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Full Length Research Paper

Evaluation of comparative antioxidant potential of aqueous and organic fractions of *Ipomoea carnea*

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The methanolic extract of *Ipomoea carnea* Jacq. was dissolved in distilled water and partitioned with *n*-hexane, chloroform, ethyl acetate and *n*-butanol successively. The antioxidant potential of all these fractions and remaining aqueous fraction was evaluated by four methods: DPPH free radical scavenging activity, total antioxidant activity, FRAP assay and ferric thiocyanate assay and total phonolics were also determined. All the fractions showed significant antioxidant potential. The results revealed that *n*-butanol fraction showed highest value of % inhibition of DPPH radical (91.11% ± 0.68), IC_{50} of *n*-butanol fraction was 74.65 ± 1.4 µg/ml, relative to butylated hydroxytoluene (BHT), having IC_{50} of 12.1 ± 0.92 µg/mL. The chloroform soluble fraction showed highest total antioxidant activity (0.9096 ± 0.1) and highest total phenolic contents (113.05 ± 1.2 mg of gallic acid equivalents) as compared to other fractions. The ethyl acetate soluble fraction exhibited highest FRAP value (511.99 ± 1.8 µg of trolox equivalents) as well as highest value of inhibition of lipid peroxidation (61.87% ± 1.2) as compared to other fractions.

Key words: *Ipomoea carnea* Jacq., DPPH assay, total antioxidant activity, FRAP value, total phenolics, Inhibition of lipid peroxidation (%).

INTRODUCTION

Free radicals and other reactive oxygen species (ROS), such as superoxide anion ($\cdot O_2^-$), hydroxyl radical ($\cdot OH$) and hydrogen peroxide (H_2O_2), and entire class of highly reactive molecules derived from the normal metabolism of oxygen or from exogenous factors and agents. Oxidative damage to crucial cellular molecules induced by ROS has been implicated as a possible factor in the etiology of several human diseases, including cancer, cardiovascular disease, and aging (Halliwel and Gutteridge, 1998). In recent years, there is an increasing interest in finding antioxidant phytochemicals, because they can inhibit the propagation of free radical reactions, protect the human body from diseases and retard lipid oxidative rancidity in foods. The most effective ones seem to be flavonoids and other phenolic compounds of many plant raw materials, particularly in herbs, seeds, and fruits. Their metal-chelating capabilities and radical-scavenging properties have enabled phenolic compounds to be thought of as effective free radical scavengers and inhibitors of lipid peroxidation (Chung et al., 2006). A large number of herbal drugs are reputed to be of excellent medicinal value, and are used for treatment of several ailments. In folk medicine, various indigenous drugs are used in single and/or in combined form to treat different types of inflammatory and arthiritic conditions, with considerable success (Paula et al., 2003). *Ipomoea carnea* Jacq. (*Convolvulaceae*) is a common weed which is locally known as 'Beshram'. Due to its high adaptability and resistance towards adverse climatic condition it may grow in all types of climate and soils, marshy as well dry (Nandkumar, 2009). The plant has allelopathic effect; boiled roots are used as laxative and it provokes menstruation. Other parts of the plant are used by traditional healers for skin diseases treatment, milky juice of plant has been used for the treatment of Leucoderma and other related skin diseases. While screening its different parts for enzymatic activity, the latex exhibited a

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considerable amount of chitinase/lysozyme activity (Patel et al., 2009). To the best of their knowledge, no salient antioxidant studies have been carried out on *I. carnea* Jacq., therefore, in the present investigation, the authors describe the comparative *in vitro* antioxidant potential of aqueous and organic fractions of this species.

MATERIALS AND METHODS

Plant material

The plant *I. carnea* Jacq. was collected from kotly, Azad Kashmir in March 2009, and identified by Muhammad Ajaib (Taxonomist), Department of Botany, GC University, Lahore.

Extraction and fractionation of antioxidants

The shade-dried ground whole plant (8.5 kg) was exhaustively extracted with methanol (12 L × 4) at room temperature. The extract was evaporated to yield the residue (815 g), which was dissolved in distilled water (1.5 L) and partitioned with *n*-hexane (1 L × 4), chloroform (1 L × 4), ethyl acetate (1 L × 4) and *n*-butanol (1 L × 4), respectively. These organic fractions and remaining water fraction was concentrated separately on rotary evaporator and the residues thus obtained were used to evaluate their *in vitro* antioxidant potential.

Chemicals and standards

DPPH$^{\cdot}$ (1,1-Diphenyl-2-picrylhydrazyl radical), TPTZ (2,4,6-Tripyridyl-s-triazine), Trolox, Gallic acid, Follin Ciocalteu reagent and BHT (Butylated hydroxytoluene) were obtained from Sigma Chemical Company Ltd. (USA) and organic solvents (*n*-hexane, chloroform, ethyl acetate, *n*-butanol), sulphuric acid, sodium phosphate, ammonium molybdate, ferric chloride and ferrous chloride from Merck (Pvt.) Ltd. (Germany).

DPPH Radical scavenging activity

The DPPH radical scavenging activities of various fractions of plant were examined by comparison with that of known antioxidant, butylated hydroxytoluene (BHT) using the method of Lee and Shibamoto (2001). Briefly, various amounts of the samples (1000, 500, 250, 125, 60, 30, 15 and 8 μg/mL) were mixed with 3 ml of methanolic solution of DPPH (0.1 mM). The mixture was shaken vigorously and allowed to stand at room temperature for one an hour. Then, absorbance was measured at 517 nm against methanol as a blank in the spectrophotometer. Lower absorbance of spectrophotometer indicated higher free radical scavenging activity. The percent of DPPH decolouration of the samples was calculated according to the formula:

$$\text{Antiradical activity} = A_{control} - A_{sample} / A_{control} \times 100$$

Each sample was assayed in triplicate and mean values were calculated.

Total antioxidant activity by phosphomolybdenum method

The total antioxidant activities of various fractions of plant were evaluated by phosphomolybdenum complex formation method (Prieto et al., 1999). Briefly, 500 μg/mL of each sample was mixed with 4 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in sample vials. The blank solution contained 4 mL of reagent solution. The vials were capped and incubated in water bath at 95°C for 90 min. After the samples had been cooled to room temperature, the absorbance of mixture was measured at 695 nm against blank. The antioxidant activity was expressed relative to that of butylated hydroxytoluene (BHT). All determinations were assayed in triplicate and mean values were calculated.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was done according to Benzie and Strain (1996) with some modifications. The stock solutions included 300 mM acetate buffer (3.1 g $CH_3COONa.3H_2O$ and 16 mL CH_3COOH), pH 3.6, 10 mM TPTZ (2,4,6-Tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$ solution. The fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution and 2.5 mL $FeCl_3.6H_2O$ solution and then warmed at 37°C before using. The solutions of plant samples and that of trolox were formed in methanol (250 μg/mL). 10 μL of each of sample solution and BHT solution were taken in separate test tubes and 2990 μL of FRAP solution was added in each to make total volume up to 3 mL. The plant samples were allowed to react with FRAP solution in the dark for 30 min. Readings of the coloured product [ferrous tripyridyltriazine complex] were then taken at 595 nm. The FRAP values were determined as micromoles of trolox equivalents per mL of sample by computing with standard calibration curve constructed for different concentrations of trolox. Results were expressed in TE μg/mL.

Total phenolic contents

Total phenolics of various fractions of plant were determined by the method of Makkar et al. (1993). 0.1 mL (0.5 mg/mL) of sample was combined with 2.8 mL of 10% Na_2CO_3 and 0.1 mL of 2 N Folin-Ciocalteu reagent. After 40 min absorbance at 725 nm was measured by UV-visible spectrophotometer. Total phenolics were determined as milligrams of gallic acid equivalents per gram of sample by computing with standard calibration curve constructed for different concentrations of gallic acid. The standard curve was linear between 50 - 400 mg/g of gallic acid. Results were expressed in GAE mg/g.

Ferric thiocyanate (FTC) assay

The antioxidant activities of various fractions of plant on inhibition of linoleic acid peroxidation were assayed by thiocyanate method (Valentao et al., 2002). The 0.1 mL of each of sample solution (0.5 mg/ mL) was mixed with 2.5 mL of linoleic acid emulsion (0.02 M, pH 7.0) and 2.0 mL of phosphate buffer (0.02 M, pH 7.0). The linoleic emulsion was prepared by mixing 0.28 g of linoleic acid, 0.28 g of Tween-20 as emulsifier and 50.0 mL of phosphate buffer. The reaction mixture was incubated for 5 days at 40°C. The mixture without extract was used as control. The 0.1 mL of the mixture was taken and mixed with 5.0 mL of 75% ethanol, 0.1 mL of 30% ammonium thiocyanate and 0.1 mL of 20 mM ferrous chloride in 3.5% HCl and allowed to stand at room temperature. Precisely, 3 min after addition of ferrous chloride to the reaction mixture, absorbance was recorded at 500 nm. The antioxidant activity was expressed as percentage inhibition of peroxidation (IP%) [IP% = {1-(abs. of sample) / (abs. of control)} × 100]. The antioxidant activity of BHT was assayed for comparison as reference standard.

Statistical analysis

All the measurements were done in triplicate and statistical analysis was performed by Microsoft excel 2003. Results are presented as average ± SEM.

RESULTS AND DISCUSSION

The 1,1-diphenyl-2- picryl hydrazyl (DPPH) radical is widely used in the model system to investigate the scavenging activities of several natural compounds such as phenolics and anthocyanins or crude mixtures such as the methanol extract of plants. DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to form a stable diamagnetic molecule. DPPH radical is scavenged by antioxidants through the donation of a proton forming the reduced DPPH. The colour changes from purple to yellow after reduction, which can be quantified by its decrease of absorbance at wavelength 517 nm. Radical scavenging activity increased with increasing percentage of the free radical inhibition (Huang et al., 2005). The significant decrease in the concentration of DPPH radical is due to the scavenging ability of *I. carnea*. As the concentration of sample increased, the percent inhibition of DPPH radical also increased. It was found that *n*-butanol soluble fraction of this plant had the highest radical-scavenging activity followed by the order of ethyl acetate soluble fraction, chloroform soluble fraction, aqueous fraction, and *n*-hexane soluble fraction (Table 1).

The *n*-butanol fraction showed highest percent inhibition of DPPH radical (91.11 ± 0.68) at concentration of 500 µg/mL in assay mixture. The IC_{50} values (concentration of sample required to scavenge 50% free radical) were calculated. This value is inversely related to the activity. In the present study, IC_{50} values of *n*-hexane, chloroform, ethyl acetate, *n*-butanol and aqueous fraction were 835.28 ± 2.1, 167.87 ± 1.5, 117.16 ± 1.9, 74.65 ± 1.4 and 395.81 ± 1 µg/mL, respectively (Table 2), relative to butylated hydroxytoluene (BHT), having IC_{50} of 12.1 ± 0.92 µg/ml. The *n*-butanol fraction showed lowest IC_{50} value which means that it has highest DPPH radical-scavenging activity. However, the radical-scavenging activity decreased little as the concentration increased further. The reason is that the interference substance(s) can not donate more protons at critical higher concentrations.

The total antioxidant capacity of the fractions was measured spectrophotometrically by phosphomolybdenum method, which is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of green phosphate / Mo (V) compounds with a maximum absorption at 695 nm (Miladi and Damak, 2008). The phosphomolybdenum method usually detects antioxidants such as ascorbic acid, some phenolics, tocopherols and carotenoids. From results, it has been observed that chloroform soluble fraction has highest total antioxidant activity (0.9096 ± 0.1) followed by *n*-hexane fraction (0.755 ± 0.04), *n*-butanol fraction (0.489 ± 0.016), ethyl acetate fraction (0.4318 ± 0.06) and aqueous fraction (0.2429 ± 0.01) (Table 2) relative to butylated hydroxytoluene (BHT), a reference standard having total antioxidant activity 1.2186 ± 0.09. It showed that chloroform fraction has highest phenolic content while aqueous fraction has lowest phenolic content.

FRAP is a simple direct test for measuring antioxidant capacity. This method was initially developed to assay plasma antioxidant capacity, but can be used with plant extracts too (Gourine et al., 2010). FRAP assay measures the reducing ability of antioxidants against oxidative effects of reactive oxygen species. Electron donating anti-oxidants can be described as reductants and inactivation of oxidants by reductants can be described as redox reactions. The antioxidative activity is estimated by measuring the increase in absorbance caused by the formation of ferrous ions from FRAP reagent containing TPTZ (2,4,6-Tri (2-pyridyl)-s-triazine) and $FeCl_3.6H_2O$. The absorbance is measured spectrophotometrically at 595 nm (Benzie and Strain, 1996). In the current study, *n*-hexane, *n*-butanol, chloroform, ethyl acetate and aqueous fractions of *I. carnea* exhibited promising antioxidant power with FRAP values 147.08 ± 1.3, 451.58 ± 2.0, 246.91 ± 1.7, 511.99 ± 1.8, 136.20 ± 2.1(µg of trolox equivalent), respectively (Table 2). Ethyl acetate fractions showed highest FRAP value, so it has highest antioxidant activity while aqueous fraction has lowest FRAP value.

Polyphenolic compounds have an important role in stabilizing lipid oxidation and are associated with antioxidant activity. The phenolic compounds may contribute directly to antioxidative action. The antioxidative activities observed can be ascribed both the different mechanisms exerted by different phenolic compounds and to the synergistic effects of different compounds. The antioxidants have different functional properties, such as reactive oxygen species scavenging e.g. quercetin and catechin, inhibition of the generation of free radicals and chain-breaking activity, e.g. *p*-coumaric acids and metal chelation. These compounds are normally phenolic compounds, which are effective proton donors, and include tocopherols, flavonoids, and other organic acid (Huang et al., 2005). It has been suggested that the phenolic content of plant materials is correlated with their antioxidant activity (Juan et al., 2010). Table 1 shows the phenolic concentration in the different fractions, expressed as milligram of gallic acid equivalents (GAEs) per gram of fraction. Among the four fractions, the chloroform soluble fraction showed the highest amount of phenolic compounds (113.05 ± 1.2 mg/g) followed by ethyl acetate soluble fraction (103.70 ± 1.2mg/g), n-butanol fraction (94.27 ± 1.27), aqueous fraction (78.59 ± 0.68), and n-hexane soluble fraction (69.36 ± 1.4 mg/g), mg of gallic acid equivalent per gram of fraction, respectively.

Table 1. Free radical scavenging activity of various fractions of *Ipomoea carnea* using 1,1-Diphenyl-2-picryl hydrazyl radical (DPPH).

S/No.	Sample	Concentration in assay (µg/4 ml)	% scavenging of DPPH ± S.E.M[a)]
1	*n*-Hexane soluble fraction	1000	55.53 ± 0.84
		500	38.37 ± 0.14
		250	30.73 ± 0.17
		125	24.36 ± 0.29
2	Chloroform soluble fraction	1000	88.87 ± 0.39
		500	74.48 ± 0.73
		250	60.74 ± 0.81
		125	50.51 ± 1.19
		60	41.52 ± 1.17
		30	34.08 ± 1.02
3	Ethyl acetate solule fraction	500	90.53 ± 0.98
		250	75.28 ± 0.15
		125	71.56 ± 0.68
		60	57.13 ± 0.63
		30	46.94 ± 1.8
4	*n*-Butanol soluble fraction	500	91.11 ± 0.68
		250	78.84 ± 0.91
		125	58.31 ± 0.29
		60	46.68 ± 0.54
		30	42.82 ± 2.0
5	Aqueous fraction	1000	75.26 ± 1.20
		500	51.41 ± 1.6
		250	44.02 ± 0.2
		125	40.92 ± 0.79
		60	37.49 ± 0.82
6	BHT[b)]	60	91.17 ± 0.13
		30	72.14 ± 0.46
		15	65.86 ± 0.47
		8	41.30 ± 0.72

[a)] Standard mean error of three assays. [b)] Standard antioxidant.

The ferric thiocyanate method measures the amount of peroxide generated at the initial stage of linoleic acid emulsion during incubation. Here, peroxide reacts with ferrous chloride to form ferric chloride, which in turn reacts with ammonium thiocyanate to produce ferric thiocyanate, a reddish pigment. Low absorbance values measured via the FTC method indicate high antioxidant activity (Kim and Kim, 2010). Table 2 shows the hydroperoxides inhibitory activity of *I. carnea* plant extracts through FTC test. As shown in the Table 2, almost all fractions significantly retarded the formation of hydroperoxides in the linoleic acid emulsion system throughout the incubation period as compared to the control sample. The ethyl acetate soluble fraction of this plant showed the highest percent of inhibition of lipid peroxidation 61.87 ± 1.2 and *n*-hexane fraction has lowest percent of inhibition of lipid peroxidation 58.24 ± 0.62. This result suggests that the ethyl acetate fraction might contain primary antioxidant compounds, which are able to react aggressively with free radicals, particularly hydroxyl radicals, thereby terminating the radical-chained reaction and retard the formation of hydroperoxides (Ismail et al., 2010). There were significant differences among the different fractions in lipid peroxidation

Table 2. IC_{50}, total phenolics, total antioxidant activity, FRAP values and lipid peroxidation inhibition values of different fractions of *I. carnea*.

S/No.	Sample	IC_{50} of DPPH assay (µg/mL) ± S.E.M [a)]	Total antioxidant activity ± S.E.M [a)]	FRAP value TE (µg/ml) ± S.E.M [a)]	Total phenolics (GAE mg/g of sample) ± S.E.M [a)]	Inhibition of lipid peroxidation (%) ± S.E.M [a)]
1	*n*-Hexane fraction	835.28 ± 2.1	0.755 ± 0.04	147.08 ± 1.3	69.36 ± 1.4	58.24 ± 0.62
2	Chloroform fraction	167.87 ± 1.5	0.9096 ± 0.1	246.91 ± 1.7	113.05 ± 1.2	60.27 ± 0.70
3	Ethyl acetate fraction	117.16 ± 1.9	0.4318 ± .06	511.99 ± 1.8	103.70 ± 1.2	61.87 ± 1.2
4	*n*-Butanol fraction	74.65 ± 1.4	0.489 ± .016	451.58 ± 2.0	94.27 ± 1.27	59.31 ± 0.34
5	Aqueous fraction	395.81 ± 1.0	0.2429 ± .01	136.20 ± 2.1	78.59 ± 0.68	61.55 ± 0.55
6	BHT [b)]	12.1 ± 0.92	1.2186 ± .09	-	-	62.91 ± 0.60

[a)] Standard mean error of three assays. [b)] Standard antioxidant.

inhibitory potential. The overall inhibitory activity of different fractions against hydroperoxides formation can be established in the following descending order: ethyl acetate fraction > aqueous fraction > chloroform fraction > *n*-butanol fraction > *n*-hexane fraction. The inhibition of lipid peroxidation by BHT (standard) was 62.91 ± 0.6 (Table 2).

Conclusion

It was concluded that *n*-butanol fraction showed highest value of % inhibition of DPPH radical (91.11% ± 0.68), IC_{50} of *n*-butanol fraction was 74.65 ± 1.4 µg/ml, relative to butylated hydroxytoluene (BHT), having IC_{50} of 12.1 ± 0.92 µg/mL. The chloroform fraction showed highest total antioxidant activity (0.9096 ± 0.1 as compared to other fractions. The ethyl acetate fractions displayed highest FRAP value (511.99 ± 1.8 µg of trolox equivalents). The chloroform soluble fraction showed highest total phenolic contents (113.05 ± 1.2 mg of gallic acid equivalents). Ethyl acetate soluble fraction showed highest value of inhibition of lipid peroxidation (61.87% ± 1.2) as compared to other fractions. So, it was concluded that although the polar fractions are rich in strong antioxidants but it is also promising to say that all the fractions of this plant are potentially valuable sources of natural antioxidants and bioactive materials, which would be expected to increase shelf life of foods and protect against peroxidative damage in living systems in relation to aging and carcinogenesis.

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REFERENCES

Benzie IEF, Strain JJ (1996). The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. Anal. Biochem.. 239: 70-76.

Chung YC, Chien CT, Yun Teng K, Chou ST (2006). Antioxidative and mutagenic properties of *Zannthxylum ailanthoides* Sieb and Zucc. Food Chem., 97: 418-425.

Gourine N, Yousfi M, Bombarda, Nadjem B, Stocker P, Gaydou EM (2010). Antioxidant activities and chemical composition of essential oil of Pistacia atlantica from Algeria. Ind. Crops Prod., 31: 203-208.

Halliwel B, Gutteridge JMC (1998). Free radicals in biology and medicine. London: Oxford University Press.

Huang DJ, Chen HJ, Lin CD, Lin YH (2005). Antioxidant and antiproliferative activities of water spinach (*Ipomea aquatica* Forsk) constituents. Bot. Bull. Acad. Sin., 46: 99-106.

Ismail HI, Chan KW, Mariod AA, Ismail M (2010). Phenolic content and antioxidant activity of cantaloupe (cucumis melo) methanolic extracts. Food Chem., 119: 643–647.

Juan MY, Chou CC (2010). Enhancement of antioxidant activity, total phenolic and flavonoid content of black soybeans by solid state fermentation with Bacillus subtilis. Food Microbiol., 27: 586-591.

Kim JSJ, Kim MJ (2010). *In vitro* antioxidant activity of Lespedeza cuneata methanolic extracts. J. Med. Plants Res., 4: 674-679.

Lee K, Shibamoto T (2001). Antioxidant property of aroma extract isolated from clove bud [Syzygiun aromaticum (L.) Merr. et Perry]. Food Chem., 74: 443-448.

Makkar HPS, Bluemmel M, Borowy NK, Becker K (1993). Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. J. Sci. Food Agric., 61: 161-165.

Miladi S, Damak M (2008). *In vitro* antioxidant activities of *Aloe vera* leaf skin extracts. J. Soc. Chim. Tunisie., 10: 101-109.

Nandkumar P (2009). Lignin from soda liquor and soda anthraquinone liquor of *Ipomoea carnea* Jacq. Aust. J. Basic App. Sci., 3: 474-476.

Patel AK, Sing VK, Yadav RP, Moir AJG, Jagannadham MV (2009). A glycosylated chitinase from the latex of *Ipomoea carnea*. Phtyochemistry, 70: 1210-1216.

Paula ACB, Hayashi LSS, Freitas JC (2003). Anti-inflammatory and antispasmodic activity of *Ipomoea imperati* (Vahl) Griseb (Convolvulaceae). Braz.. J. Med. Bio. Res.. 36: 105-112.

Prieto P, Pineda M, Aguilar M (1999). Spectrophotometric Quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal. Biochem., 269: 337-341.

Valentao P, Fernandes E, Carvalho F, Andrade PB, Seabra RM, Bastos ML (2002). Antioxidative properties of cardoon (*Cynara cardunculus* L.) infusion against superoxide radical, hydroxyl radical, and hypochlorous acid. J. Agric. Food Chem., 50: 4989-4993.