

Antioxidant and Free Radical Scavenging Activities of *Ipomoea pes-caprae* (L.) R. Br. Extracts

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Available Online: 1st November, 14

ABSTRACT

Ipomoea pes-caprae (L.) R. Br. (family Convolvulaceae), commonly known as “Ivy leaf morning glory” has long been used as a traditional medicine. This study was intended to characterize the antioxidant properties and the total phenolic compounds (TPC) of four extracts of *I. pes-caprae* obtained by hexane, dichloromethane (DCM), ethyl acetate and methanol solvents respectively. Extraction with hexane (1.3 ± 0.03 gm) and DCM (0.48 ± 0.02 gm) showed the highest yield. The highest amount (20.02 mg/g dry wt. gallic acid equivalent) of TPC was observed for methanol extract. Methanol showed the highest antioxidant activity of 208.54 ± 4.14 mg/g dry wt. ascorbic acid equivalent, followed by ethyl acetate (176.43 ± 2.4 mg/g dry wt. ascorbic acid equivalent). The reducing power ability of *Ipomoea pes-caprae* extracts steadily increased with increasing concentration of all the samples. In DPPH (2, 2-diphenyl-1-picryl hydrazyl) assay, methanol extract exhibited the most effective scavenging ability (97.85%) among the four extracts. It was observed that methanol fraction has the strongest inhibition (90.31%) against hydroxyl radical at 1000 $\mu\text{g/mL}$ concentration. The maximum hydrogen peroxide radical scavenging activity (81.56%) was exhibited by methanol fraction. In the ABTS radical cation scavenging activity, the methanol extract showed highest activity (96.43%) the concentration of 1000 $\mu\text{g/ml}$. On the contrary, maximum hemolytic activity of 32 hemolytic units (HU)/mg was observed in case of ethyl acetate extract against chicken blood. Our findings identified the appropriate solvent for extracting phenolic compounds which might provide a rich and novel source of natural antioxidants.

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Keywords: Sand Dune; *Ipomoea pes-caprae*; antioxidant; Phenolic compounds; hemolytic activity

INTRODUCTION

Natural products, such as plants extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity [1]. According to the World Health Organization (WHO), more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. The use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases [2]. Due to the increase in adverse effects and microbial resistance to the chemically synthesized drugs, mankind turned to ethnopharmacognosy. They found literally thousands of phytochemicals as safe and broadly effective alternatives with less adverse effect. Many beneficial biological activities such as anticancer, antimicrobial, antioxidant, antidiarrheal, analgesic and wound healing activity have been reported from plant extracts.

Plant provides large amounts of antioxidants, mostly phenolic compounds such as proanthocyanidins, flavonoids and phenolic acids [3, 4]. Plant phenolic compounds are synthesized via the phenyl propanoid pathway and play a role in plant defence mechanisms against biotic and abiotic stresses [5]. These molecules are widely distributed in leaves, stems, seeds and fruits of many edible plants³. Medicinal herb is considered to be a chemical factory as it contains multitude herbs which are being used in the treatment of heart disease, skin disease, high blood pressure, pain, asthma and other problems [6].

Phenols (and other antioxidant compounds) are very significant for human health. They are widely present in fruits, vegetables, and beverages (coffee, tea, fruit juices, wines) [7, 8, 9, 10, 11, 12]. In plants, phenolic acids occur mainly in bound forms, as components of complex structures such as lignins and hydrolysable tannins or derivatives of sugars and organic acids [13,14]. There is increasing evidence that consumption of a variety of phenolic compounds present in natural foods may lower the risk of serious health disorders because of the protective effect of these powerful antioxidant molecules [15,16. Besides, when added to foods, antioxidants minimize rancidity, retard the formation of toxic oxidation products, maintain nutritional quality, and increase shelf life of food products [17].

Oxidative stress refers to an imbalance between the production of free radicals and the antioxidant defense system. Reactive oxygen species (ROS) are various forms of activated oxygen which causes oxidative damage. Mechanisms responsible for the ROS-mediated

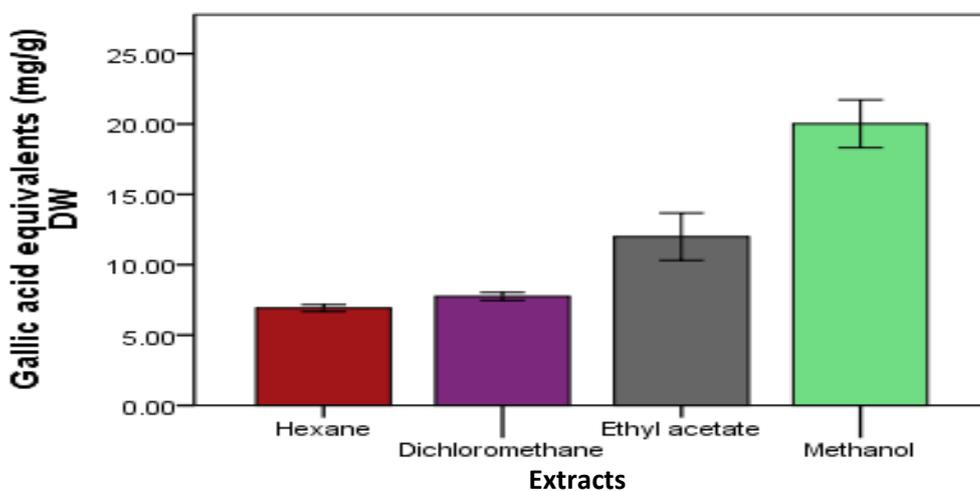


Fig.1.Total phenolic content of total extract from *Ipomoea pes-caprae*. The data are the mean \pm SD of 3 replicates

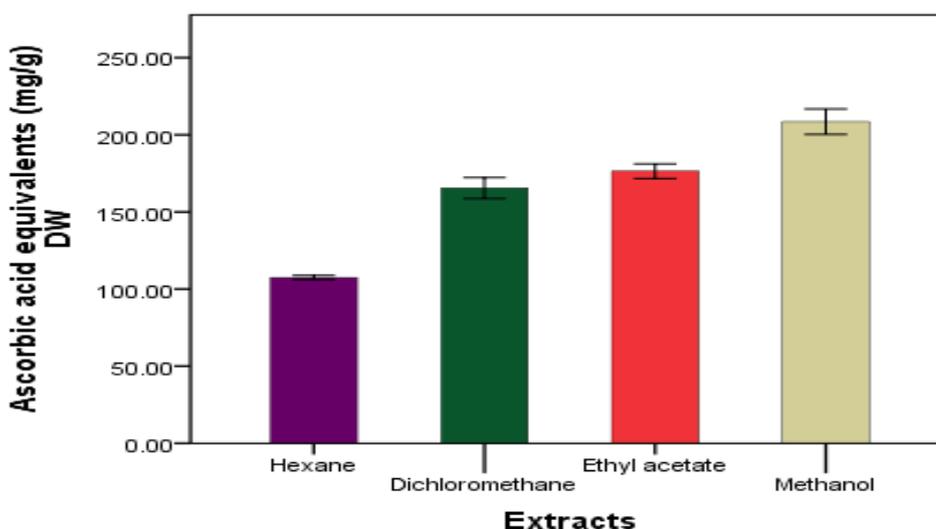


Fig.2. Total antioxidant capacity of total extract from *ipomoea pes-caprae*. The data are the mean \pm SD of 3 replicates

injuries mainly include lipid peroxidation, oxidative DNA damage and protein oxidation [18, 19]. Antioxidants are compounds that detoxify ROS and prevent their damage through multi-mechanisms. Synthetic antioxidants have been in use as food additives for a long time, but reports on their involvement in chronic diseases have restricted their use in foods. Therefore, international attention has been focused on natural antioxidants mainly from plant sources [20, 21].

The oceans are a cornucopia of still unstudied organisms. Marine organisms are a rich source of structurally novel and biologically active metabolites. Metabolites produced by these organisms may be potential bioactive compounds of interest in the pharmaceutical industry.

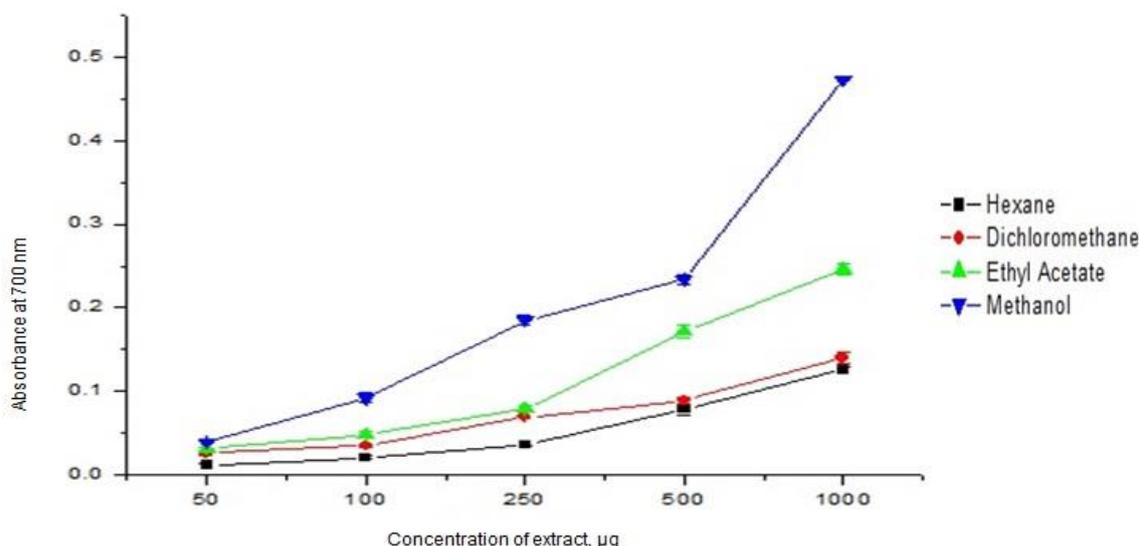


Fig. 3. Reducing power of total extract from *Ipomoea pes-caprae*. The data are the mean \pm SD of 3 replicates

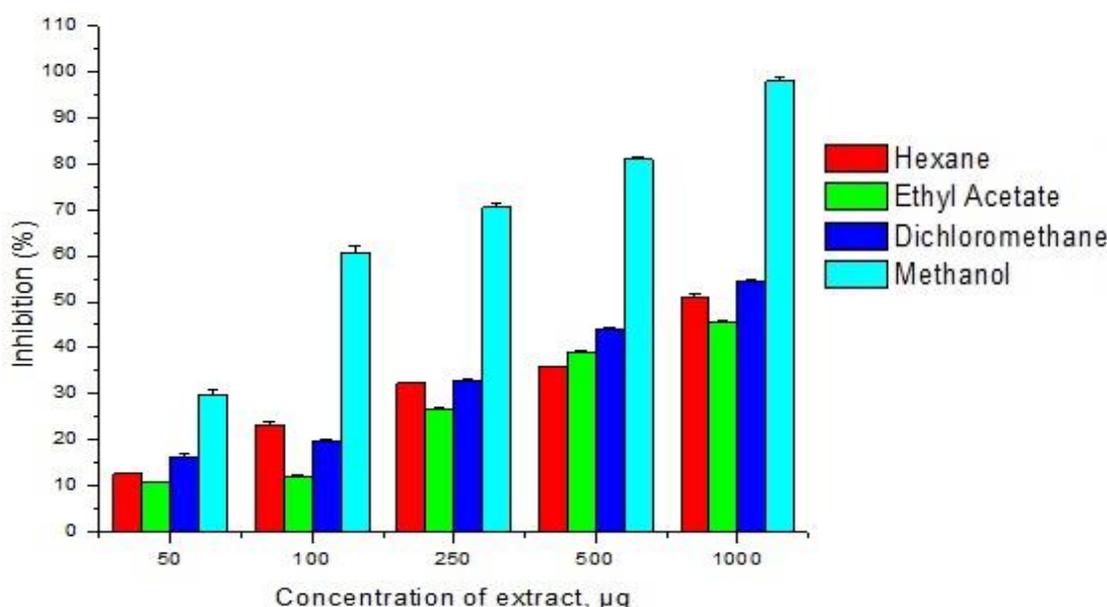


Fig.4. Scavenging effects of *Ipomoea pes-caprae* extract on DPPH radical. The data are the mean \pm SD of 3 replicates

A sand dune is a mound, hill or ridge of sand that lies behind the part of the beach affected by tides. Coastal Sand Dune (CSD) comprises a variety of flora and fauna, which play a vital role in provisioning ecological and economical services to the coastal communities [22]. The coastal communities closely associated with sand dune habitats are dependent on CSD vegetation for a variety of benefits: for food, fodder, health, manure and recreation. In fact, very few publications are available on the floral diversity of Indian sand dunes [23].

Ipomoea pes-caprae (L.) R. Br. to the family Convolvulaceae, commonly known as Ivy leaf morning glory, pharbits seeds and kaladana. It grows just above the high tide line along coastal beaches, forming large mats that assist in stabilizing sands [24]. Traditionally *I. pes-caprae* is used in different ways like; the juice from the succulent leaves has been used as a first aid to treat jellyfish stings. Leaves are used in rheumatism, and as stomachic and tonic. The extract of the leaves have the astringent, diuretic and laxative properties. It has biological activity like antispasmodic, anticancer, antinociceptive, antihistaminic, insulogenic and hypoglycemic. [25]. It is also used in inhibition of platelet aggregation, diarrhoea, vomiting, and piles [26]. Thus, the aim of the present work was to quantify phenolic compounds of *I. pes-caprae* and to evaluate its antioxidant capacity.

MATERIALS AND METHODS

Plant material and Collection

Fresh leaves of *Ipomoea pes-caprae* were collected from Vellar estuary. The collected specimens were identified based on the manual by Kathiresan [27]. Withered leaves of *Ipomoea pes-caprae* were rinsed under running tap water to eliminate dust. After that samples were washed several times with distilled water and air-dried at 25-30⁰C for about 3-5 days. The dried samples were ground to fine powder using mortar and pestle. The powder was passed through a sieve of 22 mm mesh size. The powder sample was kept in a clean, dried, air tight amber glass container to protect it from sunlight.

Moisture content determination

Fresh and dried weight of leaves is taken to calculate the moisture content of the samples.

$$\% \text{ Moisture content} = \frac{\text{Wet weight of leaves} - \text{Dried weight of leaves}}{\text{Wet weight of leaves}} \times 100$$

Dry matter determination

A published method [28] was adopted for dry matter determination. The sample was dried at 105 °C until the constant weight was obtained, cooled in a desiccator and weighed in an analytical scale. The dry matter was calculated by the following formula:

$$\text{Dry matter} = C - A/B - C$$

Where, A = The weight of empty weighing bottle, g.

B = The weight of weighing bottle with fresh sample, g.

C = The weight of weighing bottle with dried sample, g.

Solvent extraction

100 gram of ground *Ipomoea pes-caprae* was extracted using three fold volumes of solvents of different polarity in order of increasing hydrophilic property (i.e. hexane, dichloromethane (DCM), ethyl acetate and methanol respectively) for 48 h on an orbital shaker to make the extracts [29,30]. This procedure was repeated for two more times. Finally, the extracts were evaporated and concentrated. The resulting extracts were then dissolved in dimethylsulfoxide (DMSO) and kept at 4°C until further use.

Determination of Total phenolic content

Phenolic contents of all crude extracts in 10 mg/ml of DMSO were estimated by the method [31]. Briefly, 100 µl aliquot of sample was mixed with 2.0 ml of 2% Na₂CO₃ and allowed to stand for 2 min at room temperature. After incubation, 100 µl of 50% Folin-Ciocalteu's phenol reagent was added, and the reaction mixture was mixed thoroughly and allowed to stand for 30 min at room temperature in the dark. Absorbance of all the solutions was measured at 720 nm using LAMBDA 25 UV/Vis Spectrophotometers (PerkinElmer). Phenolic contents were expressed as gallic acid equivalent per gram (GE/g).

Antioxidant Assay

Determination of Total antioxidant activity

Total antioxidant activities of all crude extracts (10 mg/ml of DMSO) of *Ipomoea pes-caprae* were determined according to the method [32]. Briefly, 0.3 ml of sample was mixed with 3.0 ml reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Reaction mixture was incubated at 95°C for 90 min in water bath. Absorbance of all the sample mixtures was measured at 695 nm using LAMBDA 25 UV/Vis Spectrophotometers (PerkinElmer). Total antioxidant activity is expressed as the number of equivalents of ascorbic acid.

Reducing power ability

The reducing power of all crude extracts obtained *Ipomoea pes-caprae* was determined through the transformation of Fe³⁺ to Fe²⁺ inducing by the method prescribed [33]. Briefly, 1.0 ml of different solvent extracts containing different concentrations of sample (50, 100, 250, 500 and 1000µg) were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide (1%). Reaction mixture was incubated at 50°C for 20 min. After incubation, 2.5 ml of trichloroacetic acid (10%) was added and centrifuged at 650g for 10 min. From the upper layer, 2.5 ml solution was mixed with 2.5 ml distilled water and 0.5 ml FeCl₃ (0.1%). Absorbance of all the solutions was measured at 700 nm using LAMBDA 25 U/V is Spectrophotometers (PerkinElmer). Increased absorbance is indicated increased reducing power.

Scavenging effect on 2, 2-diphenyl-1-picryl hydrazyl radical (DPPH)

The radical scavenging ability was determined as described by Mensor *et al.* [34] with little modifications. Briefly, one ml from 0.3 mM methanol solution of DPPH was added to 2.5 ml from the samples with different concentrations of all *Ipomoea pes-caprae* extract. The samples were kept at room temperature in the dark and after 30 min the optic density was measured at 518 nm. The antiradical activity (AA) was determined by the following formula:

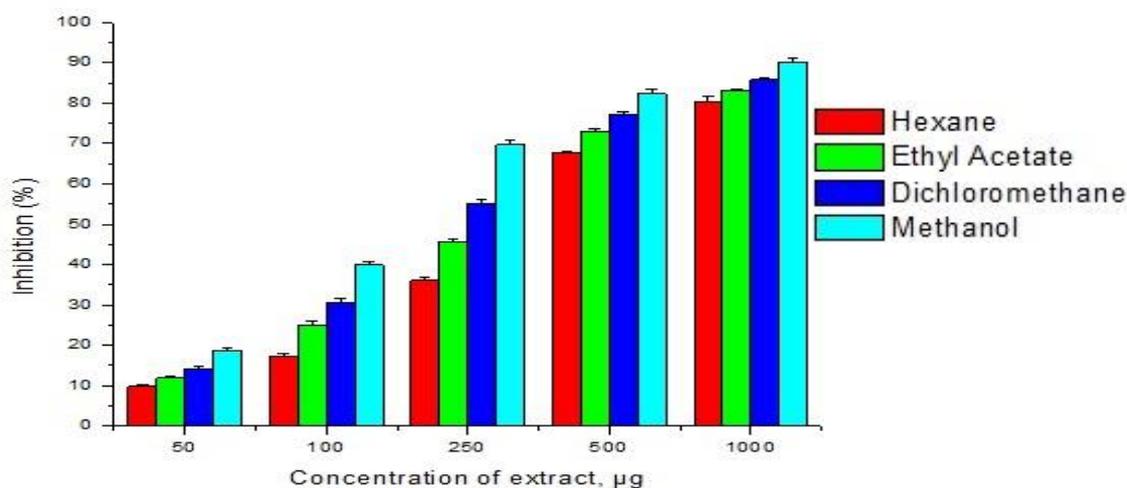


Fig.5. Inhibition of the radical degradation of 2-deoxy-D-ribose of *Ipomoea pes-caprae* extract. The data are the mean \pm SD of 3 replicates

$$AA\% = 100 - \left\{ \frac{[(Abs. Sample - Abs. Empty Sample) \times 100]}{Abs. control} \right\},$$

Where empty samples – 1 ml ethanol + 2.5 ml from various concentrations of all *I. pes-caprae* extract; control sample – 1 ml 0.3 mM DPPH + 2.5 ml methanol.

The optical density of the samples, the control and the empty samples were measured in comparison with methanol.

Detection of hydroxyl radicals by deoxyribose assay

The assay was performed as described by Halliwell, Gutteridge, and Aruoma [35] with minor changes. All solutions were freshly prepared. One ml of the reaction mixture contained 100 µl of 28 mM 2-deoxy-D-ribose (dissolved in KH_2PO_4 – K_2HPO_4 buffer, pH 7.4), 500 µl solution of various concentrations of the all *Ipomoea pes-caprae* extract, 200 µl of 200 µM $FeCl_3$ and 1.04 mM EDTA (1:1 v/v), 100 µl H_2O_2 (1.0 mM) and 100 µl ascorbic acid (1.0 mM). After an incubation period of 1 h at 37°C the extent of deoxyribose humiliation was measured by the TBA reaction. 1.0 ml of TBA (1% in 50 mM NaOH) and 1.0 ml of TCA were added to the reaction mixture and the tubes were heated at 100°C for 20 min. After cooling, the absorbance was read at 532 nm against a blank (containing only buffer and deoxyribose). All tests were performed three times. Ascorbic acid was used as a positive control. Percent inhibition in hydroxyl radical was calculated by the following expression:

$$I (\%) = [(A_0 - A_1) / A_0] \times 100,$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the sample. The data obtained at each point was the average of three measurements.

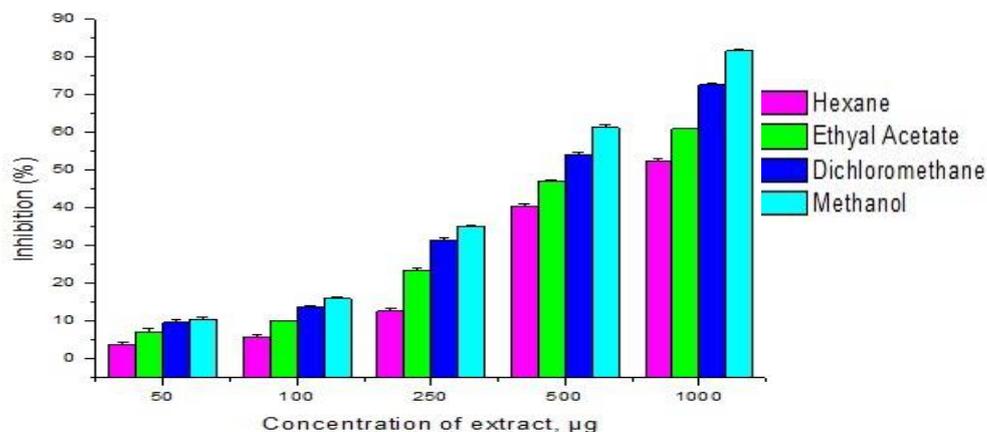


Fig.6. Hydrogen peroxide scavenging activity of *Ipomoea pes-caprae* extract. The data are the mean \pm SD of 3 replicates

Hydrogen peroxide radical scavenging assay

The ability of sand dune plant *Ipomoea pes-caprae* extract to scavenge hydrogen peroxide was determined [36]. Different concentrations of extracts were added to 2 ml solution of 10 mM hydrogen peroxide (H_2O_2) in 0.1 M phosphate buffer, pH 7.4. After 10 min, absorbance of H_2O_2 was recorded at 230 nm against blank solution without H_2O_2 . Percent inhibition in H_2O_2 was calculated using the following formula:

$$(\%) \text{ inhibition} = [(A_0 - A_1) / A_0] \times 100,$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

ABTS [2,2'- azinobis – (3-ethylbenzothiazoline-6-sulfoic acid)]

The ABTS radical anion scavenging assay was carried out by the method of Re *et.al.* [37] with some modifications. The ABTS reagent was prepared by mixing 7mM ABTS with 140Mm potassium persulphate. The mixture was kept in dark at room temperature for 16 h. The absorbance was adjusted with methanol to 0.700 ± 0.02 at 734 nm. To determine the scavenging activity, 5ml ABTS reagent was added to 1ml of different concentrations of plant samle (50, 100, 250, 500, 1000 $\mu\text{g/ml}$) and absorbance was measured at 734 nm at 7 min interval. Gallic acid was used as standard. Percentage inhibition of the sample was calculated as above.

Determination of Hemolytic activity

Preparation of normal saline (pH 7.2)

Normal saline was prepared by dissolving 0.9 gm of NaCl into 100 ml of distilled water.

Preparation of Erythrocyte suspension

Hemolytic activity of crude sample and fractions were tested on chicken blood. The blood was collected from the respective animals and by using 2.7% EDTA as anticoagulant at 5% of the volume of blood. The blood was centrifuged at 5000 rpm for 7 min with normal saline. The supernatant was discarded and the RBC pack was resuspended in normal saline. This process was repeated thrice and finally RBC concentrate was obtained. The final RBC pack was used to prepare 1% RBC suspension.

Hemolytic assay by micro titer plate

The assay was carried out according to Pani Prasad and Venkateshvaran [38] in ``U`` shaped Laxbro microtitre plates. The lyophilized plant extracts fractions were assayed. The concentration of the extracts was 10mg/ ml. One row of well was used for only one extract fraction. Initially 100 µl of normal saline was added to each well. Then 100 µl of the 1st extract fraction was added to the first well and was thoroughly mixed. From this 100 µl was transferred to the next well and this process was repeated up to the last well from which 100 µl of the

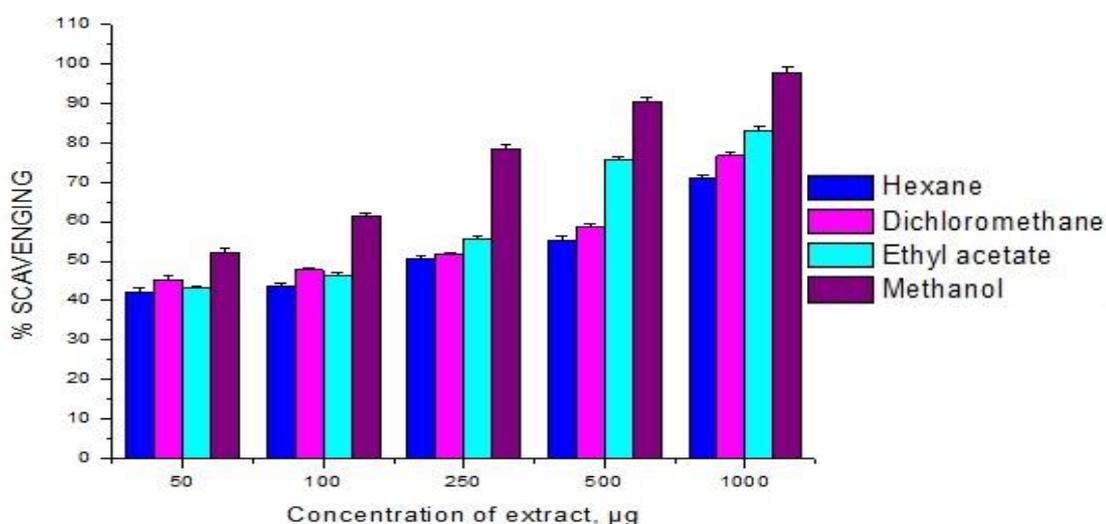


Fig. 7. Scavenging effects of *Ipomoea pes-caprae* extract on ABTS radical. The data are the mean \pm SD of 3 replicates

dilution was discarded. Then 100 µl of the prepared erythrocyte suspension was added to each well. A negative control was kept by mixing 100 µl of normal saline and 100 µl of 1% RBC suspension and positive control by mixing 100 µl of distilled water and 100 µl of 1% RBC suspension. Formation of a fine ``Button cell`` with regular margin indicates the negative reaction. A uniform red colored suspension of the lysed RBC indicates the positive result. The plates were incubated for 2 hours at room temperature and the results were read. Hemolytic activity was expressed as hemolytic Unit (HU), 1 HU being defined as the amount of protein

required to cause 50% hemolysis or the reciprocal of the highest dilution of the toxin in which a hemolytic pattern was obtained.

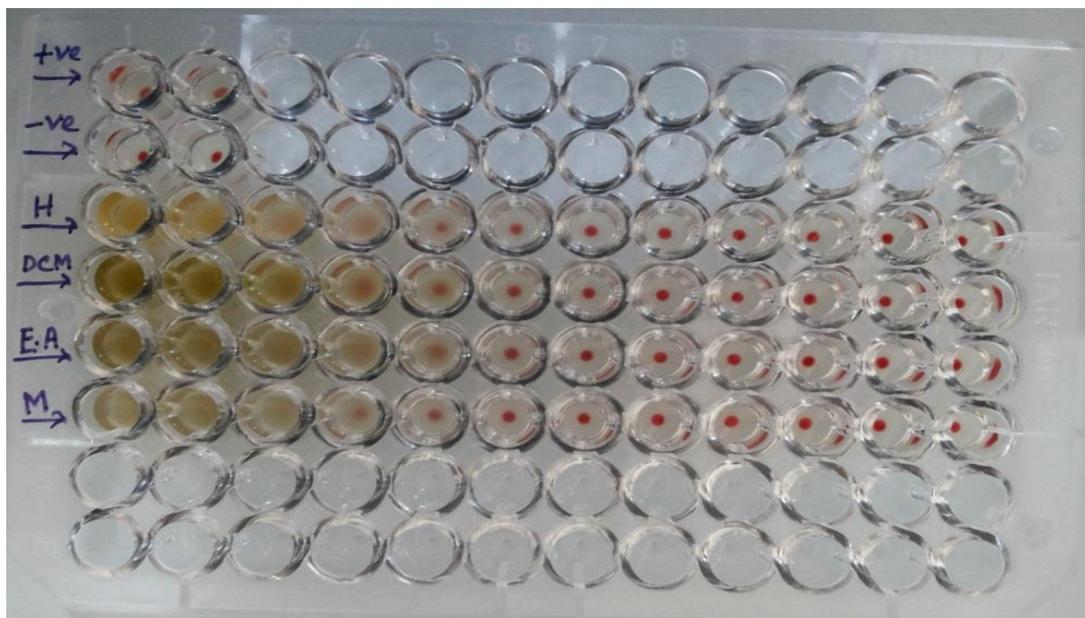


Fig.8. Micro titre plate showing Hemolytic activity of *Ipomoea pes-caprae*

RESULTS AND DISCUSSION

Moisture content determination

The Moisture content of *Ipomoea pes-caprae* was $77.95\% \pm 1.4$. It was slightly higher when compared to other sand dune plant like *Merremia umbellatan* whose moisture content was 72.6%. This could be due to the different texture of leaves as the leaves of *Ipomoea pes-caprae* are more fleshy and succulent as compared to the leaves of *Merremia umbellate* [39].

Dry matter determination

The dry matter of *Ipomoea pes-caprae* was observed to be 0.28 ± 0.02 according to the formula given by Chodak and Tarko [28]. No other information is available regarding the dry matter determination on sand dune plants.

Extraction and yield of extract

As a wide range of extract holds a better chance for the extraction and isolation of biologically active molecules for general screening of bioactivity [40], four different solvents (with different polarity) were used in the present study. The average percentage yield of each solvent was based on triplicate analysis of samples. The highest yield was found with hexane (1.3 ± 0.03 gm) and DCM (0.48 ± 0.02 gm) whereas the minimum yield was with ethyl acetate (0.27 ± 0.005 gm). The hexane solvent yielded the highest amount of extract indicating that the leaves of

Ipomoea pes-caprae contain mostly of lipophilic compounds such as waxes, chlorophyll, and fatty acids [41].

Total polyphenol content (TPC)

There are number of recent studies showing that phenolic compounds are commonly found in plants and have been reported to possess several biological activities including powerful antioxidant compounds [42, 43]. For that, total polyphenolic contents of all the four extracts of *Ipomoea pes-caprae* were assessed. The result showed significant difference in TPC among the different solvents (Figure 1.). The TPC in hexane, dichlormethane, ethyl acetate and methanol were found as 6.91, 7.75, 12 & 20.02 mg GAE/g dry weight (DW) respectively.

The highest amount of TPC was observed in methanol extract. Moreover, total phenolic content was significantly higher in *Ipomoea pes-caprae* leaf as compared to other medicinal marine plant like *Salsola kali* which exhibited 17.23 mg GAE/g DW [44] and in glycophytic species *Nigella sativa* L. which showed 10.04 mg GAE/g DW [45].

These observations indicate that the crude extract of *Ipomoea pes-caprae* possess prominent amount of phenolic content, which contributes to its biological activity. Phenolic compounds are generally more soluble in polar organic solvents [46].

Antioxidant Assay

Total antioxidant activity

The total antioxidant activity of leaves extracts using four different solvents (hexane, dichlormethane, ethyl acetate and methanol) was assessed. In the case of phosphomolybdenum method, molybdenum VI (Mo^{6+}) is reduced to form a green phosphate/ Mo^{5+} complex. Figure 2 indicates that methanol extract shows the highest antioxidant activity of 208.54 ± 4.14 mg ascorbic acid/g, followed by ethyl acetate (176.43 ± 2.4 mg ascorbic acid/g DW).

In contrast the extracts of dichloromethane and hexane exhibit less activity. Earlier, demonstrated [47] that the total antioxidant activity of higher plants extracts was ranging between 245-376 mg ascorbic acid/g. Higher activity in fractions may be due to the interferences of other compounds present in crude (methanol) extract; and, it has also been reported that solvents used for extraction have dramatic effect on the chemical species [48].

Reducing power ability

The antioxidant potential can be studied by analyzing the reducing power of the sample by reduction of ferric to ferrous in a redox-linked colourimetric reaction [49]. The reducing power indicates that the antioxidant compounds are electron donors and reduce the oxidized intermediate of the lipid peroxidation process, so that they can act as primary and secondary

antioxidants [50]. In this assay, reducing power ability of *Ipomoea pes-caprae* extracts (all the four solvents) steadily increased with increasing concentration of all the samples (Figure 3). Same trend has also been reported [47] in methanol extracts of higher plants. The maximum (0.473 ± 0.005) reducing power value was observed in methanol extracts and minimum (0.13 ± 0.004) was obtained from hexane extracts. This property is associated with the single electron transfer that is reported to be terminators of free radical chain reaction [51]. Same kinds of observations were found in other coastal medicinal plant like *Cakile maritima*, *Limoniastrum monopetalum*, *Mesembryanthemum crystallinum*, *M. edule* [42].

Scavenging effect on 2, 2-diphenyl-1-picryl hydrazyl radical (DPPH)

The antioxidant activity of plant extracts having polyphenol components is due to their ability to donate hydrogen atoms or electrons and to capture the free radicals. DPPH has been used widely as a free radical to estimate scavenging property of any antioxidant substances [52, 53]. The DPPH radical scavenging activity of *Ipomoea pes-caprae* extracts increase with increasing concentration. DPPH radical scavenging activity of all the four solvent extracts obtained results are shown in Figure 4. Among all extracts, maximum effective scavenging ability was exhibited by methanol (97.85%), followed by dichloromethane (54.47%). The other extracts showed relatively weak scavenging potentials.

It was observed that the extracts containing high level of TPC were also potent DPPH radical scavenger, suggesting that polyphenols may be the principle constituents responsible for the antiradical properties of the extracts. The similar results were obtained by some researchers that change in solvent's polarity alters its efficacy to extract a specific group of antioxidant compounds and influences the antioxidant properties of the extracts [54].

These findings may be related to the higher polyphenol contents of *Ipomoea pes-caprae*. Many other authors have reported a positive and significant relationship between the antioxidant components including phenols and polyphenols with the DPPH radical scavenging activity [55, 56].

Detection of hydroxyl radicals by Deoxyribose assay

Hydroxyl radical is an extremely responsive free radical formed in biological systems [57]. This radical has a capability to join nucleotides in DNA and cause component breakage that contributes to carcinogenesis, mutagenesis and cytotoxicity [58, 59, 60]. When Ferric-EDTA is incubated with H_2O_2 (Fenton reaction) and ascorbic acid at pH 7.4, the hydroxyl radicals formed in free solution have the ability to degrade 2-deoxy-D-ribose into fragments that on heating with TBA at low pH form a pink chromogen [61,62]. Using the principle, the hydroxyl radical scavenging activity of different fractions of *Ipomoea pes-caprae* extracts were found to

remove the hydroxyl radicals from the sugar and prevented it from degradation (Figure 5). It was observed that methanol fraction has the strongest percentage inhibition (90.31%) against hydroxyl radical at 1000 µg/mL concentration in comparison to other extracts. Hydroxyl radical scavenging activity decreases with different extracts in this following order: methanol > DCM > ethyl acetate > hexane.

Hydrogen peroxide radical scavenging assay

Hydrogen peroxide is a weak oxidizing agent and can deactivate a few enzymes directly, usually by oxidation of necessary thiol (-SH) groups. It can cross cell membranes rapidly and enter inside the cell. H₂O₂ probably reacts with Fe²⁺ and possibly Cu²⁺ ions to form hydroxyl radical which may be the origin of many of its toxic effects [63]. It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is getting accumulated inside the cells. The scavenging activity of the different fractions of *Ipomoea pes-caprae* is shown in Figure 6. In this study, all fractions exhibited appreciable scavenging activity. The maximum scavenging activity was exhibited by methanol fraction (81.56%).

ABTS [2,2'- azinobis – (3-ethylbenzothiazoline-6-sulfoic acid)]

The ABTS radical reactions involve electron transfer and the process take place in faster rate when compared to DPPH radicals. Re *et al.* [64] reported that the decolorization of the ABTS⁺ radical also reflects the capacity of an antioxidant species to donate electrons or hydrogen atoms to inactivate this radical species. In the ABTS radical cation scavenging activity, the methanol extract showed concentration dependent scavenging activity (Figure 7.). Highest activity of 96.43% was observed at the concentration of 1000 µg/ml.

Hemolytic activity

Hemolytic assay was done for all the four crude extracts of *Ipomoea pes-caprae* against chicken blood. The hemolytic factors were present in the all the four extracts. The ethyl acetate extract showed maximum of 32 hemolytic units (HU)/mg with chicken erythrocytes (Figure. 8).

It is noteworthy to mention that most of the earlier reports claim that *in vitro* hemolysis can serve as a sensitive test to express the degree of cytotoxicity. All the previous hemolytic studies on *Cyanea capillata* and *Ctanea lamarckii* [65], *Rhopilema esculentum* [66] indicated a positive result on hemolysis test. This study also showed same kind of trend. But however, the higher concentration only has the hemolytic activity. But in lower concentration, there no such hemolytic activity has found. So, *Ipomoea pes- csprae* can be a source for biologically active molecules which could evolve as a drug in near future.

CONCLUSION

As a whole, the results have demonstrated that *Ipomoea pes-caprae* exhibited differential phenolic content and is a remarkable antioxidant source, which are known to demonstrate a wide range of biological and pharmacological. The antioxidative potential of this species make it a novel natural source of numerous health benefits that could be considered in the fields of medicinal or nutraceuticals. Taken together; these information may confirm the interesting potential of sand dune plant as a valuable source for natural antioxidant molecules. The data obtained, suggests the strong potential of this plant as a source of phenolic compounds with beneficial properties, and a promising source of health products, phytochemicals for medicinal exploration and pharmaceutical industry.

ACKNOWLEDGEMENT

This work was supported by grants received under Centre of Excellence and programme support in areas of Biotechnology, Department of Biotechnology, Government of India, New Delhi.

Conflict of interest None.

Compliance with Ethics Requirements This article does not contain any studies with human or animal subjects.

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