (by p-NDA method).

Table 1: IC_{50} values of methanolic extracts of selected *Ipomoea* plants by lipid peroxidation p-NDA and hydrogen peroxide assay methods

Extracts	IC_{50}±SDM* ($\mu g\ mL^{-1}$) Lipid peroxidation	p-NDA	Hydrogen peroxide
IPL-M	395.54±1.206	384.32±2.358	148.19±2.08
IPS-M	193.56±2.646	710.22±4.625	80.87±8.62
IPR-M	358.02±7.589	242.18±5.684	444.15±37.53
I.W.-M	256.24±0.569	647.66±2.125	60.53±0.02
IBL-M	130.46±2.685	250.0±5.000	76.60±0.41
IBS-M	783.02±1.258	429.55±7.546	178.07±9.89
IBR-M	761.24±0.859	658.69±0.487	151.86±5.75
IBW-M	370.76±1.352	401.28±2.129	54.68±4.0
INL-M	230.35±4.875	784.93±1.498	36.25±3.89
INS-M	103.11±0.642	>1000	153.63±17.54
INA-M	208.33±2.453	306.56±2.584	108.68±9.87
Ascorbic acid	-	-	182.65±2.33
Rutin	94.287±4.25	>1000	35.74±3.524

*Average values of four independent determinations in quadruplicate

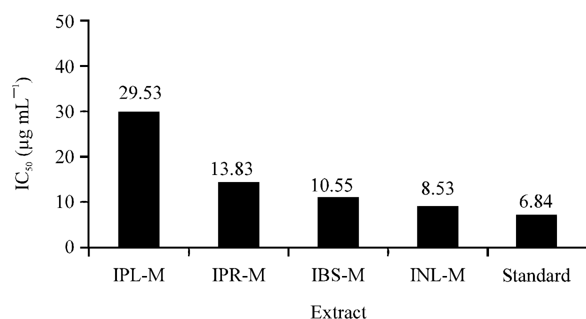

Fig. 7: IC_{50} values of methanolic extracts of selected *Ipomoea* plants for prevention of haemolysis

Inhibition of lipid peroxidation in egg lectin was also studied. Results are given in Table 1.

When tested by lipid peroxidation method, INS-M showed maximum ability to inhibit lipid peroxidation with IC_{50} value 103.11±0.642 $\mu g\ mL^{-1}$. IBL-M and IPS-M also showed good activity with IC_{50} value 130.46±2.685 $\mu g\ mL^{-1}$ and 193.56±2.646 $\mu g\ mL^{-1}$, respectively. Other methanolic extracts showed moderate activity with IC_{50} values in the range of 208.33-783.02 $\mu g\ mL^{-1}$. Rutin exhibited good antioxidant activity with IC_{50} value of 94.287±4.25 $\mu g\ mL^{-1}$.

The methanolic extracts were evaluated for their ability to scavenge hydroxyl radical by p-NDA method. Among the extracts, IPR-M and IBL-M showed maximum activity with IC_{50} value of 242.18±5.684 and 250 $\mu g\ mL^{-1}$, respectively. All other methanolic extracts except INS-M showed moderate activity with IC_{50} values in the range of 306.56-784.93 $\mu g\ mL^{-1}$.

In hydrogen peroxide method, the methanolic leaf extract of *Ipomoea nil* (INL-M) showed potent ability to inhibit the generation of reactive oxygen species, the IC_{50} value being 36.25±3.89 $\mu g\ mL^{-1}$. The extracts IBW-M, I.W.-M, IBL-M and IPS-M also showed good activity

Table 2: Total phenolic, flavonoid and antioxidant capacity of methanolic extract of selected species of *Ipomoea*

Extract	Total Phenolic content *(mg g^{-1} of dry extract)	Total Flavonoid content **(mg g^{-1} of dry extract)	Total Antioxidant capacity **(mg g^{-1} of dry extract)
IPL-M	880.0±1.532	128.780±0.231	66.182±0.048
IPS-M	756.0±0.234	49.357±0.543	116.363±0.357
IPR-M	659.0±0.544	44.143±0.034	67.000±0.246
I.W.-M	854.0±0.078	58.570±0.028	54.182±0.454
IBL-M	856.0±0.012	59.928±0.185	63.363±0.317
IBS-M	489.0±0.134	18.857±0.352	58.363±0.784
IBR-M	527.0±0.321	36.872±0.112	43.268±0.129
IBW-M	778.0±0.640	45.143±0.425	37.636±0.273
INL-M	902.0±0.098	124.780±0.244	43.090±0.549
INS-M	737.0±0.121	30.928±0.450	35.727±0.871

*Expressed in gallic acid equivalence in mg g^{-1} of extract, **Expressed in rutin equivalence in mg g^{-1} of extract

with IC_{50} values ranging between 54.68 to 80.87 $\mu g\ mL^{-1}$ where as other extracts showed moderate activity. The standard (rutin) exhibited good activity with IC_{50} value of 35.64±2.54 $\mu g\ mL^{-1}$.

Total phenolic, flavonoid and antioxidant capacity: This study was performed to check whether the antioxidant activity of methanolic extracts of selected *Ipomoea* plants is due to its phenolic or flavonoid content. The total phenolic, flavonoid and antioxidant capacity can be correlated with the antioxidant activity of the extract.

The total phenolic content of IPL-M and INL-M was found to be highest with 880.0±1.532 and 902.0±0.098 mg g^{-1} of dry extract respectively. Where as total flavonoid content for IPL-M and INL-M was found to be 128.78±0.231 and 124.78±0.244 mg g^{-1} of dry extract, respectively.

The potent antioxidant nature of flavonoids and phenolics are well known. The extracts with more of flavonoids and phenolics will naturally show better antioxidant activity. In our study also, the extracts possessing high quantities of flavonoids and phenolics (IPL-M and INL-M) showed comparatively better activity than other extracts. The results are given in Table 2.

Isolation and activity checking of free phenolics: The isolated free phenolics were evaluated for its antioxidant potential by different methods, described in 2.7. Free phenolics were able to scavenge nitric oxide radicals generated in nitric oxide method and oxygen free radicals generated by H_2O_2 method in much efficient manner, compared to crude methanolic fractions. However, free phenolics were less efficient in scavenging DPPH radicals and superoxide radicals. Hence, the potent antioxidant nature of methanolic extract of *Ipomoea pes-caprae* may not be only due to its free phenolic contents.

Polarity based fractionation of IPL-m and evaluation of its antioxidant activity: Among four fractions tested, ethyl acetate fraction showed maximum activity by DPPH

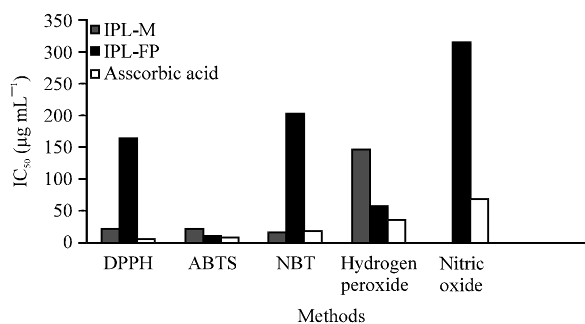

Fig. 8: Comparison of antioxidant activity of methanolic extract of leaf of *Ipomoea pes-caprae* and free phenolics isolated from it

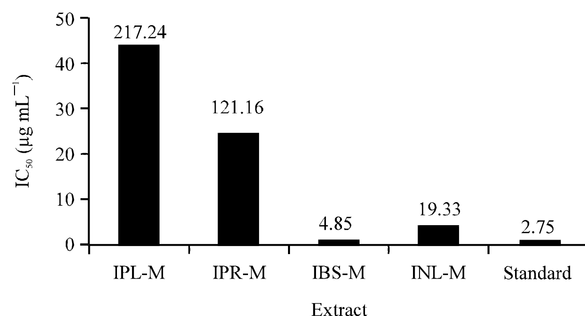

Fig. 9: Comparison of antioxidant activity of fractions of methanolic extract of leaf of *Ipomoea pes-caprae* by DPPH method

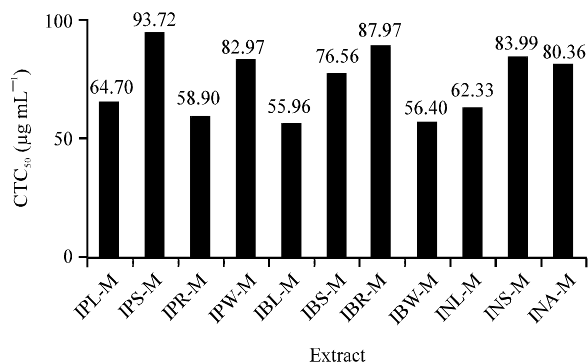

Fig. 10: CTC_{50} of methanolic extracts of selected *Ipomoea* species against HEp-2 cells by MTT assay

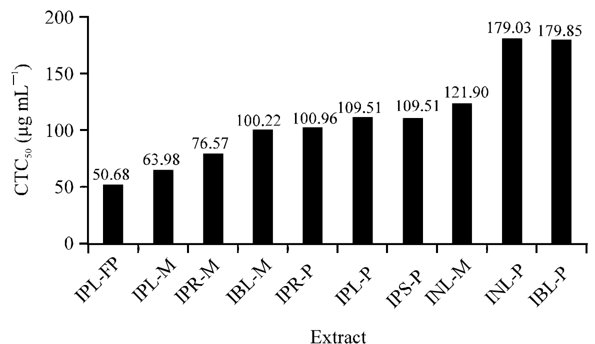

Fig. 11: CTC_{50} values of methanolic extracts of selected *Ipomoea* species against HEp-2 cells by SRB assay

method with IC_{50} value of 4.85±0.286 µg mL^{-1} which was comparable with the ascorbic acid. The methanol fraction also showed potent activity with IC_{50} value of 19.33±0.545 µg mL^{-1}. The petroleum ether and chloroform fractions showed low efficiency in scavenging DPPH free radicals. From this investigation it can be concluded that ethyl acetate and methanol fraction of *I. pes-caprae* has molecules which are potent scavengers of free radicals (Fig. 8).

***In vitro* cytotoxicity assays:** Cytotoxicity studies of all the methanolic extracts and free phenolics isolated from methanolic leaf extract of *Ipomoea pes-caprae* were conducted on HEp-2 cell culture (human laryngeal carcinoma cells). Different concentrations of the extracts prepared in maintenance medium were exposed to the cells for a period of 72 h. The viability of cells was estimated by two methods, MTT and SRB assays. The cytotoxicity studies were conducted to study the level of toxicity showed by the extracts (Fig. 9-10).

MTT assay

SRB assay: All the extracts showed good cytotoxicity with CTC_{50} value in the range of 50-100 µg mL^{-1}. Among the tested extracts, IBL-M was most potent with CTC_{50} value of 55.96±.873 µg mL^{-1} followed by IBW-M, IPR-M and IPL-M. The CTC_{50} values of MTT and SRB were comparable. Free phenolics isolated from leaves of *Ipomoea pes-caprae* showed good cytotoxic activity with CTC_{50} value of 50.68±2.228 µg mL^{-1} (Fig. 11).

CONCLUSION

Numerous species of the genus *Ipomoea* are widely used in folk medicine all over the world as powerful cathartics. Pharmacological studies have reported antimicrobial, analgesic, spasmogenic, spasmolytic, Hypotensive, insecticidal, pyschotomimetics and anticancer effects. 4-Ipomeanol is a pneumotoxic furan derivative isolated from the sweet potato *Ipomoea batatas* that has been under clinical evaluation as lung cancer specific antineoplastic agent.

The successive methanolic extract of all the selected plants of *Ipomoea* species showed potent ability to scavenge different types of free radicals generated by various *in vitro* assays. The total phenol and flavonoid content estimation of the methanolic extracts revealed

their presence in good qualities. Among different methanolic extracts tested for antioxidant activity, the leaf extract of *Ipomoea pes-caprae* was the most potent extract. The same extract contained highest content of total phenolics and flavonoids which suggests that the antioxidant nature of the extract may be due to its high phenolic and flavonoid content. Among the fractions tested of the above extract IPL-M, prepared according to their polarity, evaluated for their antioxidant activity, the ethyl acetate fraction showed best activity. Ethyl acetate fraction is believed to possess phenolics and flavonoids. This study reconfirms that the potent antioxidant activity of IPL-M is due to its high phenol and flavonoid contents. Isolated free phenolics showed better activity than crude methanolic extract by nitric oxide, ABTS and Hydrogen peroxide methods indicating that the antioxidant activity is due to free phenolics. But when tested by DPPH and alkaline DMSO method, crude extracts showed better activity indicating that there may be more than one component responsible for the activity. All methanolic extracts showed good cytotoxicity against HEp-2 cell line with CTC_{50} value in the range of 50-100 $\mu g\ mL^{-1}$.

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