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Pharmacological potential of *Ipomea pes-caprae* (L.) R. Br. whole plant extracts

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ABSTRACT

The present study was conducted to investigate phytochemical screening, antibacterial activity, MIC and MBC values and antioxidant activities of different solvent extracts viz., Hexane, Benzene, Chloroform, Ethyl acetate, Acetone and Methanol extracts of Ipomoea pes-caprae whole plant. Phytochemical screening was carried by using standard protocols, antibacterial activity was carried out by using Agar well diffusion method, MIC and MBC by broth dilution method and antioxidant activity was determined by DPPH free radical scavenging assay. The results exhibited the occurence of diffrent phytochemicals, showed significant antibacterial zone of inhibitions. MIC values ranged from 12.5 mg/ml to 75mg/ml and MBC values from 25 mg/ml to 100 mg/ml. The plant showed good antioxidant activity with Acetone extract (90.89%) and Ethyl acetate extract (77.73%). On the whole, this plant was found to be very important as a source for bioactive principle and natural antioxidants.

Key words: *Ipomoea pes-caprae*, phytochemical screening, antibacterial activity, MIC and MBC, antioxidant assay

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INTRODUCTION

Mangrove forests have played an important role in the Socio-economic life of the people. The mangrove forests have several valuable medicinal plants that are used in medicinal fields [1]. Mangroves have been a source of interest for their novel natural products as they contain biologically active antiviral, antibacterial and antifungal compounds. They provide a rich source of phytochemicals like steroids, saponins, tannins, alkaloids and triterpenes [2]. Now-a-days, the indiscriminate use of commercial antimicrobial drugs has caused multiple drug resistance in human pathogenic microorganisms [3]. In addition to this problem, hypersensitivity, immune-suppression and allergic reactions may also occur sometimes due to the adverse effects of antibiotics on the host [4]. This situation forced the scientists to search for new and effective antimicrobial agents to replace the current regimens. *Ipomoea pes-caprae* (L.) R.Br. also known as Beach Morning Glory or Goat's Foot, is a common pan tropical creeping vine and belongs to the family Convolvulaceae. The extract of the leaves has the astringent, diuretic and laxative properties and also used to cure rheumatism. It has biological activity like antioxidant, analgesic and anti-inflammatory, antispasmodic, anticancer, antinociceptive, antihistaminic, insulogenic and hypoglycemic [5]. *I. pes-caprae* has the potential in scavenging free radicals and can be a vital source of antioxidant phytochemicals. The present investigation deals with the screening of preliminary phytochemicals present in the plant extracts, antibacterial activity, MIC and MBC values and antioxidant activity of different solvent extracts of *Ipomoea pes-caprae* plant.

MATERIALS AND METHODS

Preparation of the plant extracts

Ipomoea pes-caprae (L.) R.Br. plant was collected from Nizampatnam Mangrove forest, Andhra Pradesh, India. The whole plant was thoroughly washed and dried in shade. The dried plant material was made into a coarse powder by means of electrical grinder. The dried, powdered plant material was extracted in different solvents viz., Hexane, Benzene, Chloroform, Ethyl acetate, Acetone and Methanol. The resulted extracts were filtered and then concentrated on a roto evaporator and the resultant crude extracts were preserved in sterile, air tight containers for further analysis.

Phytochemical screening

Reagents used for the different phytochemical tests:

The following reagents were prepared and tests were carried out according to standard protocols.

Mayer's reagent:

Mercuric iodide of 1.36 gm was dissolved in 60 ml of water and mixed with a solution containing 5 gm of Potassium iodide in 20 ml of water.

Dragendroff's Reagent:

Basic bismuth nitrate (1.7 gm) and tartaric acid (20 gm) were dissolved in 80 ml of water. This solution was mixed with a solution containing 16 gm potassium iodide and 40 ml water.

Fehling's solution- A:

Copper sulphate of 34.64 gm was dissolved in a mixture of 0.5 ml of sulfuric acid and sufficient water and made up to 500 ml.

Fehling's solution- B:

Sodium potassium tartarate of 176 gm and sodium hydroxide of 77 gm were dissolved in sufficient water and made up to 500 ml.

Benedicts Reagent:

Cupric sulphate (1.73 gm), sodium citrate (1.73 gm) and anhydrous sodium carbonate (10 gm) were dissolved in water and the volume was made up to 100 ml.

Molisch Reagent:

Pure α -naphthol of 2.5 gm was dissolved in 25 ml of ethanol.

Liebermann- Burchard Reagent:

Acetic acid (5 ml) was carefully mixed under cooling with 5ml concentrated sulfuric acid. This mixture was added cautiously to 50 ml absolute ethanol with cooling.

The following qualitative tests were done to find out the presence or absence of phytochemical constituents like Carbohydrates, Tannins, Steroids, Saponins, Terpenoids, Soluble starch, Flavonoids and Alkaloids.

Test for flavonoids

a) Ferric chloride test

Two ml of the test solution was boiled with distilled water and filtered. Then, few drops of 10% ferric chloride solution were added to the 2 ml of filtrate. A greenish-blue or violet coloration indicates the presence of a phenolic hydroxyl group.

b) Shinoda's test

Five grams of each extract was dissolved in ethanol individually, warmed and then filtered. Small pieces of magnesium chips were then added to the filtrate followed by few drops of conc. HCl. The pink, orange, or red to purple coloration indicates the presence of flavonoids.

c) Sodium hydroxide test
Extract of 0.2 gm was dissolved in water and filtered. To this, 2 ml of the 10% aqueous sodium hydroxide was added to produce yellow coloration. A change in color from yellow to colorless on addition of dilute hydrochloric acid was the indication for the presence of flavonoids.

d) Leadacetate test
Extract o f 0.5 gm was dissolved in water and filtered. To the 5 ml of each filtrate, 3 ml of lead acetate solution was added. Appearance of a buff-colored precipitate indicates the presence of flavonoids.

Test for alkaloids
Five grams of crude powder was stirred with 1% aqueous HCl on water bath and then filtered. To the 1 ml filtrate, few drops of Dragendroff's reagent was added. Orange- Red precipitate indicates the positive result. To another 1 ml filtrate, few drops of Mayer's reagent was added and appearance of buff- colored precipitate reveals the presence of alkaloids.

Test for soluble starch
Crude extract of 0.2 gm was boiled in 1 ml of 5% KOH, cooled and acidified with H_2SO_4. Yellow coloration indicates the presence of soluble starch.

Test for Saponins
Crude powder of 0.5 gm was shaken with water in a test tube and it warmed in a water bath. The persistent froth indicates the presence of saponins.

Test for terpenoids
Five grams of crude extract was dissolved in ethanol. To this, 1 ml of acetic acid was added followed by conc. H_2SO_4. A change in color from pink to violet confirms the presence of terpenoids.

Test for steroids
a) Salkowskii test
In 2 ml of chloroform, 0.2 gm of extract was dissolved and added the conc. H_2SO_4. The development of reddish brown color at inter phase indicates the presence of steroids.

b) Keller-Killiani test
To 0.5 ml of test solution, 2 ml of 3.5% $FeCl_3$, small amount of glacial acetic acid and 2 ml of conc. H_2SO_4 were added carefully. Appearance of reddish brown ring at inter phase is a positive indication for the presence of steroids.

c) Liebermann-Burchard test
To 0.2 gm of each extract, 2 ml of acetic acid was added and the solution was cooled well in ice followed by the addition of conc. H_2SO_4 carefully. Color development from violet to blue or bluish-green indicates the presence of a steroidal ring (i.e. aglycone portion of cardiac glycoside).

Test for carbohydrates
a) Molisch's test
Two ml of *Molisch*'s reagent was added to the extract dissolved in distilled water and 1 ml of conc. H_2SO_4 was dispensed along the walls of the test tube. The mixture was allowed to stand for two minutes and then diluted with 5 ml of distilled water. Formation of a dull violet color at the inter phase of the two layers indicates the positive test for carbohydrates.

b) Fehling's test (for free reducing sugars)
The crude extracts were treated with 5.0 ml of Fehling's solution (A & B) and kept in boiling water bath. The formation of yellow or red color precipitate indicates the presence of free reducing sugars.

c) Fehling's test (for Combined Reducing Sugars)
Extract of 0.5 gm was hydrolyzed by boiling with 5 ml of dilute hydrochloric acid and the resulting solution neutralized with sodium hydroxide solution. To this, few drops of Fehling's solution were added and then heated on

a water bath for 2 minutes. Appearance of a reddish-brown precipitate of cuprous oxide indicates the presence of combined reducing sugars.

d) Barfoed's test (for monosaccharide)
In distilled water, 0.5 gm of the extract was dissolved and filtered. To 1 ml of the filtrate, 1 ml of Barfoed's reagent was added and then heated on a water bath for 2 minutes. Reddish precipitate of cuprous oxide formation is the positive test for the presence of monosaccharide.

Test for tannins:
Crude extract of 0.5 gm was stirred with 10 ml of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2 ml of the filtrate. Occurrence of a blue-black, green or blue-green precipitate indicates the presence of tannins.

a) Borntrager's Test:
Extract of 0.2 gm was shaken with 10 ml of benzene and then filtered. To the filtrate, 5 ml of 10% ammonia solution was added and then shaken the tube well. Appearance of pink, red or violet color in the ammonical (lower) phase indicates the presence of free anthraquinones.

b) Phlonatanins test:
To 0.2gm of extract, 1% HCl solution was added. Formation of red precipitate indicates the presence of tannins.

Antibacterial activity of the plant extracts
Microorganisms used
The antibacterial activity of the crude extracts was determined by using both Gram positive and Gram negative bacteria. Nine Gram positive bacteria namely *Micrococcus luteus* MTCC 106, *Arthrobacter protophormiae* MTCC 2682, *Rhodococcus rhodochrous* MTCC 265, *Bacillus subtilis* MTCC 441, *Staphylococcus aureus* MTCC 737, *Bacillus megaterium* MTCC 428, *Enterococcus faecalis* MTCC 439, *Streptococcus mutans* MTCC 497 and *Lactobacillus acidophilus* MTCC 10307 and six Gram negative bacteria viz., *Alcaligens faecalis* MTCC 126,

Salmonella enterica MTCC 3858, *Proteus vulgaris* MTCC 426, *Proteus mirabilis* MTCC 425, *Pseudomonas aeruginosa* MTCC 1688 and *Enterobacter aerogenes* MTCC 10208, were used in this study.

Antibacterial screening by agar well diffusion method
Antibacterial screening was determined by agar well diffusion method [6]. Suspensions of different bacteria were prepared by using 24 hours old bacterial cultures. Using these bacterial suspensions, agar plates were prepared individually by following pour plate method. After solidification, 6mm diameter wells were punched in agar plate with a sterile cork borer. Streptomycin standard antibiotic was used as positive control in the concentration of 10 µg/ml DMSO. A minute quantity of sterile agar suspension was added to the well and 100 µl of the sample, which was prepared by dissolving 100 mg of sample in 1 ml of DMSO, was added to each well. In a separate well, DMSO was also dispensed to maintain the control. The plates were incubated at 37^{o} C for 24 hrs. After incubation, diameter of the zone of inhibition was measured. For each sample and bacterial species, triplicates were maintained.

Determination of MIC and MBC
Minimum inhibitory concentration (MIC) was determined by using broth dilution method [6]. Minimum Inhibitory Concentration and Minimal Bactericidal Concentration (MBC) were determined at different concentrations viz., 12.5 mg/ml, 25 mg/ml, 50 mg/ml, 75 mg/ml and 100 mg/ml on those bacterial strains which showed zones of inhibition against the plant crude extracts. Control tube was maintained for each test concentration. The lowest concentration (highest dilution) of the extract that produced no visible bacterial growth (no turbidity) in comparison with the control tubes was regarded as MIC. However, the MBC was determined by sub culturing the test dilution without any turbidity on to a fresh drug-free solid medium and incubated further for 18–24 hours. The highest dilution that yielded no single bacterial colony on a solid medium was taken as MBC.

In vitro antioxidant assay
2, 2-diphenyl-1-picryl hydrazyl (DPPH) Free radical scavenging activity
The DPPH free radical scavenging activity of the different extracts was measured following the standard method [7]. The crude extracts in different concentrations viz., 100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml and 500 µg/ml were

prepared in DMSO. One ml of each concentration was mixed with 4 ml of the 0.004% (w/v) solution of DPPH prepared in methanol. The reaction mixture was kept for incubation in dark for 30 minutes. Methanol was used as control and Ascorbic acid was used as positive control. The absorbance was measured at 517 nm. The DPPH scavenging activity (%) was calculated by using the following formula

DPPH scavenging activity (%) = $[(A_0 – As) / A_0] \times 100$,

Where, A_0 -- absorbance of the control, As -- absorbance of the plant sample

RESULTS AND DISCUSSION

Phytochemical analysis

The result of the preliminary phytochemical screening of different solvent extracts of *Ipomoea pes-caprae* whole plant revealed the presence of a wide range of phytochemical constituents including alkaloids, glycosides, saponins, flavonoids, tannins, steroids, terpenoids, carbohydrates and soluble starch (Table-1). Steroids including the cardiac glycosides and terpenoids were present in Benzene, Chloroform, Ethyl acetate and Acetone extracts. Free anthraquinones and tannins were found in Benzene, Ethyl acetate and Acetone extracts. Saponins, flavonoids, soluble starch and alkaloids were observed in Ethyl acetate, Acetone and Methanol extracts, except saponins in Methanol extract. Carbohydrates including monosaccharides were present in Ethyl acetate, Acetone and Methanol extracts. Free reducing sugars were observed in Hexane and Methanol extracts. Tannins were found in Benzene, Ethyl acetate and Acetone extracts. The reason for the biological activity of this plant may be due to the presence of various phytochemical constituents. Tannins have been reported for their wound healing, anti-inflammatory and analgesic properties [8]. Terpenoids are reported to have anti-inflammatory, anti-viral, anti-malarial, inhibition of cholesterol synthesis and anti-bacterial activity [9]. Epidemiologic studies recommend that coronary heart disease is opposed by dietary flavonoids. Plants having alkaloids are used in medicines for reducing headache and fever [10]. Saponin acts as immune modulator by inducing production of interleukins and interferons in human body [11].

Table-1. Phytochemical analysis of *Ipomoea pes-caprae* whole plant extracts in different solvents

S.No.	Phytochemicals	H	B	C	E	A	M
1.	Carbohydrates	+	--	--	+	+	+
2.	Monosaccharides	--	--	--	++	+	+
3.	Free reducing sugars	++	--	--	--	--	++
4.	Combined reducing sugars	--	--	--	--	--	+
5.	Tannins	--	+	--	+	+	--
6.	Free anthraquinones	--	+	--	+	+	--
7.	Steroids	--	+	+	+	+	+
8.	Cardiac glycosides	--	+	+	+	+	--
9.	Terpenoids	--	++	++	+	+	+
10.	Saponins	--	--	+	++	++	--
11.	Flavonoids	--	--	--	++	++	+
12.	Soluble starch	--	--	--	+	+	+
13.	Alkaloids	--	+	--	+	+	+

H - Hexane; B - Benzene; C - Chloroform; E - Ethyl Acetate; A - Acetone; M - Methanol

Antibacterial activity

Antibacterial activity of *Ipomoea pes-caprae* whole plant extracts was assessed in terms of zone of inhibition of bacterial growth and the results of antibacterial activity against test organisms including both Gram positive and Gram negative bacteria are shown in Figures 1 and 2. Ethyl acetate and Acetone extracts of *Ipomoea pes-caprae* plant have shown larger zone of inhibition against *Arthrobacter protophormiae*, *Rhodococcus rhodochrous*, *Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Proteus mirabilis*, *Proteus vulgaris*, *Enterobacter aerogenes* than the standard antibiotic. Ethyl acetate extract against *Bacillus megaterium* and Acetone extract against *Streptococcus mutans* showed greater zone of inhibition than the standard antibiotic. Both *Rhodococcus rhodochrous* and *Enterobacter aerogenes* were found resistant to standard antibiotic but, Ethyl acetate and Acetone extracts displayed good zone of inhibition on these 2 bacteria. Hexane, Benzene, Chloroform and Methanol extracts have shown relatively lesser zone of inhibition and the zones of inhibition photos are given in Plate-1. Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the in vitro antibacterial activity assay [12, 13]. The antimicrobial property of the plant products is due to the presence of specific active chemical components. Such specific antimicrobial chemicals are acquired by

plants as a self defense mechanism to combat microbial attacks and infections in general. These same plant chemicals could posses antimicrobial activities against human pathogens [14].

Table-2. MIC and MBC (mg/ml) values of *Ipomeapes-caprae* whole plant extracts

Test organisms	Ethyl acetate		Acetone	
	MIC	MBC	MIC	MBC
Micrococcus luteus MTCC 106	50	75	50	75
Arthrobacterprotophormiae MTCC 2682	25	50	25	50
Rhodococcusrhodochrous MTCC 265	25	50	25	50
Bacillus megaterium MTCC 428	25	50	50	75
Bacillus subtilis MTCC 441	25	50	25	50
Enterococcus faecalis MTCC 439	25	50	25	50
Streptococcus mutans MTCC 497	50	75	25	50
Staphylococcus aureus MTCC 737	12.5	25	25	50
Lactobacillus acidophilus MTCC 10307	50	75	50	75
Alcaligensfaecalis MTCC 126	12.5	25	50	75
Proteus mirabilis MTCC 425	25	50	25	50
Proteus vulgaris MTCC 426	12.5	25	12.5	25
Enterobacteraerogenes MTCC 10208	50	75	12.5	25
Pseudomonas aeruginosa MTCC 1688	50	75	75	100

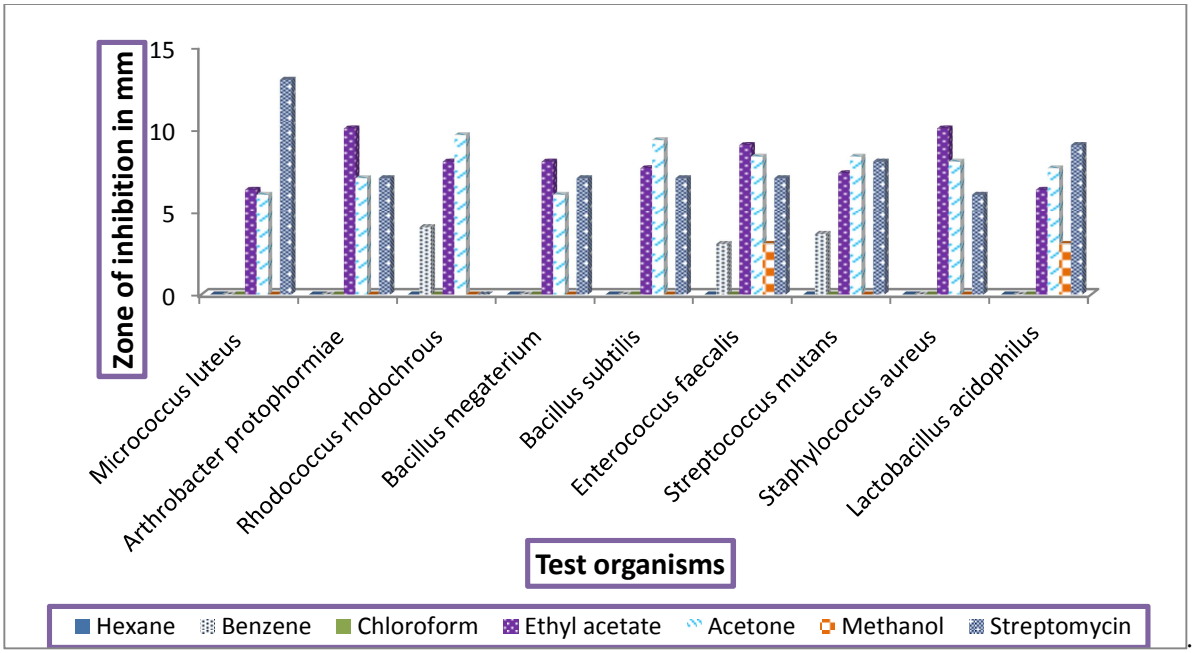


Figure-1. Antibacterial activity of different solvent extracts *Ipomoea pes-caprae* whole plant

MIC and MBC

The two most potent extracts showing antibacterial activity were selected to determine MIC and MBC and the results are presented in Table-2. The lowest MIC value (12.5 mg/ml) was observed against *Staphylococcus aureus*, *Alcaligens faecalis*, *Proteus vulgaris* by Ethyl acetate extract and MBC value was 25mg/ml. Acetone extract showed 12.5 mg/ml MIC value and 25 mg/ml MBC value against *Proteus vulgaris* and *Enterobacter aerogenes*. Ethyl acetate and Acetone extracts exhibited 25 mg/ml MIC value and 50 mg/ml MBC value against *Arthrobacter protophormiae*, *Rhodococcus rhodochrous*, *Bacillus subtilis*, *Enterococcus faecalis* and *Proteus mirabilis*. Ethyl acetate extract against *Bacillus megaterium* and Acetone extract against *Streptococcus mutans* displayed 25 mg/ml and 50 mg/ml MIC, MBC values, respectively. Ethyl acetate and Acetone extracts against *Micrococcus luteus* and *Lactobacillus acidophilus* showed MIC value of 50 mg/ml and MBC value of 75 mg/ml. MIC value of 75 mg/ml and MBC value 100 mg/ml were exhibited by Acetone extract against *Pseudomonas aeruginosa*. The variations in

the result may be due to the solvents used. Similar results have also been reported in earlier studies, with some minor differences [15, 16].

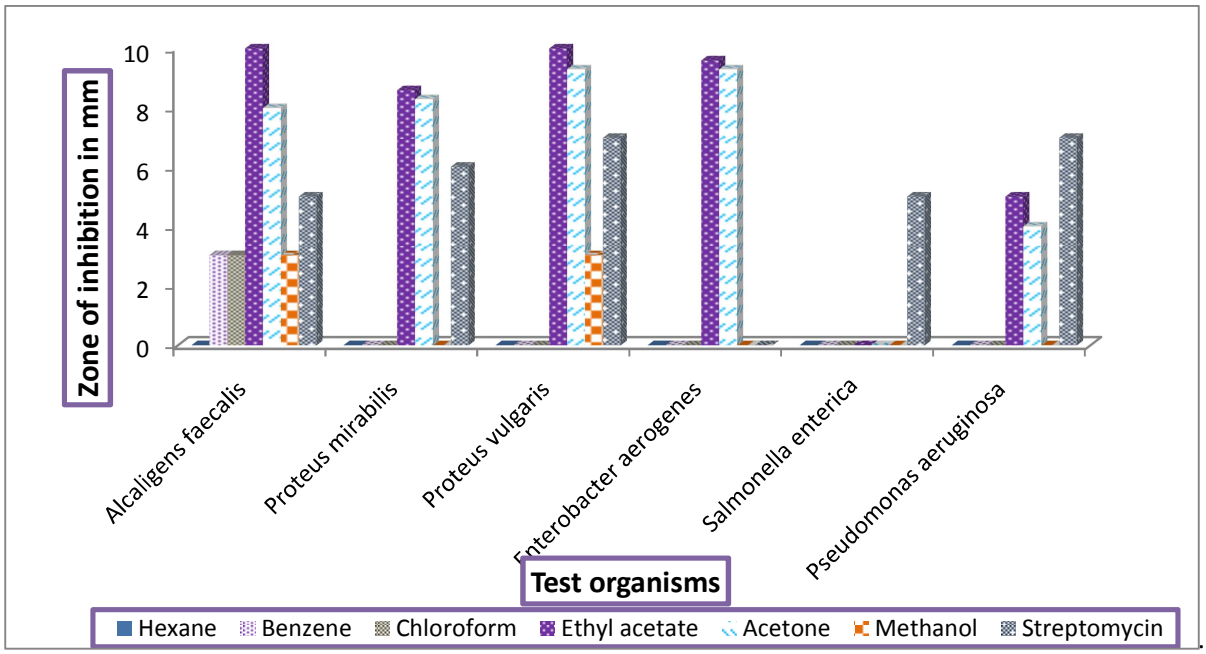


Figure-2. Antibacterial activity of different solvent extracts *Ipomoea pes-caprae* whole plant

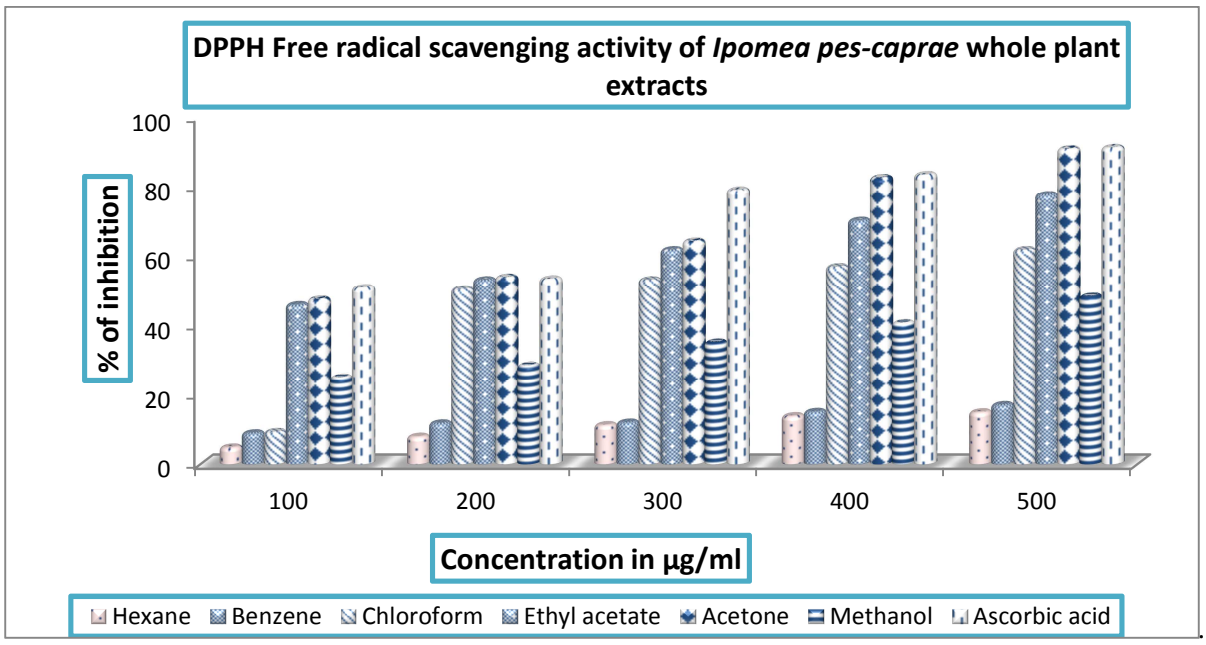


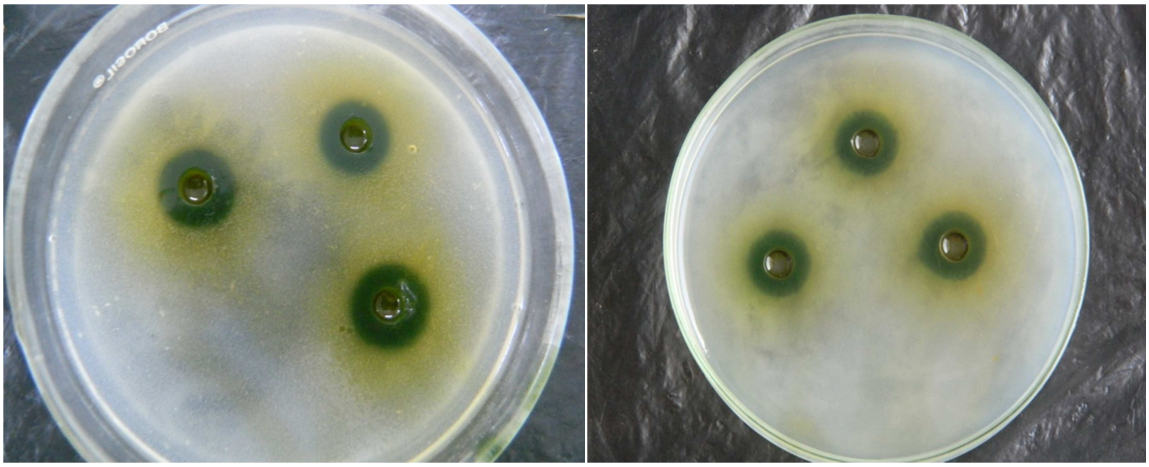
Figure-3. Antioxidant activity of different solvent extracts of *Ipomoea pes-caprae* whole plant

Antioxidant activity

The antioxidant properties of *Ipomoea pes-caprae* have been evaluated by measuring their DPPH free radical scavenging activity using different crude extracts of this plant and the results are shown in Figure-3. Acetone extract exhibited maximum free radical scavenging activity than other solvent extracts at different concentrations. High DPPH free radical scavenging activity was observed in acetone extract (90.89%) at 500 µg/ml concentration

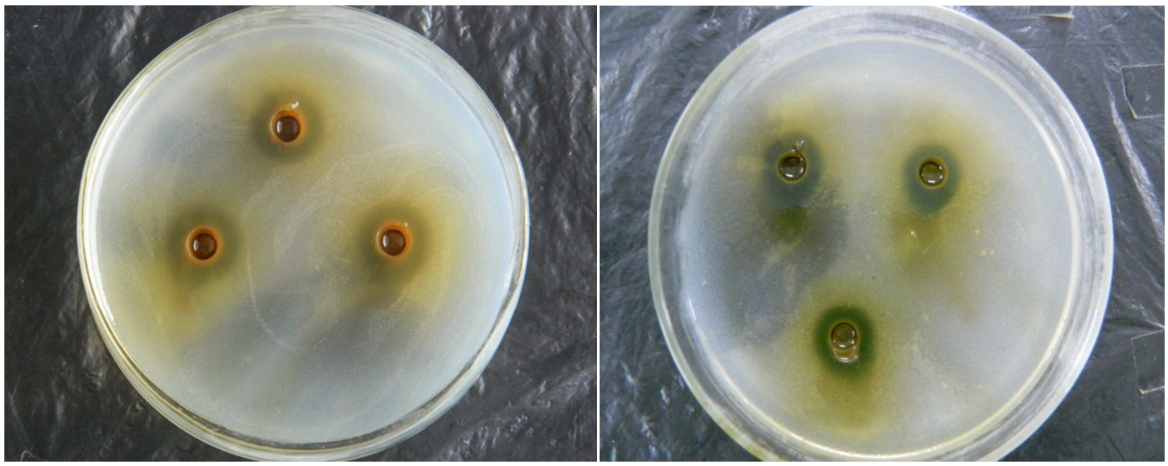
followed by Ethyl acetate extract (77.73%), Chloroform extract (61.74%), Methanol extract (48.21%), Benzene extract (16.97%) and Hexane extract (14.88%). DPPH stable free radical method is a sensitive way to determine the antioxidant activity of plant extracts. It has been shown that the scavenging effects on the DPPH radical increases sharply with increase in concentration of the samples and standards to a certain extent and hence are said to be strongly dependent on the extract concentration [17]. The therapeutic potential of natural medicinal plants as an antioxidant in reducing such free radical induced tissue injury, suggests that many plants have antioxidant activities that can be therapeutically useful [18].

Plate-1 Antibacterial activity (zones of inhibition) of *Ipomea pes-caprae* total plant extracts



a) Ethyl acetate extract against *Staphylococcus aureus*

b) Ethyl acetate extract against *Alcaligens faecalis*



c) Acetone extract against *Rhodococcus rhodochrous*

d) Ethyl acetate extract against *Arthrobacter protophormiae*

CONCLUSION

This study has revealed the presence of different phytochemicals, antibacterial and antioxidant activities of mangrove plant *Ipomoea pes-caprae* and can be suggested that the bioactive contents of the mangrove plants are promising natural antimicrobial agents and also natural antioxidants that can be harnessed as potential antibacterial and antioxidants. Further, extensive studies are recommended for this mangrove plant samples to actually identify the bioactive compounds responsible for their antibacterial and antioxidant activities.

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