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Phytochemical Screening and Antibacterial Assessment of Various Leaf Extracts of *Ipomoea pes-caprae* (L.) R. Br. Collected from the Coastal Areas of Kalpakkam, Chennai

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A B S T R A C T

Ipomoea pes-caprae (Linn.) Roth, sometimes known as beach morning glory, is a Convolvulaceae family mangrove related medicinal plant that thrives in tropical and subtropical climates. The leaves of *I. pes-caprae* collected from the coastal areas of Kalpakkam, Chennai, were prepared with various solvent extracts (hexane, chloroform, methanol) and aqueous extract and tested for phytochemical and antibacterial activity. The presence of phytochemicals such as alkaloids, glycosides, terpenoids, steroids, flavonoids, quinones, phenolic compounds, tannins, and saponins in all the extracts is shown by qualitative phytochemical analysis. The presence of alkaloids, flavonoids, phenols, and tannins can be determined via TLC profiling. Methanol was found to be the most successful of the four solvents in extracting the most secondary metabolites. On Gram-positive and Gram-negative bacteria, the antibacterial activity of different extracts was tested in vitro. The zone of inhibition found by the agar well diffusion method differed depending on the plant extract, extraction solvent, and organism examined. Against *Pseudeomonas sp*., the methanol extract produced the largest inhibitory zone (3 ≥13 mm). These findings suggested that *I. pes-caprae*, which is wildly grown, could be a source of pharmacologically active phytochemicals.

***Key words*:** Convolvulaceae, Bioactive compounds, Antibacterial activity, Traditional Medicine, Mangrove plants

Medicinal plants are widely used for curing different diseases with minimal side effects [1]. Herbal medicines inhabit distinct position, right from the ancient times to the present day [2]. India has the abundance of medicinal plants and most of which have been usually used in Siddha, Ayurveda and Unani systems of medicine and by tribal healers for generations. The bioactive phytochemical elements of mangrove plants that have a favourable physiological effect on the human body, such as anti-inflammatory, anticancer, antiviral, antimalarial, antidiabetic, and anti-hypersensitive, are used to assess their medicinal potential [3-4].

Mangrove is a term used to describe a collection of salt-tolerant and traditional woody plants with morphological differences. Mangrove plant extracts have been utilised to cure a variety of ailments since the 18th century. Plant-derived compounds have recently attracted a lot of attention due to their many applications [5]. Mangroves are rich in phytochemicals such as tannins, terpenoids, alkaloids, and flavonoids, all of which have been shown to have anti-inflammatory properties [6]. Mangroves are a special type of vascular plant that grows in saline coastal areas and is noted for its ability to withstand harsh climatic conditions. Mangrove plant extracts have been shown to be a rich source of confirmed antimicrobial activity against human, animal, and plant infections, and have been utilised in traditional medicine [7]. Under stressed situations, mangrove plants produce a variety of unique secondary metabolites which may have therapeutic significance.

Ipomoea pes-caprae (Linn.) Roth, commonly known as beach morning glory, is a Convolvulaceae family mangrove associated medicinal plant that flourishes in tropical and subtropical climates. It's an herbaceous climbing plant with heart-shaped leaves and funnel-shaped blooms. Bayhops, railroad vine, coast morning glory, goat's-foot morning glory, salsa-dapraia, and other names have been assigned to it. This plant was utilized in folk treatments for a variety of diseases [9] and stabilized sand dunes and reduced coastal erosion [8].

The plant's pharmacological profile revealed the following activities: antioxidant [10], analgesic [11], anti-inflammatory [12-13], antispasmodic [14], antinociceptive [12], antiarthritic [15], antihistaminic [16], insulinogenic and hypoglycemic activities [17], antifungal - Candida albicans and Microsporum audouinni [18] and immunostimulatory [19]. The

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presence of pharmacologically active components in *I. pes-caprae* extracts was also revealed using phytochemical screening. Saponins and tannins can be found in methanol extracts of leaves [9]. Steroids, terpenoids, alkaloids, flavonoids, and unusually high phenolic components are found in the aqueous methanol extract of the aerial part [20]. Alkaloids, saponins, tannins, anthraquinones, and flavonoids are found in both leaves and stem ethanol extracts [15]. Phytochemicals are secondary metabolites, which exhibit protective or disease preventive properties [22]. Terpenoids and alkaloids possess hypoglycemic properties; steroids and triterpenoids have analgesic effects; saponins have antioxidant and anti-inflammatory characteristics; and flavonoids have anti-allergic, anti-inflammatory, antibacterial, and anticancer properties [23]. Flavonoids, phenolic acids, and tannins are phenolic compounds that have antioxidative properties and a wide range of biological activities in plants [24]. Naphthalenone, (-)-mellein, eugenol, 4-vinyl-guaiacol, lipophilic glycosides, 2-methylpropanoic, (2S)-methylbutyric, n-hexanoic, n-decanoic, and n-dodecanoic acids are all active ingredients of the plant [25]. However, a few investigations on *I. pes-caprae* have yielded conflicting findings. Because plants synthesize these chemicals for protection under stressful situations. Phenolic compound variability could be stress-related [26].

This study aimed to conduct phytochemical screening and antibacterial potential of the leaves of the wild and locally available *I. pes-caprae* (Linn) Roth in the coastal areas of Kalpakkam, Chennai, in light of the substantial evidence on its pharmacologic and antimicrobial activities, as well as the different results observed depending on location and environmental condition.

MATERIALS AND METHODS

Collection and authentication

Ipomoea pes-caprae leaves were collected from the coastal areas of Kalpakkam, Chennai and authenticated by taxonomist. The specimens were cleaned with tap water, and then rinsed with sterile distilled water before being shade dried at room temperature. In a mechanical blender, the air-dried plant material was divided into discrete bits and homogenized. For the preparation of leaf extracts, powdered plant material was used.

Preparation of crude extracts

Using the Soxhlet Apparatus, 100 grammes of powdered plant material (leaves) were extracted for 8 hours with Methanol, Hexane, Chloroform, and Aqueous. The extracts were filtered, pooled, and the solvents were evaporated under reduced pressure at 40°C using a rotary evaporator (Heidolph, Germany), and the crude extracts were maintained at 4°C in the refrigerator for further tests.

Qualitative analysis of phytochemicals

Preliminary qualitative analysis

A qualitative chemical analysis was performed on all of the extracts. According to Harborne [27], the extracts were subjected to the following chemical analyses for the identification of various phytoconstituents such as Flavonoids, Steroids, Quinones, Glycosides, Tannins, Terpenoids, Phenols, Alkaloids, and Saponins.

Test for flavonoid
Shinoda's Test

A small amount of the powdered substance was heated and filtered with alcohol. Magnesium turnings and a few drops of strong hydrochloric acid were added to the test solution, which was then boiled for five minutes. The presence of flavonoids is indicated by the appearance of a red colour.

Test for steroids
Salkowski test

2 mL chloroform and 1 mL sulphuric acid were added to the extract. The presence of Steroids is indicated by a reddish-brown development in the bottom layer.

Test for quinones

1 mL concentrated H_2SO_4 was added to the extracts. The presence of quinones is indicated by the formation of a red colour.

Test for glycosides
Killer Killiani test

A solution of 1% ferric sulphate in 5% glacial acetic acid was added to the extract. A few drops of concentrated Sulphuric acid added. The presence of glycosides is evidenced by a brown ring formation.

Test for tannins
Braymer's test

1 mL Ferric chloride (5%) was added to the extracts. The formation of bluish black or greenish black precipitate showed the presence of tannins.

Test for terpenoid
Salkowski test

5ml of each extract was mixed with 2ml of chloroform in a separate test tube, and 3ml of concentrated sulphuric acid was carefully added. The presence of terpenoid is evidenced by a reddish-brown appearance.

Test for phenols
Ferric Chloride test

A few drops of neutral 5% ferric chloride solution are added to the extract. The presence of phenolic compounds is indicated by a dark green colour.

Test for alkaloids
Dragondroff's test

1 ml of Dragondroff's reagent was added to 1 ml of plant extract. The presence of yellow precipitate reveals that alkaloids are present.

Test for saponins
Froth Foaming test

Distilled water is used to dilute the extract (2ml). The suspension is vigorously shaken. The presence of saponins is indicated by the formation of a foam layer.

Thin layer chromatography (TLC)

TLC is a chromatographic technique which is used to separate compounds in a mixture. TLC is carried out on a sheet of aluminium foil that has been covered with a thin coating of adsorbent silica gel (commercially available 60 F254) (Merck). Capillary tubes were used to spot samples prepared in various solvents onto the TLC plate as a single spot [28]. The different mobile phase used are:

- Hexane:Ethyl Acetate (1:1)
- Hexane:Ethyl Acetate (7:3)
- Hexane:Ethyl Acetate (3:7)

- Ethyl Acetate (100%)

The plates are stained using the following reagents: Anisaldehyde reagent, Dragondroff's reagent, Potassium permanganate and Sulphuric acid.

TLC plates were viewed in UV chamber. The solvent front and the compound front was measured and the R_f value was calculated using the formula:

R_f = distance travelled by the compound/Distance travelled by the solvent

Antibacterial activity by agar well diffusion method

Microorganisms employed

Gram positive and Gram-negative microorganisms were used to test the antibacterial activity of the crude extracts. *Staphylococcus aureus* was used as Gram positive bacteria, whereas *Pseudomonas aeruginosa, Escherichia coli,* and *Klebsiella sp.* were used as Gram negative bacteria.

Agar well diffusion method

The agar well diffusion method was used to determine antibacterial screening [29]. Using 24-hour-old bacterial cultures, suspensions of several microorganisms were made. After that, using 0.5 McFarland standards, each strain was adjusted to a concentration of 10^8 cells/ml [30]. Plates of Muller-Hinton agar were prepared. After solidification, the bacterial cultures were swab cultured, and 6mm diameter wells in agar plates were punched with a sterile cork borer. For gram negative culture, chloramphenicol antibiotic was used as a positive control in a concentration of 10 µg /ml DMSO, and for gram positive bacterial culture, Ampicillin in a concentration of 10 µg /ml DMSO was used. 100 µl of the sample, which was prepared by dissolving 100 mg of sample in 1 ml of DMSO, was added to the well. To maintain the control, DMSO was administered in a separate well. The plates were incubated for 24 hours at 37°C. The diameter of the inhibition zone was measured after incubation. Triplicates of each sample and bacterial type were maintained.

RESULTS AND DISCUSSION

Plants are a rich source of potential therapeutic bioactive compounds that can be used in the development of novel chemotherapeutic drugs [31]. *Ipomoea pes-caprae* leaves were collected from the coastal areas of Kalpakkam, Chennai (Fig 1) and validated by taxonomists. Shade-dried leaves were pulverised in a mechanical blender. Powdered plant material (Fig 2) was used for the preparation of leaf extracts.

Fig 1 *Ipomoea pes-caprae*

Fig 2 *Ipomoea pes-caprae* leaf powder

Preparation of leaf extract

Using the Soxhlet Apparatus, 100 grams of powdered plant material were extracted systematically with Methanol, Hexane, Chloroform, and Aqueous, and the crude extracts were maintained at 4°C in the refrigerator for further experiments (Fig 3).

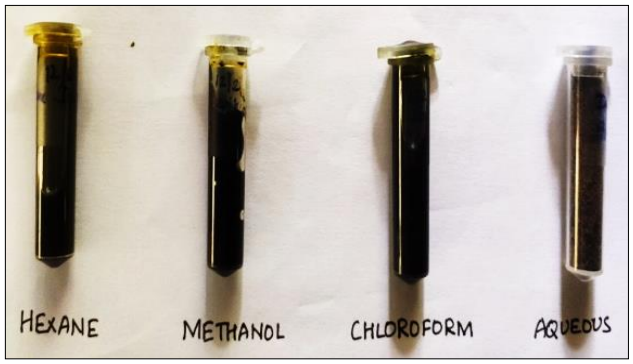


Fig 3 Leaf extracts of different solvents

Phytochemical analysis of Ipomoea Pes-caprae leaf extracts

Plant species' therapeutic effectiveness is attributed to phytochemical constituents. As a result, preliminary phytochemical screening of Ipomoea pes-caprae was carried out in this work. Phytochemicals such as alkaloids, cardiac glycosides, terpenoids, steroids, flavonoids, quinones, phenolic compounds, tannins, and saponins were detected in hexane, chloroform, Methanol, and aqueous extracts of the leaf of Ipomoea pes-caprae (Table 1, Fig 4). Phytochemicals such as terpenoids, steroid, quinones, glycosides, alkaloids, and saponin were identified in the hexane extracts, while Flavonoid, quinones, terpenoids, phenolic compounds, tannin, and saponin were observed in the aqueous extracts. Alkaloids, cardiac glycosides, phenolic compounds, quinones, tannins, terpenoids, and saponins were revealed in chloroform extracts.

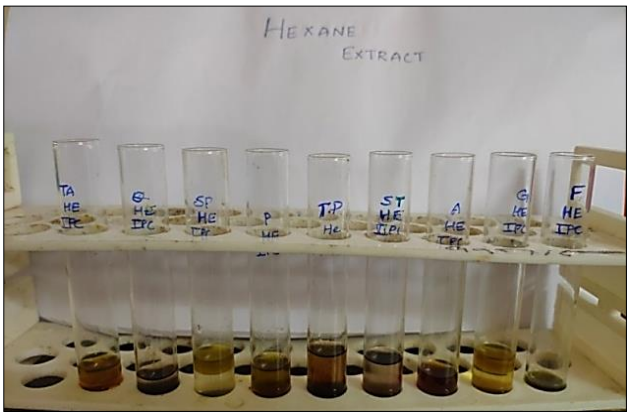
Strong phytochemicals such as terpenoids, steroids, quinones, phenolic compounds, tannins, and flavonoids were found in methanol extracts of *Ipomoea pes-caprae*. The findings reveal that certain phytoconstituents have antibacterial, anti-inflammatory, and anti-oxidant properties, which could lead to the discovery of novel compounds. Similar findings have been found in other research. De Souza *et al.* [20] found alkaloids in the plant's aerial section, while Venkataraman *et al.* [13] recognized alkaloids in the plant's leaves. These findings contradicted the findings of Ganjir *et al.* [9], who found that these compounds were not present in plant leaves. Flavonoids and phenolic acids have recently been

demonstrated in *I. pes-caprae* ethanol and methanol extracts [26]. The presence of a variety of phytochemicals in *I. pes caprae* leaves collected along the coast revealed that the plant had been exposed to herbivores, pathogens, and abiotic stresses [32]. Secondary metabolite synthesis and accumulation apparently happened in the plant for maximum protection [33]. This finding is similar to that of Venkataraman *et al.* [13], who used plant samples from coastal regions as well. However, these findings differ significantly from those of Ganjir *et al.* [9], who used plant samples from the Regional Plant Resource Centre of Bhubaneswar's medicinal germplasm garden. To survive in a coastal habitat, wild *I. pes caprae* produced more secondary metabolites than the cultivated plant in a controlled environment.

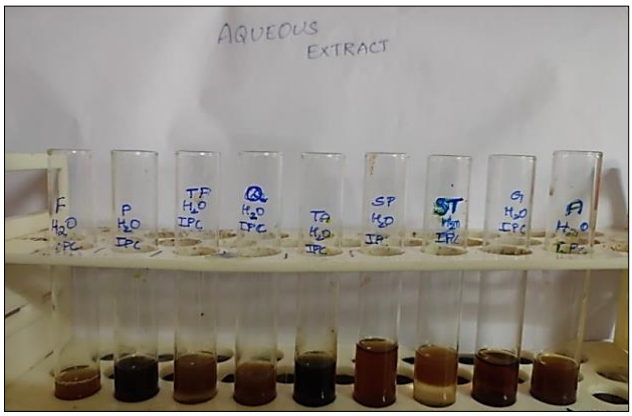
Table 1 Phytochemical analysis of *Ipomoea Pes-caprae* leaf extracts

Phytochemicals / Extracts	Name of the test	Hexane	Chloroform	Methanol	Water
Flavonoid	Shinoda's Test	-	-	+	+
Steroids	Salkowski test	+	-	+	-
Quinones	Test for Quinones	+	+	+	+
Glycosides	Killer Killiani test	+	+	-	-
Tannins	Braymer's test	-	+	+	+
Terpenoid	Salkowski test	+	+	+	+
Phenols	Ferric Chloride test	-	+	+	+
Alkaloids	Dragondroff's Reagent	+	+	-	-
Saponins	Froth foaming test	+	+	-	+

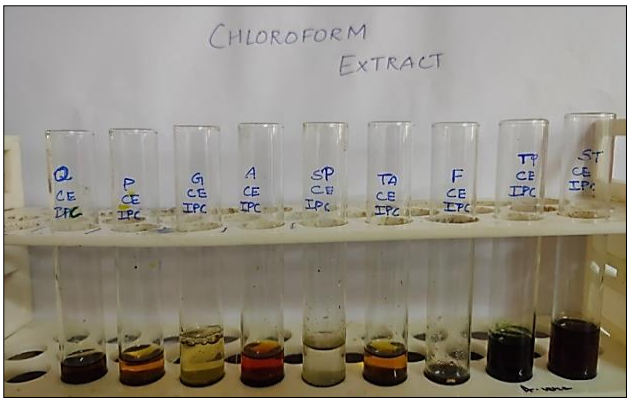
+ indicates presence, - indicates absence



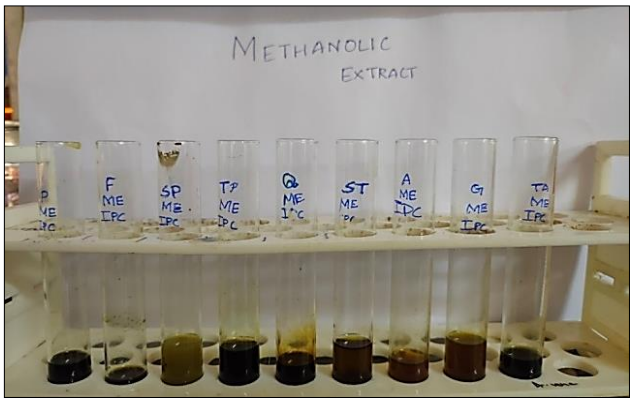
a. Hexane extract



b. Aqueous extract



c. Chloroform extract



d. Methanol extract

Fig 4 Phytochemical analysis of *Ipomoea Pes-caprae* leaf extracts

TLC Profiling

The result of TLC profiling was summarized in the following (Fig 5-8). Alkaloid (Rf 0.56) was found in hexane extract, while alkaloid (Rf 0.56), terpenoids (Rf 0.8), and tannin (Rf 0.92) were found in chloroform extract. Flavonoid (Rf value 0.918), tannins (Rf 0.92), and phenol (Rf 0.8) were found in methanol extract, however only quinones (Rf 0.737) were found in aqueous extract. TLC profiling of all plant extracts revealed the presence of several metabolites such as alkaloids, flavonoids, phenols, and tannins in the current study. Methanol was found to be the most successful in extracting secondary metabolites among the four solvents tested (Chloroform, Hexane, Methanol, and Aqueous). Similar findings have been seen in other research.

In both the leaf and stem of *I. pes-caprae*, the extractive value was highest in methanol solvent [34]. The presence of glycosidic resins, coumarins, steroids, flavonoids, and triterpenes in *Ipomoea pes-caprae* characterized the phytochemical profile [35]. The presence of high amounts of phytoconstituents such as alkaloids, phenols, steroids, tannins, and flavonoids in *Ipomoea pes-caprae* leaf and stem satisfy pharmacognostic standards [34].

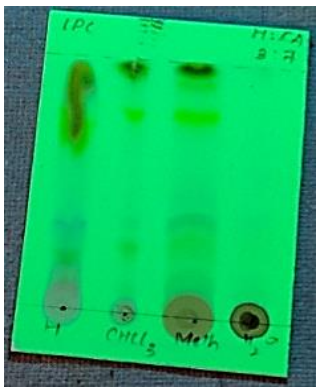


Fig 5 TLC Plates under UV light with mobile phase Hexane:Ethyl Acetate (3:7)

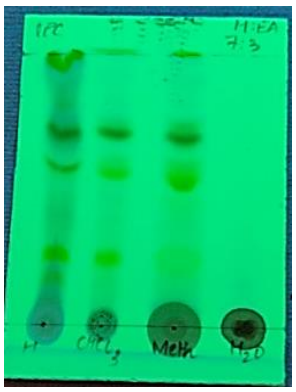


Fig 6 TLC Plates under UV light with mobile phase Hexane:Ethyl Acetate (7:3)

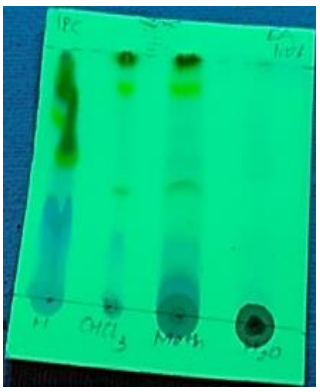


Fig 7 TLC Plates under UV light with mobile phase Ethyl Acetate (100%)

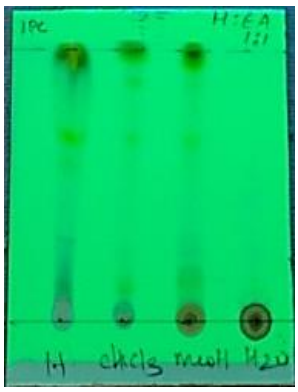


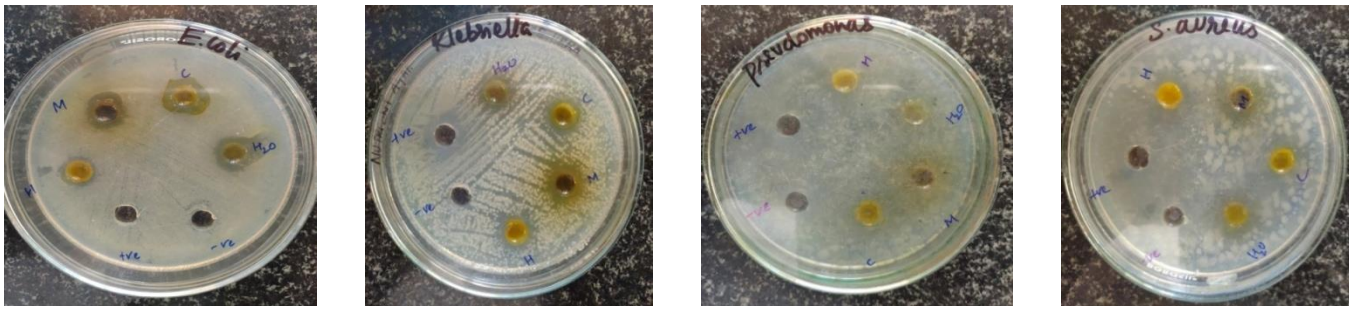
Fig 8 TLC Plates under UV light with mobile phase Hexane:Ethyl Acetate (1:1)

Antibacterial activity by Well Diffusion Method

The (Table 2, Fig 9-10) showed the antibacterial activity of various extracts of *Ipomoea pes-caprae* leaves. The mean zone of inhibition for bacteria was found to be between 2 and 11 mm for Hexane extracts and 3 and 11 mm for chloroform extracts. Aqueous extracts had a mean zone of inhibition of 3 - 8 mm, while methanol extracts had a mean zone of inhibition of 3 - 13 mm. *Pseudomonas aeruginosa* was inhibited by the methanol extract as much, with a zone of inhibition of 13 mm. Other research has found that methanolic extracts have a higher antibacterial activity [36]. Hexane, dichloromethane, and ethyl acetate extracts showed no bactericidal action, although methanol extracts exhibited more bactericidal action [37]. Sharmin *et al.* [35] found that the methanolic crude extract and its aqueous soluble fractions had significant antibacterial activity, with average zones of inhibition ranging from 8-12 mm each. Rashid *et al.* [38], found that crude *Ipomoea turpethum* extracts in Petroleum ether, chloroform, and ethyl acetate had antimicrobial activity against gram positive bacteria (*Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus, Sarcina lutea, Streptococcus hemolyticus, Pseudomonas aeruginosa, Sarcina sarcinacea*) and gram-negative bacteria (*E. coli, Shigella dysenteriae, Shigella shiga, Shigella boydii, Shigella sonnei, Shigella flexneriae, Salmonella typhi*).

Table 2 Antibacterial activity of *I. Pes-caprae* leaf extracts

Extracts / Mircroorganisms	*Klebsiella*	*Pseudomonas*	*E. coli*	*S. aureus*
Hexane	4mm	11mm	2mm	6mm
Aqueous	3mm	7mm	4mm	8mm
Chloroform	3mm	11mm	5mm	7mm
Methanol	5mm	13mm	3mm	6mm



a. *E. coli* b. *Klebsiella sp.* C. *Pseudomonas sp.* d. *S. aureus*

Fig 9 Antibacterial activity of *I. Pes-caprae* leaf extracts by well diffusion method

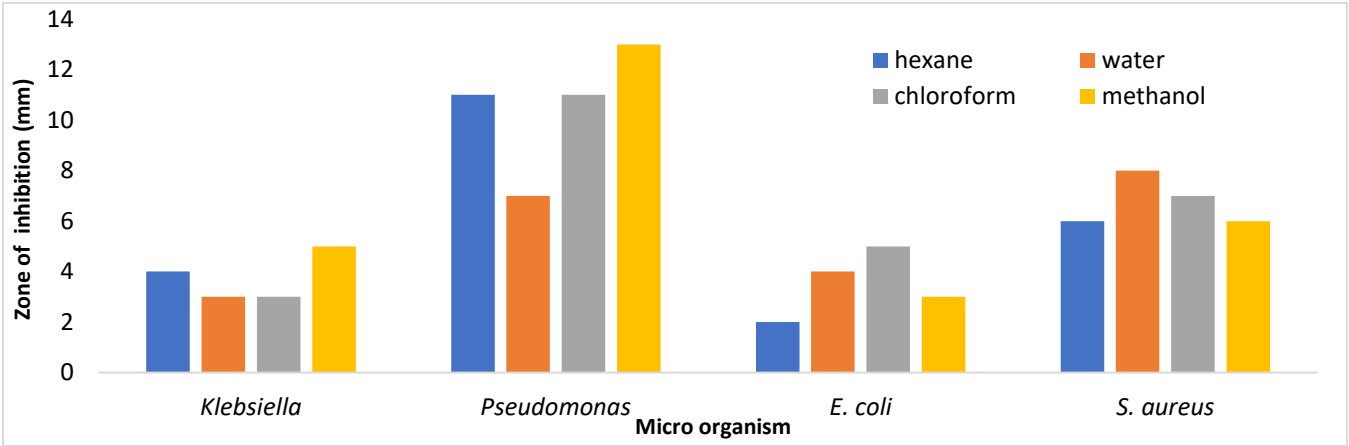


Fig 10 Antibacterial activity of various leaf extracts of *I. pes-caprae*

CONCLUSION

The pharmacological potential of the mangroves from Indian region still remains largely unexplored. The present study shows that, the mangrove species of *Ipomoea pes-caprae* are considered to be the highly potential candidate for the blockbuster of bacterial infections and they have been reported for the various pharmacological activities. The current investigation was found to be useful in identifying many components present in various extracts of *I. pes-caprae* leaves. Traditional practitioners employ the plant for a variety of diseases due to the presence of numerous bioactive components. *I. pes-caprae* leaf extracts contain a wide range of bioactive compounds with excellent therapeutic value, according to TLC profiling. Further research into these phytochemicals will open the way for the development of low-cost drugs with fewer negative effects. The people will be sufficiently motivated to safeguard the plant from extinction as a result of anthropogenic activities if they are aware of its value. The preservation of the coast and the availability of a potent herbal plant are two advantages of protecting the plant and its habitat.

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