OPEN ACCESS

Haya: The Saudi Journal of Life Sciences

Abbreviated Key Title: Haya Saudi J Life Sci
ISSN 2415-623X (Print) | ISSN 2415-6221 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article

Antimicrobial Activity of *Sesuvium portulacastrum* (L.) Against Selected Pathogens

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DOI: 10.36348/sjls.2023.v08i09.003 | **Received:** 05.09.2023 | **Accepted:** 08.10.2023 | **Published:** 11.10.2023

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Abstract

This work aims to evaluate the antimicrobial potential of halophytic plant, *Sesuvium portulacastrum* (L.) against, some plant and human pathogens. Plant parts of *S. portulacastrum* were collected from the mangrove habitats of Coringa Reserve Forest near Kakinada, Andhra Pradesh, India. Plant parts are dried and extracts were obtained successfully with hexane, chloroform, methanol and water, using Soxhlet extraction apparatus. Agar well diffusion method has been used to determine the antimicrobial activity of plant extracts against some gram positive bacteria (*Bacillus subtilis, Bacillus megaterium* and *Lactobacillus acidophilus*), gram negative bacteria (*Escherichia coli, Enterobacter aerogenes, Enterobacter cloace* and *Klebsiella pneumonia*) and fungal species (*Candida albicans, Mucar recemosus, Rhizoctonia solani, Rhizopus stolonifer* and *Saccharomyces cerevisiae*). The hexane, chloroform extracts showed minimum antimicrobial activity when compared to methanolic and water extracts. Water extract of *S. portulacastrum* showed appreciable antimicrobial activity against all bacterial and fungal strains. It reveals that this halophytic species has antimicrobial compounds which can act against microorganisms and they can be used in the treatment of infectious diseases caused by pathogenic microorganisms.

Keywords: Halophytes**,** Antimicrobial Activity, Agar Well Diffusion Method, *Sesuvium portulacastrum.* Godavari Estuary.

INTRODUCTION

Several halophytes are extensively used in the traditional medicine, only some of them were tested for biological activities (Funnel *et al.,* 2004). Halophytes are a distinctive group of vascular plants that occur in estuarine habitats 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, terpenes, tannins and glycosides etc. (Bandaranayake 2002; Patra and Thatoi, 2011). A number of mangrove associates contain poisonous substances, which also show biological activities such as antifungal, antibacterial properties (Bandaranayake, 1998). Antimicrobial properties of different halophytes of Godavari estuary were studied by Prasanna Lakshmi and Narasimha Rao, (2013); Prasanna Lakshmi *et al.*, (2015); Prasanna Lakshmi and Narasimha Rao, (2023).

Sesuvium portulacastrum (L.) known as 'sea purslane' belongs to the family Aizoaceae and it is a perennial herb that grows naturally in the sub-tropical, Mediterranean coastal and warmer areas around the world (Thomas, 2014). Plant populations of *Sesuvium portulacastrum* were distributed along the estuarine habitats of Godavari estuary (Umamaheswara Rao and Narasimha Rao, 1988; Narasimha Rao and Subba Rangaiah, 2010; Narasimha Rao, 2012; Prasanna Lakshmi, 2015), Andhra Pradesh, India. Traditionally, *S. portulacastrum*, is consumed by the native people as a salty, leafy vegetable. (Divya *et al.*, 2023). *S. portulacastrum* has a long history of use in folk medicine where, in Zimbabwe and South Africa use the plant to treat various infections and kidney problems (Rojas *et al.*, 1992; Magwa *et al.*, 2006, Lokhande *et al.*, 2011). In traditional medicine, it has been used for the treatment of fever, kidney disorders and scurvy, epilepsy, conjunctivitis, dermatitis, haematuria, leprosy and also used to cure toothache (Bandaranayake, 1998). The present study an attempt was made to evaluate the

antimicrobial activity of halophytic plant *S. portulacastrum* against some plant and human pathogens.

MATERIALS AND METHODS

Plant Material

Plant materials of *S. portulacastrum* (Fig.1) were collected during 2010 to 2011 from mangrove habitats of Coringa mangrove reserve forest near Kakinada, Andhra Pradesh. Plant materials were identified with the help of authentic specimens available in the Department of Botany, Andhra University, Visakhapatnam.

***Sesuvium portulacastrum* (L.)**

Test Microorganisms

The selected bacterial strains were obtained from Microbial Type Culture (MMTC) from Institute of Microbial Technology, Chandigarh, India. The microorganisms including bacteria and fungi were presented in the Table-1 and Table-2 respectively.

Table 1: Details of the bacterial strains used in bioassay

S. No	Name of the Bacterial Strains	MMTC Catalogue No.
1.	*Bacillus subtilis*	B 2274
2.	*Bacillus megaterium*	B 2444
3.	*Lactobacillus acidophilus*	B 5463
4.	*Escherichia coli*	B 9637
5.	*Enterobacter aerogenes*	B 2822
6.	*Enterobacter cloacae*	B 7982
7.	*Klebsiella pneumonia*	B 2405

Table 2: Details of the fungal stains used in bioassay

S. No	Name of the Fungal Strains	MMTC Catalogue No.
1.	*Candida albicans*	F 0227
2.	*Mucar racemosus*	F 7382
3.	*Rhizoctonia solani*	F 5642
4.	*Rhizopus stolonifer*	F 2591
5.	*Saccharomyces cerevisiae*	F 0174

Preparation of Plant Extracts

The epiphytes and other deposits are removed from the halophytic plant and then the specimens are shade dried. The shade dried plant materials were chopped into small pieces and coarsely powdered. The coarsely powdered material was weighed and extracted with hexane, chloroform, methanol and water in sequential order of polarity using a soxhlet extractor for five to six hours at temperature not exceeding the boiling point of the solvent. For each gram of dry material 2 ml of solvent was used. The extracted solvents were filtered though Whatman no-1 filter paper and subsequently concentrated under reduced pressure (in vacuo at 40^0C) using a rotary evaporator. The residue obtained was designated as crude extract and was stored in a freezer at -20^0 C until bioassayed. The plant extract residues obtained were redissolved in 0.1% Dimethyl Sulfoxide (DMSO) to get different concentrations (100 mg/ml, 300 mg/ml and 500 mg/ml) and filtration through a 0.45 µm membrane filter and stored in sterile brown bottles at 20^0 C until bioassayed.

In Vitro Antibacterial Activity Assays

The antimicrobial activity of the hexane, chloroform, methanol and water extracts of each sample was evaluated by using Agar Well Diffusion Method of

Murray *et al.*, (1995) modified by Olurinola, (1996). 20ml of sterilized agar medium (Nutrient Agar Media for bacteria and Potato Dextrose Agar or PDA for fungi) was dispensed into sterile universal bottles. These were then inoculated with 0.2 ml of bacterial cultures, media was mixed gently and poured into sterile petri dishes and it is allowed to solidify. Then the 4 uniform wells were made in each petri dish by using a sterilized number 3-cup borer (6mm diameter). The wells were filled with 50—µl of the extract concentration of 100mg/ml, 300mg/ml, 500mg/ml and control (DMSO) and allowed diffusion for 45 minutes. The plates were incubated at 37° C for 24 hours for bacteria and 25° C for 48 hours for fungi. The zones inhibition was measured with antibiotic Zone Scale in mm and the experiment was carried out in duplicates.

RESULTS & DISCUSSION

Hexane, chloroform, methanol and water extracts of *S. portulacstrum* leaves and stem exhibited the different degree of growth inhibition against tested bacterial and fungal strains in the present study. The data (values of Inhibition Zones (IZ)) pertaining to the antimicrobial potential of the leaves and stem of four solvents such as hexane, chloroform, methanol and water (100 mg/ml, 300 mg/ml and 500 mg/ml) presented in tables 3 and 4 respectively. In the present investigation there was a gradual increase in the zone of inhibition from 100 to 500mg/ml, with highest at 500 mg/ml concentration of plant extract. Hence only 500 mg/ml dosage level results were analyzed. Negative results do not indicate the absence of bioactive constituents, nor is that the plant inactive.

Antimicrobial Activity of *Sesuvium portulacastrum* Leaves

Fig. 2.1 represents the comparison among four solvents of *S. portulacastrum* leaves of 100mg/ml of hexane, chloroform, methanol and water extracts. Fig. 2.2 represents the comparison among four solvents of *S. portulacastrum* leaves of 300mg/ml of hexane, chloroform, methanol and water extracts. Fig. 2.3 represents the comparison among four solvents of *S. portulacastrum* leaves of 500mg/ml of hexane, chloroform, methanol and water extracts.

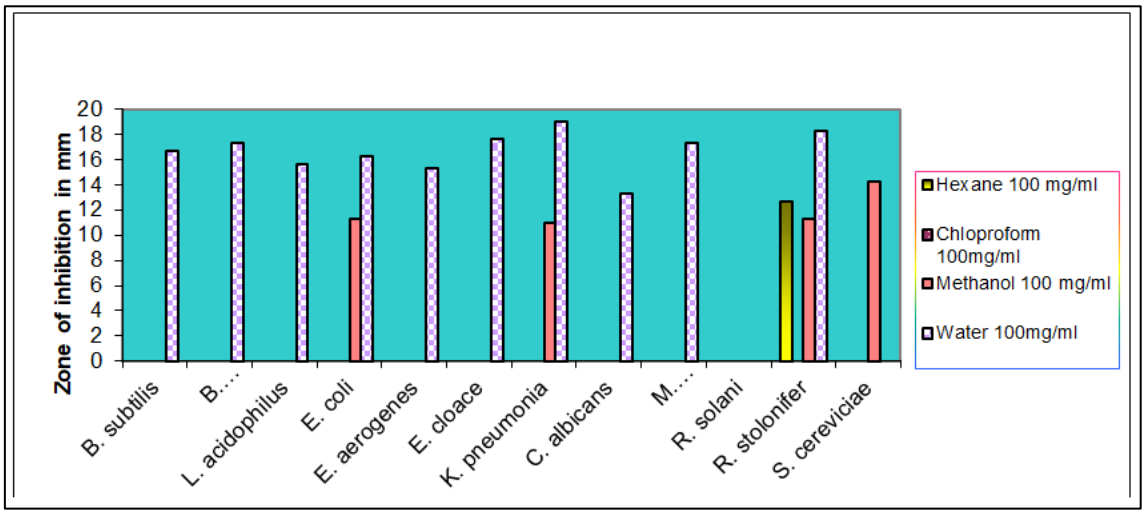

Fig 2.1: Antimicrobial Activity of *Sesuvium portulacastrum* leaves (100 mg/ml)

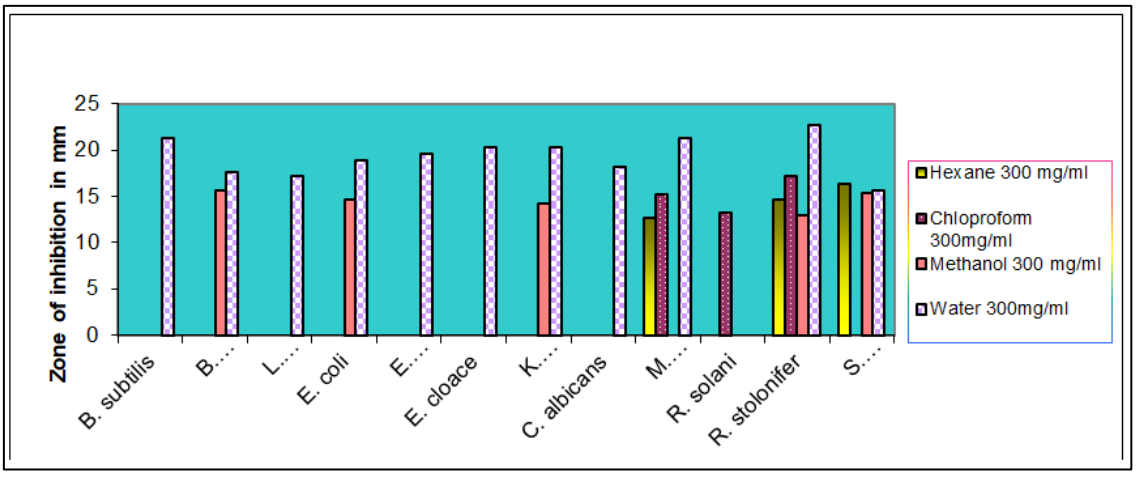

Fig 2.2 Antimicrobial Activity of *Sesuvium portulacastrum* leaves (300 mg/ml)

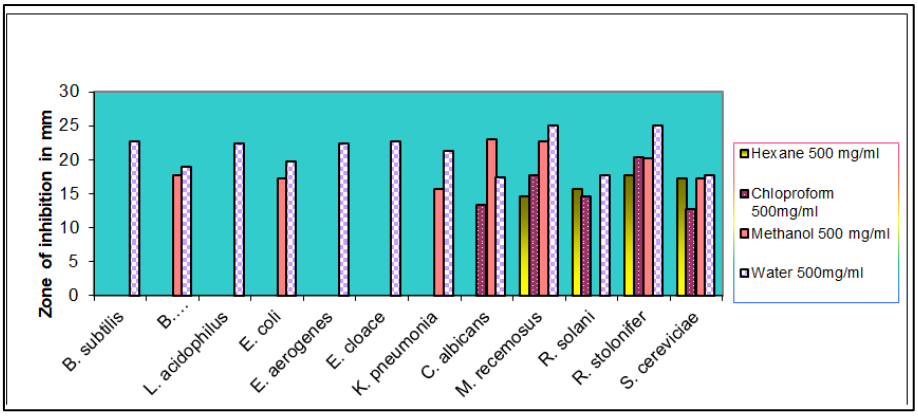

Fig 2.3 Antimicrobial Activity of *Sesuvium portulacastrum* leaves (500 mg/ml)

Hexane & Chloroform Extracts of 500 mg/ml

Moderate level of antimicrobial activity was found with the hexane and chloroform extracts of *S. portulacastrum* leaves, against fungal strains such as *R. stolonifer* followed by *S. cerevisiae* and *R. solani*. Absence of antibacterial activity observed against all bacterial strains and fungal strain such as *C. albicans* (Table-3 & Fig 2.3).

Methanol Extracts of 500 mg/ml

Highest level of antimicrobial activity was found with the methanol and water extracts of *S. portulacastrum* leaves, against fungal strains such as *C. albicans* (23 mm) followed by *M. recemosus* (22.7 mm). Absence of antibacterial activity found against bacterial strains such as *B. subtilis, L. acidophilus, E. aereogenes*, and *E. cloacae* and fungal strain *R. solani* resistant to this extract (Table-3 & Fig 2.3).

Water Extracts of 500 mg/ml

Highest level of antimicrobial activity was recorded for the water extracts of *S. portulacastrum* leaves, against bacterial strains such as *B. subtilis* (22.7 mm) and *E. cloacae* (22.7 mm) and fungal strains such as *M. recemosus* (25 mm) followed by *R. stolonifer* (25 mm). Moderate level of antimicrobial activity was found against bacterial strains such as *E. coli* (19.7 mm) followed by *B. megaterium* (19 mm) and fungal strains such as *R. solani* (17.7 mm) and *S. cerevisiae* (17.7 mm) (Table-3 & Fig 2.3).

The results in the present study are in accordance with the antimicrobial activity studies made by Magwa *et al.,* (2006) against *Acetobacter calcoacetica, Bacillus subtillis, Clostridium sporogenes, Clostridium perfringens, Escherichia coli, Salmonella typhii, Staphylococcus aureus* and *Yersinia enterocolitica*, and antifungal activity against *Candida albicans, Aspergillus niger, Aspergillus flavus* and *Penicillium notatum*. In another study Vadlapudi *et al*., (2009) obtained similar results with methanol extracts against *Acremonium strictum, Aspergillus niger, Candida albicans, Ervinia carotovara, Fusarium oxysporum, Pseudomonas marginales* and *Ustilago maydis.*

Antimicrobial Activity of *Sesuvium portulacastrum* Stem

Fig. 3.1 represents the comparison among four solvents of *S. portulacastrum* stem of 100mg/ml of hexane, chloroform, methanol and water extracts. Fig. 3.2 represents the comparison among four solvents of *S. portulacastrum* stem of 300mg/ml of hexane, chloroform, methanol and water extracts. Fig. 3.3 represents the comparison among four solvents of *S. portulacastrum* stem of 500mg/ml of hexane, chloroform, methanol and water extracts.

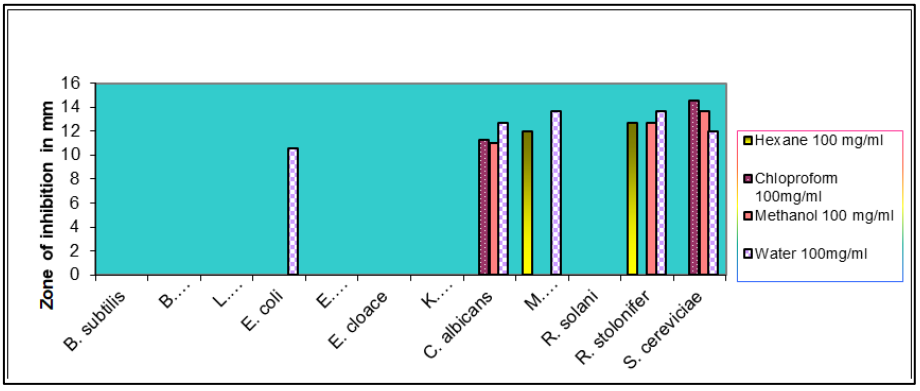

Fig 3.1 Antimicrobial Activity of *Sesuvium portulacastrum* Stem (100 mg/ml)

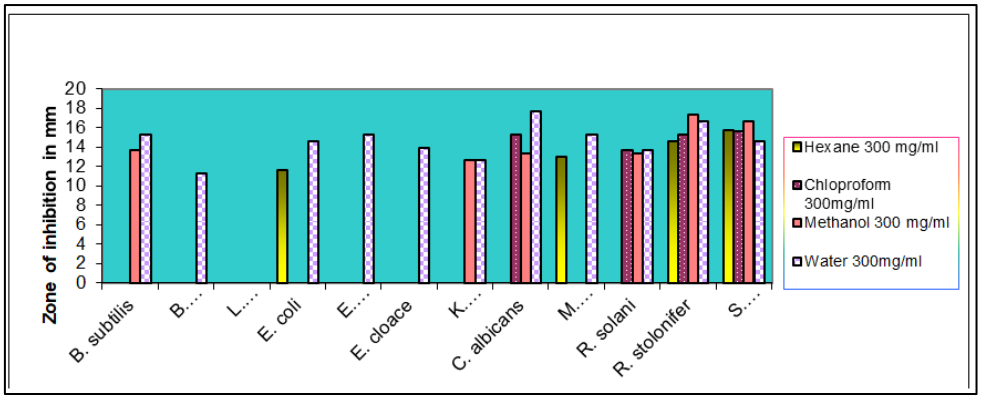

Fig 3.2 Antimicrobial Activity of *Sesuvium portulacastrum* Stem (300 mg/ml)

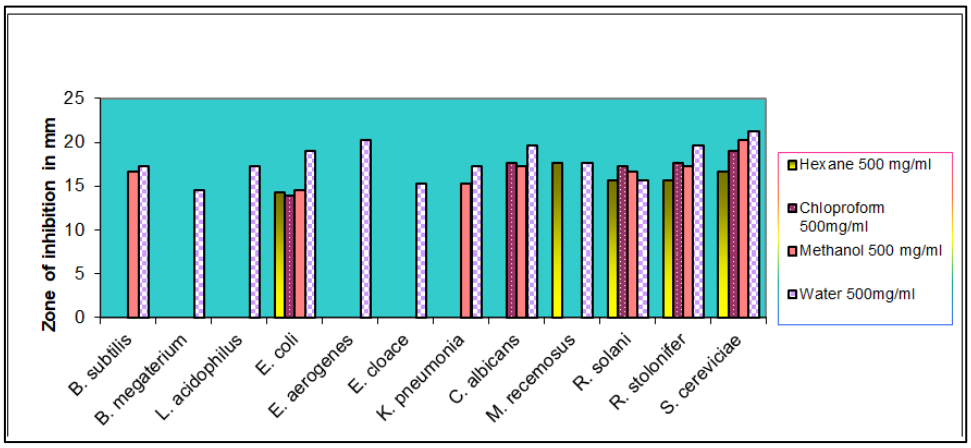

Fig 3.3 Antimicrobial Activity of *Sesuvium portulacastrum* Stem (500 mg/ml)

Hexane & Chloroform Extracts of 500 mg/ml

Moderate level of antimicrobial activity was found with the hexane and chloroform extracts of *S. portulacastrum* stem, against fungal strains such as *R. solani, R. stolonifer* and *S. cerevisia.* Less antibacterial activity observed against *E. coli* (14.3 mm). Absence of antibacterial activity found against *B. subtilis, B. megaterium, L. acidophilus, E. aereogenes, E. cloacae* and *K. pneumonia* (Table- 4 & Fig. 3.1).

Methanol Extracts of 500 mg/ml

Moderate level of antimicrobial activity was found with the methanol extracts of *S. portulacastrum* stem, against bacterial strain such as *B. subtilis* (16.7 mm) and fungal strain such as *S. cerevisiae* (20.3 mm). Less antibacterial activity observed against *E. coli* (15 mm) and *K. pneumonia* (15 mm). Absence of antibacterial activity found against *B. megaterium, L. acidophilus, E. aereogenes, E. cloacae* and fungal strain such as *M. recemosus* (Table- 4 & Fig. 3.1).

Water Extracts of 500 mg/ml

Highest level of antimicrobial activity was recorded for the water extracts of *S. portulacastrum* stem, against fungal strain such as *S. cerevisiae* (21.3 mm). Moderate level of antimicrobial activity was found against bacterial strains such as *E. aereogenes* (20.3 mm) followed by *E. coli* (19 mm) and *B. subtilis* (17.3 mm) and fungal strains such as *C. albicans* (19.7 mm) and *R. stolonifer* (19.7 mm). Less antibacterial activity observed against *B. megaterium* (14.6 mm) (Table- 4 & Fig. 3.1).

Similar studies were made by Al-Azzawi *et al*., (2012), reported antimicrobial screening of *S. portulacastrum* of ethanol, aqueous, dichloromethane for extraction. Among the solvents, ethanol was considered as best and showed good activity against *staphylococcus aureus* and *E. coli*. Another study by Lincy, *et al*., (2013) reported *in vitro* antibacterial activity of leaf of *S. portulacastrum* (petroleum ether, benzene, ethyl acetate, methanol, and ethanol extracts). They concluded that ethanol extract shows very good activity than the remaining tested solvents. Similarly, the methanol extract of the leaves exhibited more action against the bacterial strains, while the stem extract did not show any activity against *K. pneumonia* (Alshrari *et al*., 2020).

In the present study water extracts showed strong activity against bacterial and fungal organisms tested compared to hexane, chloroform and methanol extracts. More substances dissolve in water than any other liquid. For this reason, water is often called the universal solvent. The reason for water's excellent dissolving capability rates to its polarity.

Table 3: Antimicrobial activity of *Sesuvium portulacastrum* leaf extract in four different solvents- hexane, chloroform, methanol and water

Microorganisms	100mg/ml				300mg/ml				500 mg/ml				Standard
	H	C	M	W	H	C	M	W	H	C	M	W	
Bacillus subtilis **GP**	-	-	-	16.7± 1.2	-	-	-	21.3± 1.5	-	-	-	22.7± 1.2	32
Bacillus megaterium **GP**	-	-	-	17.3± 1.6	-	-	15.7± 0.6	17.7± 1.2	-	-	17.7± 1.2	19.0± 1.0	28
Lactobacillus acidophilus **GP**	-	-	-	15.7± 0.6	-	-	-	17.3± 1.6	-	-	-	22.3± 1.6	30
Escherichia coli **GN**	-	-	11.3± 0.6	16.3± 0.6	-	-	14.6± 0.6	19.0± 1.0	-	-	17.3± 1.6	19.7± 0.6	34
Enterobacter aerogenes **GN**	-	-	-	15.3± 1.2	-	-	-	19.7± 0.6	-	-	-	22.3± 1.6	31
Enterobacter cloacae **GN**	-	-	-	17.7± 1.2	-	-	-	20.3± 1.5	-	-	-	22.7± 1.2	32
Klebsiella pneumonia **GN**	-	-	11.0± 1.0	19.0± 1.0	-	-	14.3± 0.6	20.3± 1.5	-	-	15.7± 0.6	21.3± 1.5	31
Candida albicans **FS**	-	-	-	13.3± 0.6	-	-	-	18.3± 0.6	-	13.3± 0.6	23 ±1.0	17.3± 1.6	35
Mucar recemosus **FS**	-	-	-	17.3± 1.6	12.7± 1.2	15.3± 1.2	-	21.3± 1.5	14.6± 0.6	17.7± 1.2	22.7± 1.2	25.0± 2.0	32
Rhizoctonia solani **FS**	-	-	-	-	-	13.3± 0.6	-	-	15.7± 0.6	14.6± 0.6	-	17.7± 1.2	34
Rhizopus stolonifer **FS**	12.7± 1.2	-	11.3± 0.6	18.3± 0.6	14.6± 0.6	17.3± 1.6	13.0± 1.0	22.7± 1.2	17.7± 1.2	20.3± 1.5	20.3± 1.5	25.0± 1.0	30
Saccharomyces cerevisiae FS	-	-	14.3± 0.6	-	16.3± 0.6	-	15.3± 1.2	15.7± 0.6	17.3± 1.6	12.7± 1.2	17.3± 1.6	17.7± 1.2	29

Volume per well: 50µl; Borer size used: 6mm; **H**-Hexane, **C**-Chloroform, **M**-Methanol and **W**-Water.
GP = Gram positive; **GN** = Gram negative; **FS** = Fungal species and (-) indicates 'No inhibition'.
Diameter of zone of inhibition (mm) including disc diameter of 6mm: mean of three assays ± standard deviation.

Table 4: Antimicrobial activities of *Sesuvium portulacastrum* stem extract in four different solvents- hexane, chloroform, methanol and water

Microorganisms	100mg/ml				300mg/ml				500 mg/ml				Standard
	H	C	M	W	H	C	M	W	H	C	M	W	
Bacillus subtilis **GP**	-	-	11.3± 0.6	-	-	-	13.7± 0.6	15.3± 1.2	-	-	16.7± 1.2	17.3± 1.6	32
Bacillus megaterium **GP**	-	-	-	-	-	-	-	11.3± 0.6	-	-	-	14.6± 0.6	28
Lactobacillus acidophilus **GP**	-	-	-	-	-	-	-	-	-	-	-	17.3± 1.6	30
Escherichia coli **GN**	-	-	-	10.6± 0.6	11.7± 1.2	-	-	14.6± 0.6	14.3± 0.6	14.0± 1.0	14.6± 0.6	19.0± 1.0	34
Enterobacter aerogenes **GN**	-	-	-	-	-	-	-	15.3± 1.2	-	-	-	20.3± 1.5	31
Enterobacter cloacae **GN**	-	-	-	-	-	-	-	14.0± 1.0	-	-	-	15.3± 1.2	32

Microorganisms	100mg/ml				300mg/ml				500 mg/ml				Standard
	H	C	M	W	H	C	M	W	H	C	M	W	
Klebsiella pneumonia **GN**	-	-	-	-	-	-	12.7± 1.2	12.7± 1.2	-	-	15.3± 1.2	17.3± 1.6	31
Candida albicans **FS**	-	11.3± 0.6	11.0± 1.0	12.7± 1.2	-	15.3± 1.2	13.3± 0.6	17.7± 1.2	-	17.7± 1.2	17.3± 1.6	19.7± 0.6	35
Mucar recemosus **FS**	12.0± 1.0	-	-	13.7± 0.6	13.0± 0.0	-	-	15.3± 1.2	17.7± 1.2	-	-	17.7± 1.2	32
Rhizoctonia solani **FS**	-	-	-	-	-	13.7± 0.6	13.3± 0.6	13.7± 0.6	15.7± 0.6	17.3± 1.6	16.7± 1.2	15.7± 0.6	34
Rhizopus stolonifer **FS**	12.7± 1.2	-	12.7± 1.2	13.7± 1.2	14.6± 0.6	15.3± 1.2	17.3± 1.6	16.7± 1.2	15.7± 0.6	17.7± 1.2	17.3± 1.6	19.7± 0.6	30
Saccharomyces cerevisiae **FS**	-	14.6± 0.6	13.7± 0.6	12.0± 1.0	15.7± 0.6	15.7± 0.6	16.7± 1.2	14.6± 0.6	16.7± 1.2	19.0± 1.0	20.3± 1.5	21.3± 1.5	29

Volume per well: 50µl; Borer size used: 6mm; **H**-Hexane, **C**-Chloroform, **M**-Methanol and **W**-Water.
GP = Gram positive; **GN** = Gram negative; **FS** = Fungal species and (-) indicates 'No inhibition'.
Diameter of zone of inhibition (mm) including disc diameter of 6mm: mean of three assays ± standard deviation.

CONCLUSION

The results revealed that water extracts of *S. portulacastrum* plant extracts have greater potential compounds against microorganisms and that they can be used as novel antimicrobial agents. The variation of antimicrobial activity of present study might be due to distribution of antimicrobial substances, which varied from fraction to fraction of the crude extract. Further studies are needed to identify the pure component and establish the exact mechanism of action for antibacterial action of the plant extract.

ACKNOWLEDGEMENTS

We thank to the Head of the Department of Pharmaceutical Sciences, Andhra University for their kind cooperation and valuable support to carry out this work. K. Prasanna Lakshmi is grateful to UGC SAP for the grant of Junior Research Fellowship.

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