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IN VITRO PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITYOF *SESUVIUM PORTULACASTRUM(L.)L.*AND *SUAEDA MARITIMA* L.

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ABSTRACT

Background*:* *Sesuvium portulacastrum* (L.) L**.** and *Suaeda martima* (L.) are two important medicinal plants inhabited in mangrove regions. **Objective:** The objective of this study is to screening of phytochemicals, and to measure the antimicrobial activity against 2 Gram positive bacteria, 2 Gram negative bacteria and 3 fungal strains. **Materials and Methods:** Hexane, Ethyl acetate and Methanol were utilized for extraction. Bacterial Strains *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Pseudomonas aeruginosa* and fungal strains *Aspergillus niger*, *Aspergillus flavus*, were used to test the antimicrobial activity. **Results:** The activity increased in dose dependent manner thus hexane concentrate *of Sesuvium portulacastrum* display most noteworthy action against *P.aeruginosa* and *E.coli* and *Aspergillus flavus* demonstrated maximum. *Suaeda maritima* show highest zone against *S.aureus* and *P.aeruginosa* demonstrated least movement. Hexane was demonstrated best dissolvable for *Sesuvium portulacastrum* and Ethyl acetate for *Suaeda maritima*. Ethyl acetate was finest for the fungal activity of both plants. Phytochemical analysis of both plants showed presence of alkaloids, steroids, tannins, glycosides, ascorbic acid etc. **Conclusion:** These outcomes bolster the thought that therapeutic plants may have a job as pharmaceutical and additives.

KEYWORDS: *Sesuvium portulacastrum*, *Suaeda maritima*, Medicinal plants, Phytochemical screening, Hexane, Ethyl acetate.

INTRODUCTION

Mangroves are distinctive group of vascular plants that occur in saline coastal habitats (coringa estuary) and are known to tolerate extreme environmental conditions. Mangrove plants have primary and secondary metabolites such as proteins, carbohydrates, carotenoids, hydrocarbons, aliphatic alcohols, polyunsaturated fatty acids, lipids, pheromones, phorbol esters, phenolics, steroids, terpens, tannins and glycosides etc.[1] These metabolites were described for bioactive substances as bactericidal, fungicidal, pharmaceutical agents for animal and human beings.[2] Application of chemotherapeutants has created problems via toxicity, resistance, residue leftover and possibly some public health and environmental consequences. Therefore, new drugs have to be found in order to combat such consequences and it is essential to find new compounds that have antimicrobial properties. Among the mangrove associated plants, *Sesuvium portulacastrum* (L.) L. is known as "Sea purslane" belongs to family Aizoaceae and grows in the Mediterranean coast and sub- tropical areas around the world. In traditional medicine, *Sesuvium* has been used for the treatment of conjunctivitis, leprosy, dermatitis and toothache[2] *Suaeda maritima* (Amaranthaceae) is a small mangrove plant commonly known as annual seabite found extensively in the tidal forests and swamps of the Krishna-Godavari area. This plant is also well- distributed in a number of other countries of temperate and tropical Asia. It is edible and consumed by local people as leaf vegetable dish called as "Goilakuru". This plant also has been traditionally used to treat sores and stings from marine creatures, and ulcers, as a purgative and an emetic. Clinical trials carried out on this plant have shown its potential anti-HIV, anticancer, anti-neurodegenerative, antibacterial and antiviral properties. The aim of this study was to screen for the phytochemicals present in the plant and study the two medicinal plant extracts against a diverse range of organisms comprising Gram-positive and Gram-negative bacteria and fungi. Therefore the present study deals with antimicrobial activity of Hexane, Ethyl acetate and methanol extracts of these two medicinal plants against 4 bacteria and 3 fungalspecies.

MATERIALS AND METHODS

Plants extraction preparation

Plant materials of two plant species included in this study were collected from coringa wildlife sanctuary and estuary situated in Andhra Pradesh, India. The collected plants were watery washed, disinfected, rinsed with

distilled water and finally dried in shade. The dried plant material of each plant species was grounded into fine powder to pass 100 mm sieve. 50 g of the fine powder was subjected to soxhlet extraction by using Hexane, Ethyl acetate and Methanol for 48 hrs resulting extracts in different solvents were evaporated and dried at 40 °C under reduced pressure using rotatory vacuum evaporator. The extract yields were weighted, stored in small bottles in fridge at 5 °C and these crude extracts were tested for standard strains of microorganisms.

Phytochemical screening for different compounds[3,4]

Test for Flavonoids

0.5 g of various extract was shaken with petroleum ether to remove the fatty materials (lipid layer). The defatted residue was dissolved in 20 ml of 80% ethanol and filtered. The filtrate was used for the following tests: (a) 3 ml of the filtrate was mixed with 4 ml of 1% aluminium chloride in methanol in a test tube and the colour was observed. Formation of yellow colour indicated the presence of flavonols, flavones and chalcones. (b) 3 ml of the filtrate was mixed with 4 ml of 1% potassium hydroxide in a test tube and the colour was observed. A dark yellow colour indicated the presence of Flavonoids. (c) 5 ml of the dilute ammonia solution was added to the portion of the aqueous filtrate of each plant extract followed by the addition of concentrated H_2SO_4. The appearance of the yellow colouration indicated the presence of flavonoids.

Test for alkaloids

0.5 to 0.6 g of various extract was mixed in 8 ml of 1% HCl, warmed and filtered. 2 ml of the filtrate were treated separately with both reagents (Maeyer's and Dragendorff`s), after which it was observed whether the alkaloids were present or absent in the turbidity or precipitate formation.

Test for Glycosides

5ml each of various extract were hydrolysed separately with 5 ml each of conc. HCl and boiled for few hours on a water bath and hydrolysates were subjected to the following test: A small amount of alcoholic extract of samples was dissolved in 1ml water and then aqueous 10% sodium hydroxide was added. Formation of a yellow colour indicated the presence of glycosides

Test for steroids

0.5 g of the various solvent extract fraction of each plant was mixed with 2 ml of acetic anhydride followed by 2 ml of sulphuric acid. The colour changed from violet to blue or green in some samples indicated the presence of steroids.

Test for Phenols

To 1ml of various solvent extracts of sample, 2ml of distilled water followed by a few drops of 10% aqueous ferric chloride solution were added. Formation of blue or green colour indicated the presence of phenols.

Test for Saponins

0.5 g of various solvent extract was dissolved in boiling water in a test tube. Test cooling aqueous extracts were mixed vigorously to froth and the height of the froth was measured to determine the saponin contents in the sample. 2.0 g of the powdered plant material was boiled in distilled water in a test tube in boiling water bath and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and was shaken vigorously to the formation of stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously for the formation of emulsion thus a characteristic of saponins.

Test for Resins

1ml of various solvent extract was treated with few drops of acetic anhydride solution followed by one ml of conc. H_2SO_4. Resins give coloration ranging from orange to yellow.

Test for Tannins

0.25 g of various solvent extract was dissolved in 10 ml distilled water and filtered. 1% aqueous Iron chloride ($FeCl_3$) solution was added to the filtrate. The appearance of intense green, purple, blue or black color indicated the presence of tannins in the test samples.

Test for Quinones

1ml of each of the various extracts was treated separately with alcoholic potassium hydroxide solution. Quinines give coloration ranging from red to blue

Antibacterial activity of the plant extracts Bacterial strains

The antibacterial potency of each plant extract was evaluated using five bacterial strains Two strains of Gram positive (*Staphylococcus aureus*, *Streptococcus pyogenes*) and two strains of Gram negative *(Escherichia coli*, *Pseudomonas aeruginosa*) bacteria. Three fungal strains (*Aspergillus niger*, *Aspergillus flavus, Aspergillus fumigatus*).The bacterial and fungal strains were provided from the microbial type culture (MTCC), Institute of Microbial technology, Chandigarh, India (**Table2**).

Assay for antimicrobial testing

Isolated test bacteria were grown overnight on nutrient agar plates and fungi were grown on Sabouraud dextrose agar plates. Bacterial inoculums were prepared from overnight grown cultures (24 h) in liquid broth (Hi Media, Mumbai, India), and the turbidity was adjusted equivalent to 0.5 McFarland units (approximately 10^8 CFU/ml for bacteria and fungi inoculums turbidity was equivalent at 10^5 or 10^6 CFU/ml). The microorganisms were inoculated into liquid broth and incubated at 35 ± 2°C for 4 h. The positive control was taken streptomycin (10 µg/ml) for antibacterial activity and Ketocanozole (10 µg/ml) for antifungal activity. The DMSO was taken as negative control to determine possible inhibitory activity of the dilutant of extract. The

susceptibilities of the isolated pathogens were determined by the modified Olurinola. 1996[5] agar well diffusion method with Muller Hinton agar plates. Aliquots of inoculums were spread over the surface of agar plates with a sterile glass spreader. To test the antimicrobial activity all extracts were dissolved in DMSO to make a final concentration of 25 and 50µl. After culture medium poured in the Petri plates remained placed at room temperature for settling and then kept refrigerator for 30 minutes. After this procedure was done, took 3 number cup borer (6 mm) sterilized properly by flaming and then used to make uniform cups/wells in each Petri plate. Then cups/wells filled with different extracts and allow to diffusing the extract into the medium for about 45 minutes. These plates were incubated for a period of 24 h at 37°C in incubator for bacteria and at 30°C for 24-48 h in B.O.D incubator for fungi. Each experiment was done in triplicate and mean values were taken. Antimicrobial activity was measured in the diameter (mm) of the clear inhibitory zone formed around the well.

RESULTS

The phytochemical analysis of the two plants are shown in **Table1** while The antimicrobial activity of *S. portulacastrum* and *S. maritima* extracts are shown in **Table 2 and Table 3** respectively. The results showed that the antimicrobial activities of the crude extracts were increased with increasing concentration. Although the antimicrobial activity of the extracts tested is variable, two gram – positive bacteria (*Staphylococcus aureus, streptococcus pyogenes*), two gram –negative bacteria (E. coli, Pseudomonas aeruginosa) and three strains of fungi (*Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus*) were inhibited by theextracts.

Table 1: Phytochemical analysis of *S.maritima* and *S.portulacastrum* extracts in different solvents.

Name of the Test	*S.martiima*			*S.portulacastrum*		
	Ethanol	**Methanol**	**Hexane**	**Ethanol**	**Methanol**	**Hexane**
Flavonoids	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+
Glycosides	-	-	-	+	+	+
Steroids	+	+	+	+	+	+
Tannins & Phenols	+	+	+	+	+	+
Saponins	-	-	-	+	+	+
Resins	+	+	+	+	+	+
Quinones	+	+	+	+	+	+

Table: 1 shows the presence of phytochemical compounds like alkaloids, steroids, phenols, resins, tannins, quinones are present in *S.protulacastrum* whereas in *S.maritima* along with above mentioned compounds, glycosides and saponins are also present.

Table 2: Details of the bacterial and fungal strains used in bioassay.

S.No	Name of the Bacterial/ fungal trains	MMTC Catalogue No.
Gram positive Bacteria		
1	*Staphylococcus aureus*	MTCC 3160
2	*Streptococcus pyogenes*	MTCC 442
Gram negative Bacteria		
3	*Escherichia coli*	MTCC 443
4	*Pseudomonas aeruginosa*	MTCC 424
Fungal pathogens		
5	*Aspergillus niger*	MTCC 961
6	*Aspergillus flavus*	MTCC 3396
7	*Aspergillus fumigatus*	MTCC 2584

Table3:Antimicrobial activity of different solvent extracts of *Sesuviumportulacastrum*(L.) L.

Organism	Solvent	DMSO	25µl	50µl	Control
					Streptom ycin (10 µg/ml)
Staphylococcus aureus	EA	1.6±0.01	0.63±0.04	0.8±0.04	ND
	ME	0.8±0.08	ND	ND	ND
	HE	1.6±0.08	ND	ND	ND
Streptococcus pyogenes	EA	1.6±0.01	ND	0.76±0.04	ND
	ME	1.6±0.04	ND	0.7±0.08	ND
	HE	1.5±0	1.1±0.12	**1.5±0**	0.7±0.08

Escherichia coli	EA	1.8±0.08	1.7±0.04	**2.3±0.04**	0.4±5.5
	ME	1.7±0	0.8±0.08	0.9±0.08	ND
	HE	1.83±0.04	1.7±0.08	**2.1±0.12**	0.7±0.08
Pseudomonas aeruginosa	EA	1.5±0.08	ND	ND	0.5±0.0
	ME	1.9±0.08	0.6±0.12	0.9±0.08	0.4±0.04
	HE	1.9±0.08	1.5±0.08	**2.3±0.08**	0.7±0.08
					Ketocano zole (10 µg/ml)
Aspergillus niger	EA	1.3±0.08	0.6±0.04	ND	ND
	ME	0.8±0.04	0.9±0.08	1.4±0.08	ND
	HE	0.7±0.08	1.3±0.16	**2.1±0.08**	ND
Aspergillus flavus	EA	1.3±0.08	ND	ND	0.8±0.08
	ME	ND	1.0±0.04	1.7±0.08	ND
	HE	0.7±0.04	1.16±0.12	**1.36±0.04**	ND
Aspergillus fumigatus	EA	1.8±0.09	1.3±0.04	ND	0.7±0.08
	ME	0.8±0.08	ND	1.0±0.09	ND
	HE	0.7±0.04	1.16±0.12	**1.36±0.04**	ND

Note: expand the abbreviations EA=,ME=,HE=,DMSO=Negativecontrol,ND=,andControl=Positivecontrol.

Table4: Antimicrobialactivityofdifferentsolventextractsof *Suaeda maritima* L.

Organism	Solvent	DMSO	25µl	50µl	Control
					Streptomycin (10 µg/ml)
Staphylococcus aureus	EA	1.2±0.08	1.1±0.12	1.6±0.12	ND
	ME	1.7±0.08	1.9±0.12	2.0±0.12	1.0±0.12
	HE	1.6±0.04	1.3±0.08	**2.2±0.12**	ND
Streptococcus pyogenes	EA	1.2±0.04	0.7±0.08	1.2±0.09	ND
	ME	0.8±0.04	0.6±0.08	1.2±0.09	ND
	HE	1.3±0.04	1.6±0.12	**2.1±0.08**	0.7±0.08
Escherichia coli	EA	1.7±0.04	1.8±0.08	**2.3±0.08**	0.7±0.04
	ME	1.6±0.08	0.7±0.08	1.0±0.09	ND
	HE	1.4±0.04	1.7±0.12	**2.1±0.09**	0.4±0.08
Pseudomonas aeruginosa	EA	1.6±2.22	0.83±0.08	1.5±0.12	0.4±0.04
	ME	0.8±0.04	ND	0.3±0.08	ND
	HE	1.8±0.04	0.5±0.04	0.8±0.08	ND
					Ketocanozole (10 µg/ml)
Aspergillus niger	EA	1.4±2.22	0.83±0.12	**2.1±0.12**	ND
	ME	1.3±0.04	ND	0.8±0.04	ND
	HE	0.8±0.04	0.4±0.08	1.0±0	ND
Aspergillus flavus	EA	1.3±0.08	ND	ND	ND
	ME	0.7±0.08	ND	ND	ND
	HE	0.7±0.08	ND	**ND**	ND
Aspergillus fumigatus	EA	1.9±0.08	1.3±0.04	**1.7±0**	ND
	ME	0.9±0	ND	ND	ND
	HE	2.0±0.08	ND	1.1±0.12	ND

Table: 2 show the antimicrobial activity of *S. portulacastrum* where, among three solvents used Hexane exhibit better activity followed by ethyl acetate and methanol. Highest zone of inhibition were observed in Hexane extract against *Pseudomonas aeruginosa* (2.3 ± 0.08 at 50µl concentration) followed by ethyl acetate extract against *E. coli* (2.3 ± 0.04 at 50µl concentration). Lowest activity recorded in ethyl acetate extract against *Staphylococcus aureus* (0.8 ± 0.04 at 50µl concentration). While *Pseudomonas aeruginosa*, *Staphylococcus aureus* strains appear to be resistance to the tested concentration in Ethyl acetate, Methanol and Hexane solvents since no inhibition zone were observed. Coming to the fungal strains among tested solvents Hexane gives better activity followed by Methanol, Ethyl acetate. *Aspergillus niger* showed highest zone of inhibition (2.1 ± 0.08 at 50µl concentration) in Hexane extract. Lowest recorded with *Aspergillus fumigatus* (1.0

± 0.09 at 50µl concentration) in Methanol extract. While *Aspergillus flavus* strains appear to be resistant in Ethyl acetate extract since there is no inhibition zone wasrecorded. In *S.maritima* (**Table: 3)** all the extracts were found to possess various degree of antimicrobial activity against gram positive Bacterial, gram negative Bacterial and fungal strains. Among the tested extracts Hexane exhibited highest antibacterial potential of activity against all the tested bacterial species irrespective of their gram nature. However, Ethyl acetate was found to be active after hexane. Methanol exhibited mixed response. Further, the higher zone of inhibition recorded in hexane extract against *Staphylococcus aureus* (2.2 ± 0.12 at 50µl concentration) followed by *Streptococcus pyogenes* and *E.coli* (2.1 ± 0.08 at 50µl concentration). Ethyl acetate exhibited higher zone of inhibition in *Ecoli* (2.3 ± 0.08 at 50µl concentration) among others. Minimum inhibition was noted in Methanol extract against *Pseudomonas aeruginosa* (0.3 ± 0.08 at 50µl concentration). Antifungal activity of *E.agallocha* the results revealed that Ethyl acetate extract gives somehow better results than Hexane and Methanol. Methanol solvent proved weak solvent than remaining solvents. Highest zone of inhibition noted in Ethyl acetate extracts against *Aspergillus niger* (2.1 ± 0.12 at 50µl concentration) and least also recorded from methanol extract against *Aspergillus niger* (0.8 ± 0.04 at 50µl concentration), *Aspergillus flavus* in methanol. Hexane extracts were resistant and methanol of *Aspergillus fumigatus* also resistant because there were no zones of inhibition observed.

DISCUSSION

Mangroves are inimitable assembly of vascular plants that happen in saline seashore front natural surroundings and are known to endure outrageous ecological conditions. The antimicrobial activity exhibited by the mangrove plant parts could be due to the presence of phytochemicals like alkaloids, tannins, flavonoids and sugars present in the plant extract. Primary and secondary metabolites are very important for the regular mechanism/survival of the species and also it can be used as therapeutic agents. Potential antimicrobial agents from mangrove species were due to the presence of phytoconstituents.

Al – Azzawi.A *et.al*.,2012.[6] reported antimicrobial screening of *S.portulacastrum* in the United Arab Emirates, They were used leaves as their interested part for activity, and used Ethanol, Aqueous Dichloromethane for extraction. Among used solvents Ethanol was considered as best and given good activity against *staphylococcus aureus* and *E.coli*. Satish.P *et.al*. 2016.[7] reported antibacterial activity of SAH marsh plant extracts and they conclude that *S.portulacastrum* did not show any activity against three tested human pathogens. Lincy, M. P *et. Al*., 2013.[8] reported in vitro antibacterial activity of leaf of *S.portulacastrum*. In their investigation they were used petroleum ether, Benzene, Ethyl acetate, Methanol and Ethanol for extraction. They concluded that Ethanol extract shows very good activity than remaining tested solvents. Abirami.H and Rameshwari.R 2013.[9] reported Antibacterial and Antifungal screening of *S.portulacastrum* extracts against lather contaminating organisms. They used leaf, Methanol, Chloroform, Petroleum ether and Ethyl acetate for extraction. They concluded form their findings that chloroform was found best and *Klebsiella pneumonia* showed least activity in petroleum ether and *Staphylococcus aureus*, *E.coli* recorded highest activity in chloroform extract. They also concluded that *S.portulacastrum* inhibited the reproductive activity in *A.niger* and *A.flaus*. K.Feroz khan and G. Sankar reported antibacterial activity of salt marsh plants against Marine ornamental fish pathogens. They used solvents like Aqueous, Methanol, and Diethyl ether for extraction. Among tested Methanol extract showed more significant activity than remain tested.

In the present study we were taking whole plant of *S.portulacastrum* for antimicrobial activity. For Extraction were used Ethyl acetate, Methanol and Hexane solvents. From our findings it was concluded that Hexane was proved better than remaining tested solvents. *Pseudomonas aeruginosa* was exhibit highest zone of inhibition in hexane extract. *Aspergillus niger* gives highest inhibition zone in hexane extract. So this was the first result that hexane proved better.

Satish.P et.al. 2016[10] reported antibacterial activity of SAH marsh plant extracts and they conclude that *S. maritima*, they took leaves and stems as interested parts, where methanolic extract showed highest zone of inhibition against *E.coli* and *Pseudomonas aeruginosa*. Young-Gun Moon.et. al., 2008.[11] reported antimicrobial activity of the roots of *S.maritima* against *Bacillus cereus*, *Bacillus subtillis*, *Staphylococcus aureus*. The highest antibacterial activity against bacteria test was found in the methanol extract. Bulti Nayak et.al.2018.[12] reported antimicrobial property of *Suaeda martima* against Gram- negative and Gram-positive isolates .Among the different solvents n-hexane extract from root showed highest antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas sp*. And some fungicidal activity was noted against *Saccharomyces cerevisiae*.

So by reviewing all the literature, in this present study whole plant was used, and Ethyl acetate, Methanol and Hexane were utilized for extraction. Among tested solvents Hexane gives better activity against *Staphylococcus aureus* (2.2mm), and Ethyl acetate activity against *E.coli* (2.3mm), and for fungi *A.niger* (2.1mm). This is also first record with hexane because no one get better activity with hexane so far. From our finding also concluded that Ethyl acetate was good for the fungal activity of both the plants.

CONCLUSION

Sesuvium portulacastrum (L.) L. and *Suaeda maritima* L. extracts had the ability to inhibit bacterial growth. In the present study whole plant were assessed to test antimicrobial activity against human pathogens. By the results it was concluded that among the tested extracts Hexane proved better for *S. portulacastrum* and Ethyl acetate for *Suaeda maritima* L. Among tested pathogens *P.aeruginosa* and *S.aureus* display highest inhibitory activity for 2 plants respectively. All these extracts were not effective than antibiotics to combat the pathogenic microorganisms studied. This indicated that these plants have potentially antibacterial properties and could be used in the development of novel antibacterial agents.

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CONFLICT OF INTEREST

None

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