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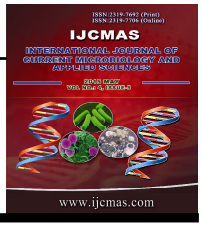

Original Research Article

Evaluation of *in vitro* Antioxidant Activity of *Ipomoea carnea* Jacq.

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Keywords

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Nitric oxide,
Ipomea carnea Jacq,
Superoxide

A B S T R A C T

Free radicals are implicated for many diseases including ageing, diabetes mellitus, cancer, arthritis *etc*. Alcoholic extract of *Ipomoea carnea* Jacq. flower was studied for its *in vitro* antioxidant activity using different models of screening *viz.* DPPH radical scavenging, ABTS radical scavenging, iron chelating activity, nitric oxide scavenging assay, and alkaline DMSO assay. The extracts showed good dose dependant free radical scavenging property in all the models. Phytochemical analysis revealed the presence of major phytocompounds like alkaloids, glycosides, phenolics and saponins. Its antioxidant activity was estimated by IC50 value and the values are 99.4 µg/ml (DPPH radical scavenging), 12.57 µg/ml (ABTS radical scavenging), 220.1 µg/ml (Iron chelating activity) and 20.8 µg/ml (nitric oxide scavenging) and 15.91 µg/ml (alkaline DMSO). The antioxidant property may be related to the polyphenols and flavonoids present in the extract. The findings indicated promising antioxidant activity of alcoholic extract of *Ipomoea carnea* Jacq, needs further exploration for their effective use in both modern and traditional system of medicines.

Introduction

A wide range of parts of the medicinal plant is used for extract as raw drugs and they possess varied medicinal properties. The different parts are used include fruit, flower, twigs, root and stem exudates and modified plant organs (Uniyal *et al.*, 2006). Some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local use.

Phenols, sometimes called phenolics, are one of the main secondary metabolites present in the plant kingdom. They are commonly found in both non-edible and edible plants, and have been reported to have multiple biological effects, including antioxidant. Flavonoids the most common group of polyphenolic compounds that are found ubiquitously in plants. Flavonoids and

other plant phenolics are especially common in flowering tissues, leaves and woody parts such as stems and bark. They are important in plant for normal growth development and defense against infection and injury (Kahkonen *et al.,* 1999).

Ipomoea carnea is popularly known as Besharam, Behaya in India and Morning glory in English. It is a large diffuse shrub with milky juice. The flowers are pale rose, pink or light violet in lax, dichotomously branched axillary and terminal, pedunculate cymes; fruits have a glabrous capsule; seed is silky. The plant belongs to family Convolvulaceae. In India it is distributed particularly in Chhattisgarh and Madhya Pradesh (Dwivedi, 1999, 2003; Ekka and Dixit, 2007). This plant is spread all over the world including American tropics, Argentina, Brazil and Bolivia, Pakistan, Srilanka etc, (Bhattacharyya, 1976; Mahapata, 1978; Sharma, 1978; Austin and Ghazanfar, 1979; Dassanayake and Fosberg, 1980). It is used in different traditional medical systems including Ayurveda, Siddha and Unani. The plant part is used for Safed Dag (Leucoderma), Cyclophosphamide as aphrodisiac, purgative and cathartic (Phillips *et al.,* 1994; Varghese *et al.,* 1996; Ved *et al.,* 2004).

Antioxidants are added as redox systems possessing higher oxidative potential than the drug that they are designed to protect or as chain inhibitors of radical inducted decomposition. In general, the effect of antioxidants is to break up the chains formed during the propagation process by providing a hydrogen atom or an electron to the free radical and receiving the excess energy possessed by the activated molecule. It has been suggested that fruits, vegetables, natural plants contain a large variety of substance called phytochemicals which are present in plants and are the main source of antioxidant in the diet, which could decrease the potential stress caused by reactive oxygen species. The natural antioxidants may have free-radical scavengers, reducing agents, potential complexes of prooxidant metals, quenches of singlet oxygen etc (Lachman *et al.,* 1986; Ebadi, 2002).

The antioxidants can interfere with the oxidation process by reacting with free radicals (Gupta *et al.,* 2004). Recently interest has increased considerably in finding natural occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants which are being restricted due to their side effects such as carcinogenicity (Kumaran and Karunakaran, 2007). Antioxidants principles from natural resources possess multifacetedness in their multitude and magnitude of activity and provide enormous scope in correcting imbalance (Shriwaikar *et al.,* 2006). Food industry uses natural antioxidants as a replacement of conventional synthetic antioxidants (Govindarajan *et al.,* 2003).

Materials and Methods

Chemicals

Ascorbic acid, rutin, gallic acid, hydrogen peroxide, potassium ferricyanide, trichloroacetic acid, ferric chloride, folin-ciocalteu reagent, indigosulphonic acid, α-α diphenyl β picryl hydrazyl (DPPH), Riboflavin**,** Nitro Blue Tetrazolium (NBT) and Dimethyl Sulphoxide (DMSO) were all purchased from SD-fine chemicals, India, all other reagents used were of analytical grade.

Instrument

UV spectrophotometer (Shimadzu-UV), centrifuge machine (Eltek-research centrifuge-TC).

Plant material

The flowers of *Ipomoea carnea* was collected locally from the college campus of Kumbakonam - Tamilnadu (India) and were authentified by Dr. S. Madhavan, Botany Department from Mannai Rajagopalasamy Government Arts College-Mannargudi, Tamilnadu, India.

Extraction of plant material

The *Ipomoea carnea* flowers are graded, cleaned and disintegrated to required mesh (20-60). It is then extracted with 55% v/v alcohol using soxhlet apparatus for 6 hours. The micella is concentrated under reduced pressure to 30% solids. It is filtered, weighed and used for the study (Rajpal, 2002).

Preparation of *Ipomoea carnea* flower stock solution

Alcoholic extracts of *Ipomoea carnea* flowers was prepared at the concentration of 1,000 µg/ml in methanol. From the stock solution different concentration viz. 10, 20, 40, 60, 80 and 100 µg/ml were prepared in methanol and used for antioxidant studies.

Preliminary phytochemical screening

Preliminary phytochemical screening of the ***Ipomoea carnea*** flowers extract was carried out for the detection of the various plant constituents (Khandelwal, 2004).

Determination of total phenolics

The total phenolic contents of flower extracts of *I. carnea* were determined according to the method described by Malik and Singh (1980). Aliquots of the extracts were taken in a 10 ml glass tube and made up to a volume of 3 ml with distilled water. Then 0.5 ml folin ciocalteau reagent (1:1 with water) and 2 ml Na_2CO_3 (20%) were added sequentially in each tube. A blue color was developed in each tube because the phenols undergo a complex redox reaction with phosphomolibdic acid in folin ciocalteau reagent in alkaline medium which resulted in a blue colored complex, molybdenum blue. The test solutions were warmed for 1 minute, cooled and absorbance was measured at 650 nm against the reagent used as a blank. A standard calibration plot was generated at 650 nm using known concentrations of catechol. The concentrations of phenols in the test samples were calculated from the calibration plot and expressed as mg catechol equivalent of phenol/g of sample.

Determination of total flavonoids

The aluminum chloride method was used for the determination of the total flavonoid content of the sample extracts5. Aliquots of extract solutions were taken and made up the volume 3ml with methanol. Then 0.1ml $AlCl_3$ (10%), 0.1ml Na-K tartarate and 2.8 ml distilled water were added sequentially. The test solution was vigorously shaken. Absorbance at 415 nm was recorded after 30 minutes of incubation. The concentrations of flavonoid in the test samples were calculated from the calibration plot and expressed as mg quercetin equivalent /g of sample.

Total antioxidant capacity

For total antioxidant capacity assay, 0.3 ml of the *Ipomoea carnea* flower extract (10 mg/ml) dissolved in water and mixed with 3ml of reagent solution (0.6 M sulfuric acid, 28mM sodium phosphate and 4 mM ammonium molybdate) in Eppendorf tube. The tubes were capped and incubated in a thermal block at 95°C for 90 min. After 90 min, the mixture was cooled to room

temperature, the absorbance was measured at 695 nm against reagent blank. Methanol (0.3 ml) in the place of extract is used as the blank. Ascorbic acid was used as the standard and the total antioxidant capacity is expressed as equivalents of ascorbic acid (Kumaran and Karunakaran, 2007).

DPPH radical scavenging activity

Plant extract and standard ascorbic acid solution (0.1 ml) of different concentrations viz. 10, 20, 40, 60, 80 and 100 μg/ml was added to 3 ml of a 0.004% methanol solution of DPPH. An equal amount of methanol and DPPH served as control. After 30 minutes incubation in the dark, absorbance was recorded at 517 nm, and the percentage inhibition activity was calculated from [(A0–A1)/A0]×100, where A0 is the absorbance of the control, and A1 is the absorbance of the extract/standard. The antioxidant activity of the extract was expressed as IC50. The IC50 value was defined as the concentration (in μg/ml) of extracts that inhibits the formation of DPPH radicals by 50%. All the tests were performed in triplicate and the graph was plotted with the average of three observations (Sreejayan and Rao, 1996; John and Steven, 1984; Kumaran and Karunakaran, 2007).

Superoxide radical scavenging activity

Each 3ml reaction mixture contained 50 mM sodium phosphate buffer (pH 7.6), 20 μg riboflavin, and 12 mM EDTA and 0.1 mg NBT and 1ml of sample solution. Reaction was started by illuminating the reaction mixture with different concentrations of plant extract and standard ascorbic acid solution viz. 20, 40, 60, 80, 100 and 120 μg/ml for 5min. Immediately after illumination, the absorbance was measured at 590 nm. Identical tubes with reaction mixture and 1ml of methanol were kept in the dark along and served as control. The percentage inhibition of superoxide anion generation was calculated from [(A0–A1)/A0]×100, where A0 is the absorbance of the control, and A1 is the absorbance of the extract/standard. The antioxidant activity of the extract was expressed as IC50. All the tests were performed in triplicate and the graph was plotted with the average of three observations (Govindarajan *et al.,* 2003; Kumaran and Karunakaran, 2007).

ABTS radical scavenging assay

To the reaction mixture containing 0.3 ml of ABTS radical, 1.7 ml phosphate buffer and 0.5 ml extract was added at various concentrations from 2 to 200 μg/ml and the blank was carried out without drug. Absorbance was recorded at 734 nm. Experiment was performed in triplicate (Sreejayan and Rao, 1996, John and Steven, 1984).

Iron chelating activity assay

The method of Benzie and Strain (1996) was adopted for the assay. The principle is based on the formation of O-Phenanthroline-Fe^{2+} complex and its disruption in the presence of chelating agents. The reaction mixture containing 1 ml of 0.05% O - Phenanthroline in methanol, 2 ml ferric chloride (200μM) and 2 ml of various concentrations ranging from 10 to 1000μg was incubated at room temperature for 10 min and the absorbance of the same was measured at 510 nm. EDTA was used as a classical metal chelator. The experiment was performed in triplicates

Nitric oxide radical scavenging

Sodium nitroprusside 5 mM was prepared in phosphate buffer pH 7.4. To 1 ml of various

concentrations of test compound, sodium nitroprusside 0.3 ml was added. The test tubes were incubated at 25 ºC for 5 hr after which, 0.5 ml of Griess reagent was added. The absorbance of the chromophore was read at 546 nm. The experiment was performed in triplicate (Sreejayan and Rao, 1996).

Statistical analysis

All results are expressed as mean ± S.E.M. Linear regression analysis (Origin 6.0 version) was used to calculate the IC50 values.

Result and Discussion

Free radical is a molecule with an unpaired electron and is involved in parasitic and bacterial infections, inflammation, lung damage, cardiovascular disorders, reperfusion injury, neoplastic diseases, aging and atherosclerosis (Roy and Burdon, 1994). They are also involved in autoimmune disorder like rheumatoid arthritis (Rao and Raman, 2004). The results demonstrated that the alcoholic extracts of *Ipomoea carnea* flower extract possess free radical scavenging activity *in vitro* models like DPPH•, ABTS•+,superoxide radical scavenging activity, ion chelating activity assay and Nitric oxide radical scavenging activity.

Phytochemical screening

Ipomoea carnea flower shows alkaloids, flavonoids, carbohydrates, glycosides, saponins, phytosterols, tannins, triterpenoid, protein and amino acids positive. Phenolic compounds are known as powerful chain breaking antioxidant and very important plant constituents because of their scavenging ability, which is due to their hydroxyl groups (Hatano *et al.,* 1989; Shahidi and Wansundeara, 1992). The total phenolic content of alcoholic flower extract of *Ipomoea carne* was found to be 75 mg catechol equivalent/g dry material. Flavonoids are a group of polyphenolic compounds, which exhibit several biological effects such as anti-inflammatory, anti-allergic, anti-cancer, anti-ulcer, anti-viral, and anti-hepatotoxic activities. They are capable of effectively scavenging the reactive O2 species because of their phenolic hydroxyl groups and so they are potent antioxidants (Cao *et al.,* 1997; Umamaheswari and Chatterjee, 2008). The total flavonoids content of alcoholic flower extract of *Ipomoea canea* was determined to be 428 mg quercetin equivalent /g dry material.

Total antioxidant capacity

The total antioxidant capacity in the *Ipomoea carnea* flower extract measured spectrophotometrically was 15.47 mg/gm expressed as ascorbic acid.

The IC50 value was calculated from the plotted graph (Figure 1) of scavenging activity against the concentrations of the samples. IC50 of total antioxidant necessary to decrease the initial DPPH radical by 50%. Triplicate measurements were carried out and IC50 was calculated for the alcoholic extracts based on the percentage of DPPH radicals scavenged. It was observed that free radicals were scavenged by the test compounds in a concentration dependent manner up to the given concentration in all the models. Ascorbic acid was used as the reference compound (positive control) with concentrations 10 to 100 μg/ml.

Free radical scavenging activity (DPPH•)

In the present study, the percentage of scavenging effect on the DPPH• radical was

concomitantly increased with the increase in the concentration of alcoholic extracts from 10 to 120 µg/ml. The percentage of inhibition exists from 11.31 at 10µg/ml to 82.30 at120 µg/ml for alcoholic extracts (Figure 2). From the results it is known that the species, *Ipomoea carnea* possess hydrogen donating capabilities for alcoholic extract and does scavenging free radicals. Furthermore, it was noticed that the flower extract has more pronounced scavenging activity than that of the standard ascorbic acid. Scavenging of DPPH• radical is related to the inhibition of lipid peroxidation (Rekka and Kourounakis 1991; Bhuiyan *et al.,* 2009). DPPH• is usually used as a substance to evaluate the antioxidant activity (Tara Chand *et al.,* 2012). Antioxidants either transfer an electron or a hydrogen atom to DPPH•, thus neutralizing its free radical character (Pan *et al.,* 2008; Raquibul Hasan *et al.,* 2009). The DPPH• assay has been largely used as a reliable and reproducible parameter to search the *in vitro* general antioxidant activity of pure compounds as well as plant extracts. The reducing capacity of compounds could serve as indicator of potential antioxidant property (Meir *et al.,* 1995; Koleva *et al.,* 2002).

ABTS radical scavenging activity

The presence of specific chemical compounds in the extracts of *Ipomoea carnea* may inhibit the potassium persulfate activity and hence reduced the production of ABTS. This study reports that the alcoholic extract of *Ipomoea carnea* has highest antioxidant activity. The ABTS radical scavenging activity of *Ipomoea carnea* flower extract shown in figure 4. It was able to scavenge ABTS radical in a concentration range of 2–128 µg/ml, and the IC50 value of extracts was12.57 µg/ml and ascorbic acid was 30.4 µg/ml. The ABTS method is one of the most often used method for the determination of total antioxidant capacity, it's based on neutralization of radical formed by a single electron oxidation of a synthetic ABTS to a strongly absorbing ABTS•+ radical according to the reaction ABTS-e- → ABTS•+. A stable ABTS radical, which has blue-green absorption, was produced by oxidation of ABTS with potassium persulfate prior to the addition of antioxidants (Stratil *et al.,* 2006).

Superoxide radical scavenging activity

Superoxide radical reduced NBT to a blue colour formazon that can be measured at 560 nm. Maximum scavenging activity was observed at 120 µg/ml concentration (Figure 3) and the IC50 value of flower extract and ascorbic acid were found to be 15.91µg/ml and 35.99 µg/ml respectively. Superoxide can cause oxidation or reduction of solutes depending on their reduction potential. Aerobic and anaerobic organisms possess superoxide dismutase enzymes, which catalyze the breakdown of superoxide radical (Shirwaiar *et al.,* 2007). In our study, alkaline DMSO used for superoxide generation indicates that *Ipomoea carnea* is a potent superoxide scavenger.

Iron chelating activity

Iron binding capacity of the alcoholic extract of *Ipomoea carnea* and the metal chelator

EDTA at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values were presented in Figure 5. Maximum chelating of metal ions at 1000µg/ml for flower extract and EDTA was found to be 49.12% and 97.90% respectively. The IC 50 value of flower extract and EDTA was recorded as 220µg/ml and 63µg/ml respectively. Iron is essential for life because it is required for oxygen transport, respiration and activity of many enzymes.

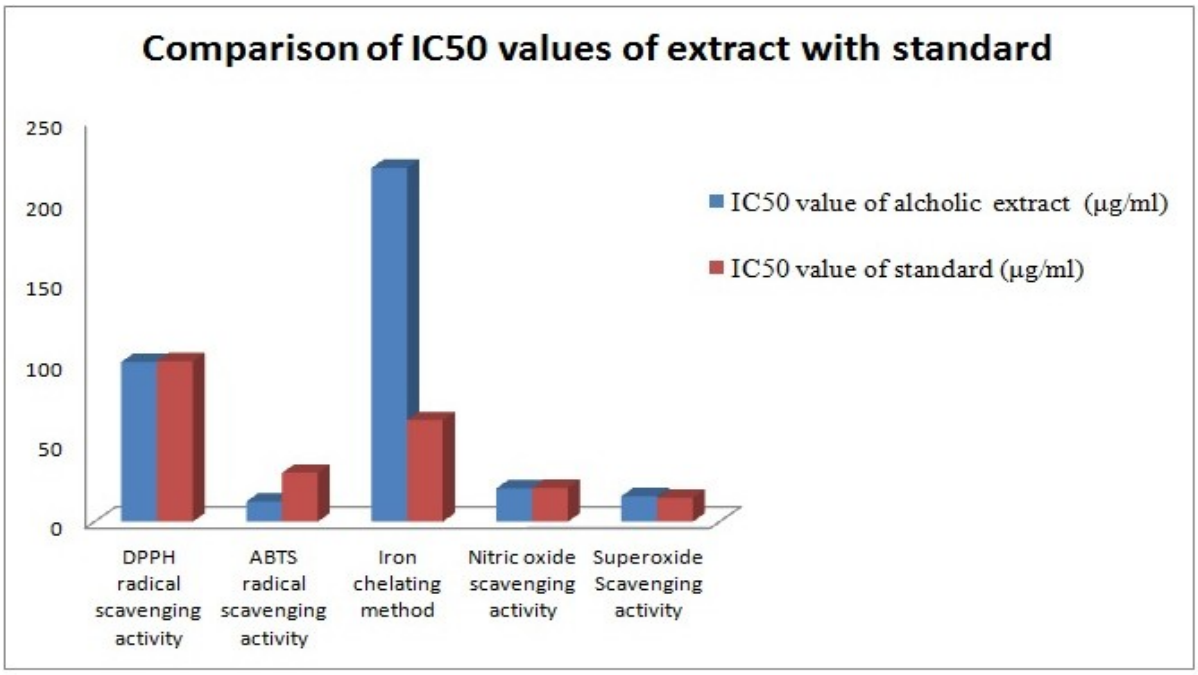

Figure 1: Comparison of IC50 values of alcoholic extract and standard

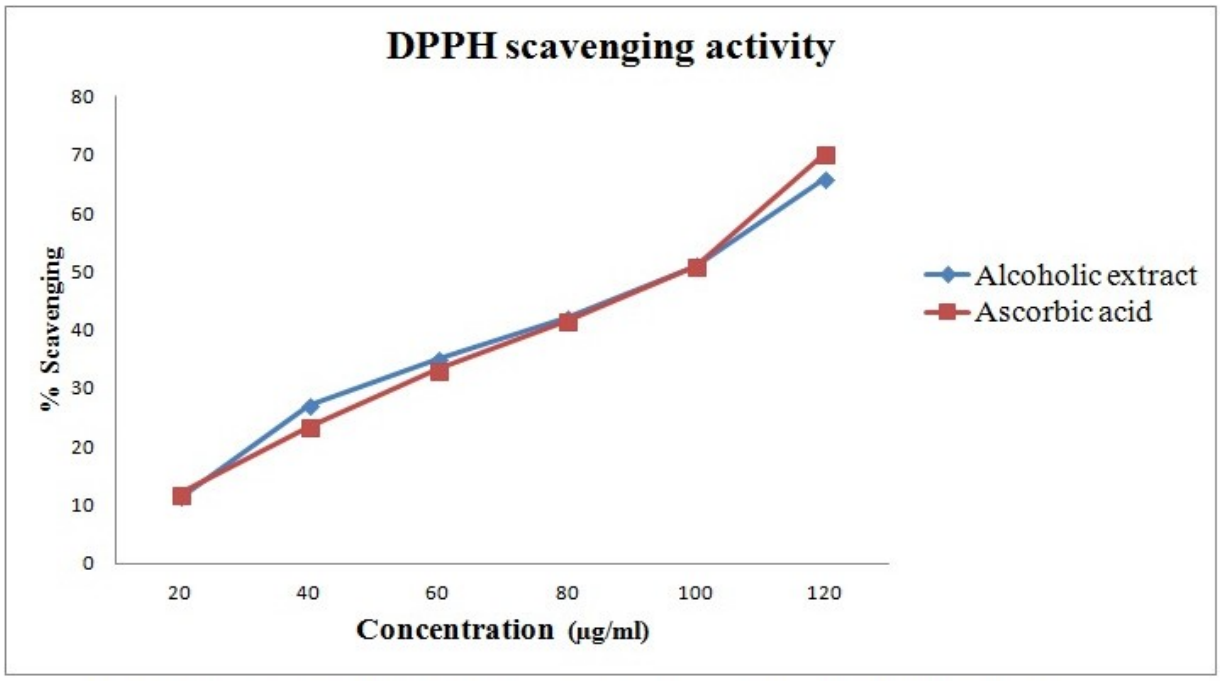

Figure 2: Scavenging ability of *Ipomoea carnea* flower extract on DPPH radical.

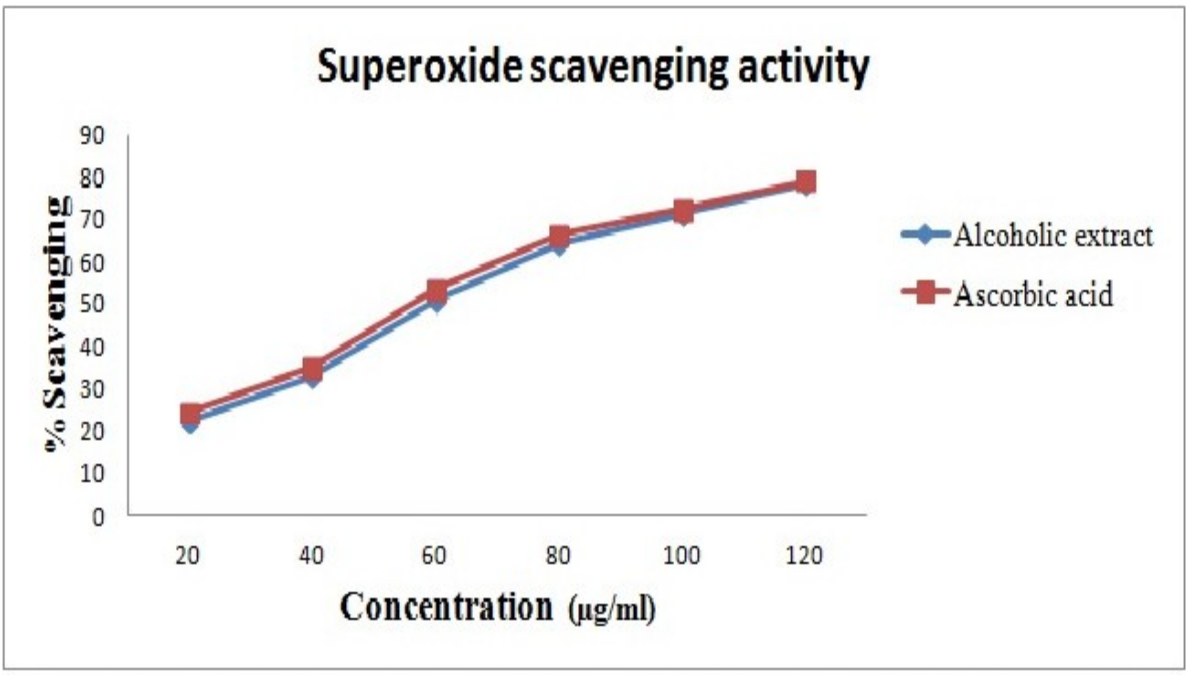

Figure 3: Scavenging ability of *Ipomoea carnea* flower extract on Super oxide radical.

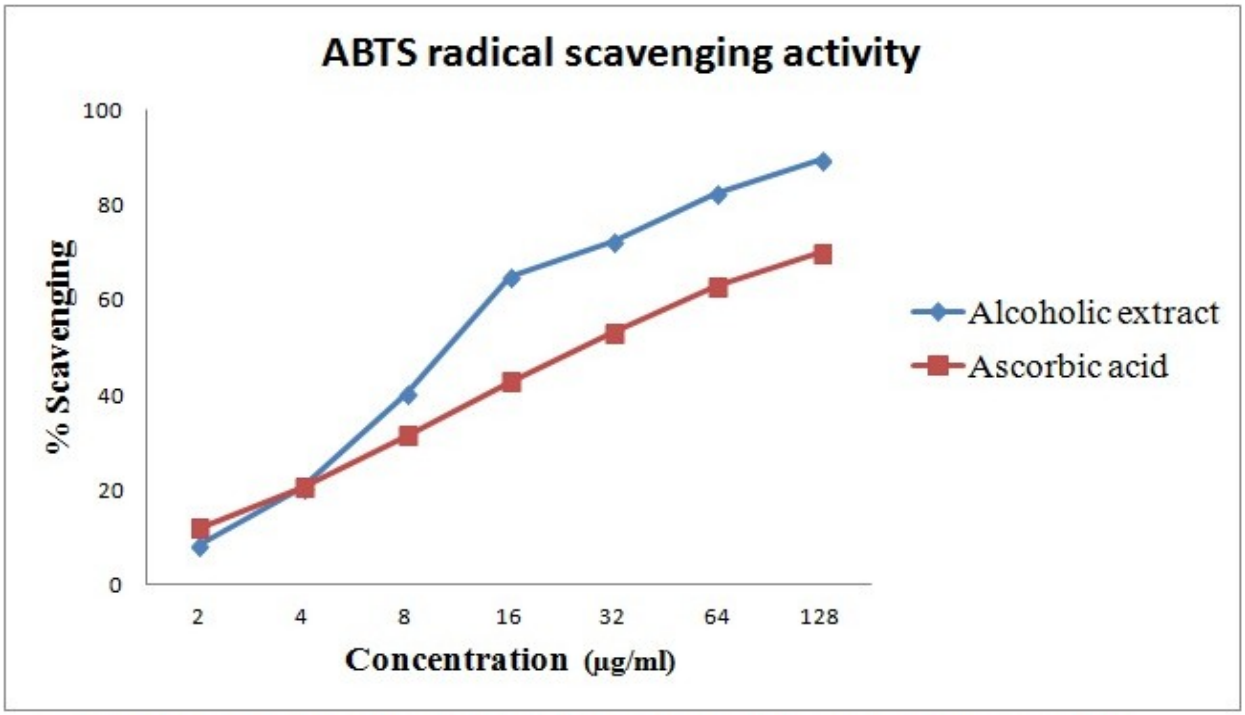

Figure 4: ABTS radical scavenging activity of *Ipomoea carnea* flower extract.

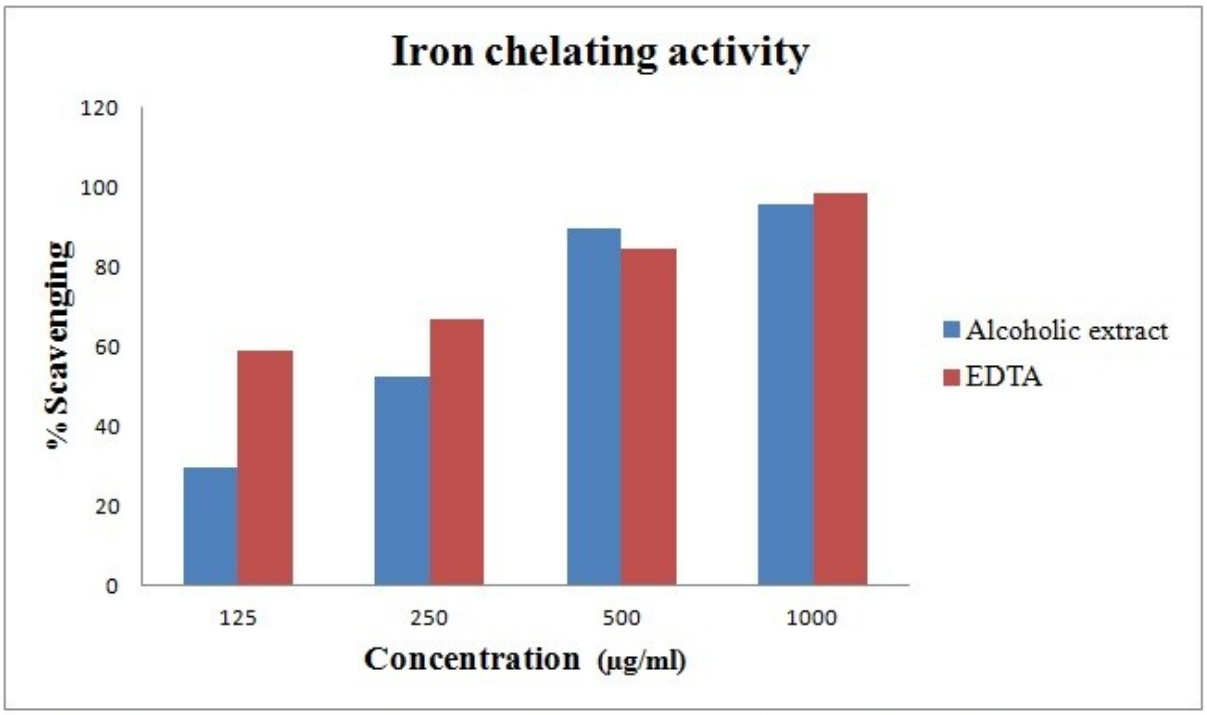

Figure 5: Effect of alcoholic extract of *Ipomoea carnea* flower on Iron-chelating method.

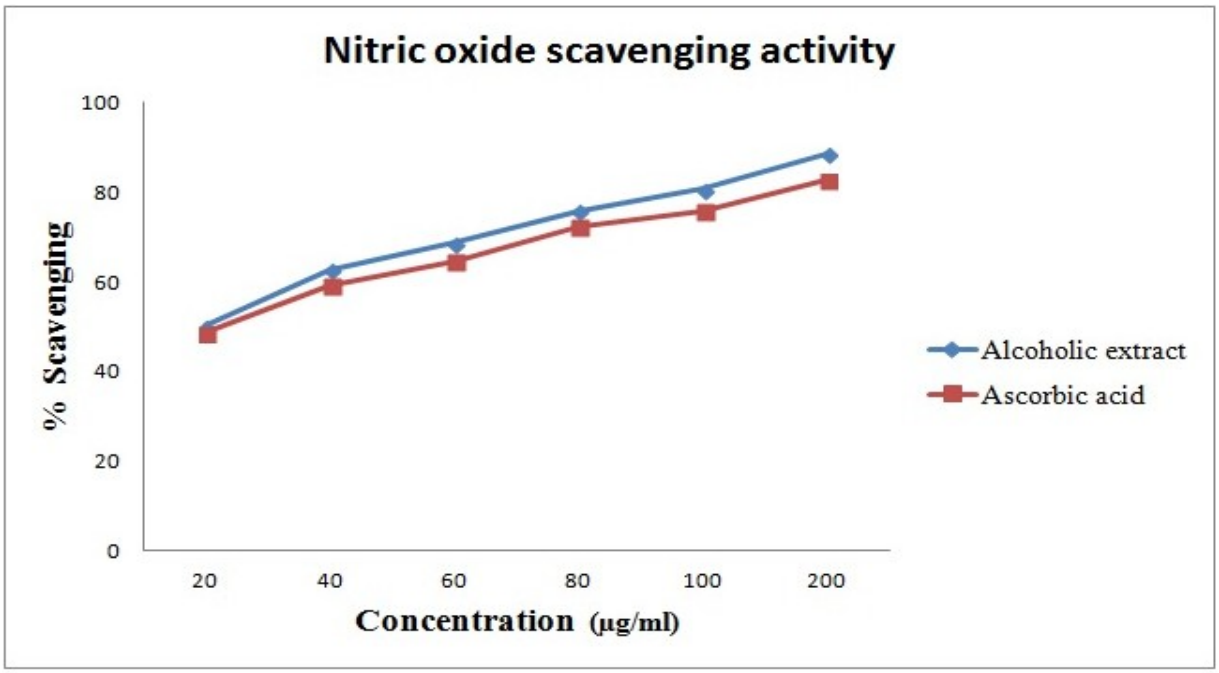

Figure 6: Scavenging ability of *Ipomoea carnea* flower extract on Nitric oxide radical

However, iron is an extremely reactive metal and catalyzes oxidative changes in lipids, proteins and other cellular components (St Angelo 1992; Smith *et al.*, 1992). It causes lipid peroxidation through the Fenton and Haber-weiss reaction

(Halliwell and Gutteridge, 1990) and decomposes the lipid hydroxide into peroxyl and Alkoxyl radicals that can perpetuate the chain reactions (Halliwell, 1991).

Nitric oxide scavenging activity

The extract also showed a moderate nitric oxide-scavenging activity between 20 and 200 µg/ml in a dose-dependent manner (IC50 = 20.8 µg/ml) (Figure 6). *Nitric oxide* (NO) is a free radical produced in mammalian cells, involved in the regulation of various physiological processes. In addition to nitric oxide, reactive oxygen species is also implicated in cancer, inflammation and other pathological conditions (Moncada *et al.*, 1991). The plant products may have the property to counteract the effect of NO formation and in turn may be of considerable interest in preventing the ill effects of excessive NO generation in the human body. Further, the scavenging activity may also help to arrest the chain of reactions initiated by excess generation of NO that are detrimental to human health. The flower extract showed a moderate nitric oxide-scavenging activity. The % inhibition was increased with increasing concentration of the extract. Curcumin, a natural antioxidant was used as a positive control for comparison (Sreejayan and Rao, 1996).

Searching plant sources may bring new natural products into cosmetic, pharmaceutical, and food production. From the results obtained in the present study, it is concluded that an alcoholic extract of *Ipomoea carnea* flower, which contains large amounts of phenolic compounds, exhibits free radical scavenging activities and high antioxidant activities. Further studies are warranted for the isolation and purification of antioxidant compounds, and *in vivo* studies are needed for understanding their mechanism of action of antioxidants.

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