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Antimicrobial Screening of the Solvent Extracts of Halophytic Plant *Suaeda maritima* (L.) Demort. Against Selected Pathogens

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Abstract **Original Research Article**

The antimicrobial activity of leaf and stem extracts of halophytic plant *Suaeda maritima* (L.) Demort. against, some plant and human pathogens. Plant parts of *S. maritima* were collected from the mangrove habitats of Pandi and Pora regions of Godavari estuary, near Amalapuram, Andhra Pradesh, India. Plant parts are dried and extracts were obtained successfully with hexane, chloroform, methanol and water, using Soxhlet extraction apparatus. The antimicrobial activity of the plant extracts on various test organisms including multiple antibiotic resistant bacteria were investigated by Well Diffusion Method under *in vitro* conditions. The plant extracts have shown promising antimicrobial activity against all tested organisms. Among the four extracts, water extract of *S. maritima* showed appreciable antimicrobial activity against all bacterial and fungal strains. It reveals that halophytic plant has antimicrobial compounds which can act against microorganisms and they can also be used in the treatment of infectious diseases caused by resistant pathogenic microorganisms.

Keywords: Halophytes**,** Antimicrobial Activity, Well Diffusion Method, *In vitro* Screening, *Suaeda maritima*.

INTRODUCTION

Halophytes have long been used for medicinal purposes [Banerjee *et al.*, 2008; Agoramoorthy *et al.*, 2008; Muthazhagan *et al.*, 2014; Ferreira, *et al.*, 2022]. In recent decades, there has been a resurgence of interest in the utilization of halophytes for medicinal purposes and the screening of halophytes for antimicrobial compounds [Alanis, 2005; Meot- Duros, 2008; Ksouri *et al.*, 2013; Buhmann and Papenbrock, 2013; Faustino *et al.*, 2019]. Antimicrobial activity is associated with secondary metabolites, such as phenolic acids, flavonoids and tannins. Halophytes possess alkaloids, phenols, steroids, terpenoids, tannins, etc. [Combs and Anderson, 1949; Patra and Thatoi, 2011; Qadir *et al.*, 2017]. The main function of an antimicrobial agent is to inhibit growth or kill the microorganisms [Laxminarayan *et al.*, 2013].

Suaeda maritima (L.) Demort. is an annual herb belongs to the family Chenopodiaceae and adapted to estuarine soil and lives in salt marshes or estuarine habitats. The plant is distributed throughout the east and west coast of India including Godavari estuary, Kakinada, Andhra Pradesh [Umamaheswara Rao and Narasimha Rao, 1988; Prasanna Lakshmi, 2015]. Polyphenols from *S. maritima* cures hepatitis and it has antiviral, hepato-protective, anti- inflammatory and antioxidant activities [Magwa *et al.*, 2006; Patra and Thatoi, 2011; Ravikumar *et al.*, 2011]. The leaves of *S. maritima* are known for curing liver, heart, and lipid disorders [Bandaranayake, 1998]. Investigations revealed that the plant contain triterpenoid, sterols, alkaloids, acids, glycosides (Krishchenko *et al.*,1984; Kapadia *et al.*, 1985; Miftakhova *et al.*, 1999), triterpenoid saponins [Segal *et al.*, 1969; Brutko *et al.*, 1968], coumarins [Rizk *et al.*, 1985], alkaloids [Sadykov *et al.*, 1978] and Alpha amyrin, [Ghosh *et al.*, 1985]. α and β amyrins are two structural isomers possessing a wide spectrum of pharmaceutical and biological functions like anti-microbial, insecticidal [Bandeira *et al.*, 2006; Ekalu *et al.*, 2019], anti- arthritic, anti-inflamatory, anti-nociceptive, anti- depressant, anti-hyperglycemic [Siani *et al.*,1999; Oliveira *et al.*, 2005; Holanda *et al.*, 2008; Barros *et al.*, 2011; Santos *et al.*, 2012; Aragao *et al.*, 2015; Carvalho *et al.*,2017; Pinto *et al.*, 2017], anti-ulcer and gastroprotective activities [Prabhakar *et al.*, 2017]. In this present study an attempt has been made to evaluate the antimicrobial activity of leaf and stem extracts of halophytic plant *Suaeda maritima* against some pathogenic microbes.

MATERIALS AND METHODS

Plant Material

Plant materials of *Suaeda maritima* (Fig.1) were collected during 2010 to 2011 from mangrove habitats of Pandi and Pora regions of Godavari estuary, near Amalapuram, Andhra Pradesh, India. Plant materials were identified with the help of authentic specimens available in the Department of Botany, Andhra University, Visakhapatnam.

Test Microorganisms

The selected bacterial strains were obtained from Microbial Type Culture (MMTC) from Institute of Microbial Technology, Chandigarh, India. The microorganisms (including fungi and bacteria) selected were *Bacillus subtilis* [B 2274], *Bacillus megaterium* [B 2444], *Lactobacillus acidophilus* [B 5463], *Escherichia coli* [B 9637], *Enterobacter aerogenes* [B 2822], *Enterobacter cloace* [B 7982], *Klebsiella pneumonia* [B 2405], *Candida albicans* [0227], *Mucar recemosus* [7382], *Rhizoctonia solani* [5642], *Rhizopus stolonifer* [2591] and *Saccharomyces cerevisiae* [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 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 and 500mg/ml and allow diffusion for 45 minutes. The plates were incubated at 37º C for 24 hours for bacteria and 25º 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. maritima* 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 1, 2 and figures 2 and 3 respectively. In the present investigation, the highest mean zones of inhibition were recorded at the concentration of 500 mg/ml. 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 with water as solvent. Negative results do not indicate the absence of bioactive constituents, nor is that the plant inactive.

Antimicrobial Activity of *Suaeda Maritima* Leaves Hexane Extracts of 500 mg/ml

Highest level of antimicrobial activity was found with the hexane extracts of *S. maritima* leaves, against bacterial strains such as *E. cloacae* (22.7 mm) followed by *K. pneumonia* (22.3 mm) whereas fungal strains such as *R. stolonifer* (24.3 mm) followed by *S. cerevisiae* (22.7 mm) and *M. recemosus* (21.3 mm). Moderate level of antimicrobial activity observed against fungal strains such as *C. albicans* (17.7 mm) and *R. solani* (16.7 mm). Less antibacterial activity observed against *B. subtilis* (14.6 mm) followed by *B. megaterium* (12.7 mm) (Table-1).

Chloroform Extracts of 500 mg/ml

Highest level of antimicrobial activity was recorded for the chloroform extracts of *S. maritima* leaves, against bacterial strains such as *E. aereogenes* (22.3 mm) whereas fungal strains such as *R. stolonifer* (24 mm). Moderate level of antimicrobial activity observed against bacterial strain such as *E.coli* (19.7 mm) whereas fungal strains such as *C. albicans* (20.3 mm). Less antibacterial activity observed against *E. cloacae* (15.3 mm) and *B. megaterium* (14 mm). Absence of antibacterial activity found against bacterial strain such as *K. pneumonia* whereas fungal strain such as *M. recemosus* (Table-1).

Methanol Extracts of 500 mg/ml

Highest level of antimicrobial activity was found the methanol extracts of *S. maritima* leaves, against bacterial strains such as *K. pneumonia* (21.3 mm) and *B. megaterium* (19 mm) whereas fungal strains such as *M. recemosus* (22.7 mm). Moderate level of antimicrobial activity observed against bacterial strain such as *E. aerogenes* (18.7 mm) whereas fungal strains such as *C. albicans* (19 mm) and *R. solani* (17.7 mm).

Absence of antibacterial activity observed against *E. coli* and *B. cloacae* (Table-1).

Water Extracts of 500 mg/ml

Highest level of antimicrobial activity was recorded for the water extracts of *S. maritima* leaves, against bacterial strains *B. megaterium* (26.7 mm) followed by *E. coli* (25.3 mm) whereas fungal strains such as *S. cerevisiae* (24.3 mm), *M. recemosus* (22.3 mm), *R. solani* (22.7 mm) and *C. albicans* (19 mm). Moderate level of antimicrobial activity was found against fungal strain such as *R. stolonifer* (20.3 mm) (Table-1 and Fig. 2)

The results in the present study are in accordance with the antimicrobial activity studies made by Bilal and Hossain [2019] against several pathogenic Gram-positive and Gram-negative bacterial strains and Patra *et al.*, [2011] obtained similar results by methanol and ethanol extracts of *S. maritima* against 10 bacterial strains.

Antimicrobial Activity of *Suaeda Maritima* Stem

Hexane Extracts of 500 mg/ml

Highest level of antimicrobial activity was found with the hexane extracts of *S. maritima* stem, against bacterial strains such as *L. acidophilus* (22.7 mm) followed by *B. subtilis* (22.3 mm) and *E. aereogenes* (22.3 mm) whereas fungal strain such as *R. stolonifer* (20.7 mm). Moderate level of antimicrobial activity observed against bacterial strains such as *B. megaterium* (18.7 mm) followed by *E. cloacae* (18.7 mm) and *K. pneumonia* (18.7 mm) whereas fungal strain such as *C. albicans* (19.7 mm), *R. solani* (18.3 mm), *M. recemosus* (16.7 mm) and *S. cerevisiae* (17 mm) (Table-2).

Chloroform Extracts of 500 mg/ml

Highest level of antimicrobial activity was recorded for the chloroform extracts of *S. maritima* stem, against bacterial strains such as *E. cloacae* (22.7 mm) and *B. subtilis* (20.7 mm) whereas fungal strain such as *R. stolonifer* (21.3 mm). Moderate level of antimicrobial activity observed against bacterial strains such as *B. megaterium* (20 mm) followed by *K. pneumonia* (20.3 mm) and *L. acidophilus* (19.7 mm) whereas fungal strains such as *C. albicans* (18.7 mm) followed by *S. cerevisiae* (17.7 mm) and *R. solani* (17 mm) (Table-2).

Methanol Extracts of 500 mg/ml

Highest level of antimicrobial activity was found with the methanol extracts of *S. maritima* stem, against bacterial strains such as *L. acidophilus* (21.3 mm) and*B. subtilis* (21 mm). Moderate level of antimicrobial activity observed against bacterial strains such *B. megaterium* (20.3 mm) followed by *K. pneumonia* (19.7 mm) and *E. cloacae* (19 mm) whereas fungal strains such as *R. solani* (18.3 mm) followed by *C. albicans* (16.7 mm) and *S. cerevisiae* (16.3 mm). No activity observed in fungal strain such as *R. stolonifer* (Table-2).

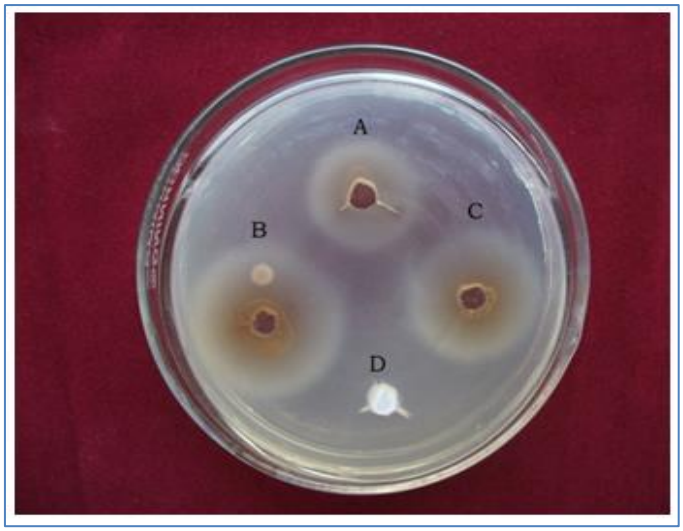
Water Extracts of 500 mg/ml

Highest level of antimicrobial activity was recorded for the water extracts of *S. maritima* stem, against bacterial strains such as *E. aereogenes* (25.3 mm) followed by *B. megaterium* (23.7 mm) and *E. coli* (24.3 mm) whereas fungal strains such as *R. stolonifer* (22.3 mm) followed by *S. cerevisiae* (22.7 mm) and *C. albicans* (22.3 mm). Moderate level of antimicrobial activity was found against bacterial strain such as *E. cloacae* (18.7 mm) (Table-2 and Fig. 3)

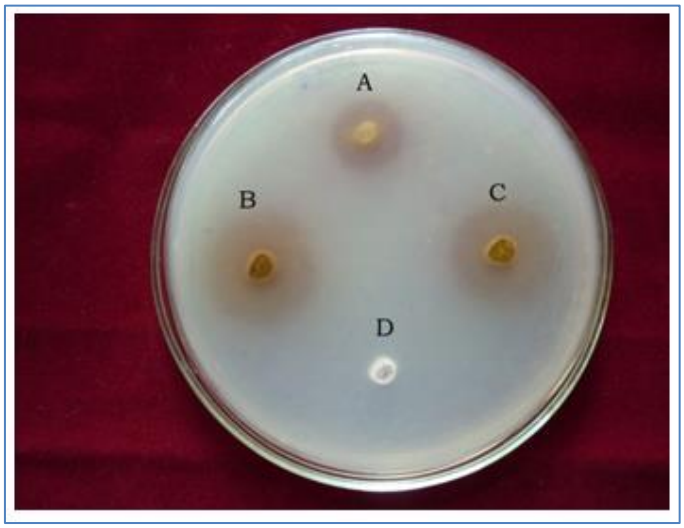
Similarly the studies made by Beulah *et al.*, [2021] on four extracts (hexane, diethyl ether, ethanol, and water) of *Suaeda maritima* leaves, among them ethanolic and water extracts showed highest antibacterial activity against *Staphylococcus. aureus*, *B. subtilis*, *K. pneumonia*, and *Pseudomonas aeruginosa* and another study by Nayak *et al.*, (2018) proved the n-hexane extract of the *S. maritima* showed highest antimicrobial activity against gram positive, gram negative bacteria and fungi. Identical results were obtained by Prasanna Lakshmi and Narasimha Rao, [2013] in their studies on *S. monoica* against Human and Plant Pathogens.

The above results revealed that water extracts of *S. maritima* 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.

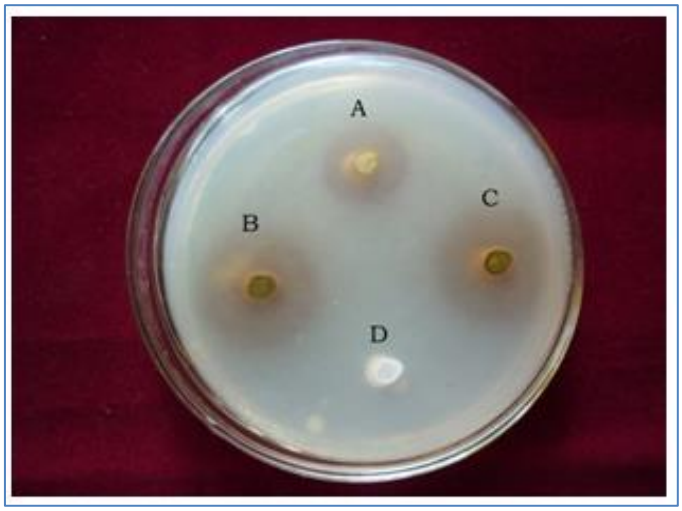
Fig. 1: *Suaeda maritima* L (Dumort)



A. Bacillus subtilis

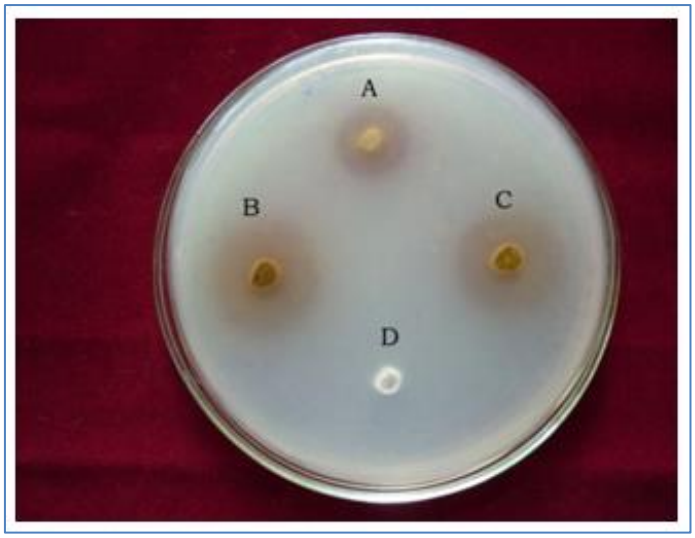


B. Candida albicans

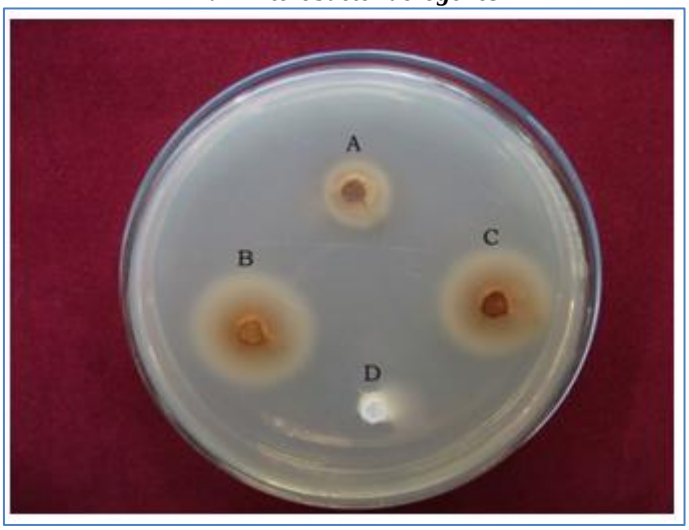


C. Rhizopus stolonifer

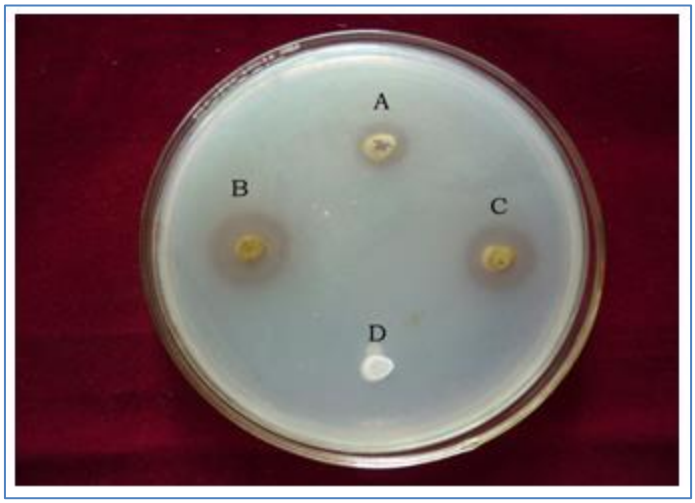
Fig. 2: Zone formation of the water extracts of S. *maritima* Leaves against the microbes A. *Bacillus subtilis, B. Candida albicans and C. Rhizopus stolonifer*



A. Enterobacter aerogenes



***B.* Saccharomyces cerevisiae**



C. Enterobacter cloacae

Fig. 3: Zone formation of the water extracts of *S. maritima* stem against the microbes A. *Enterobacter aerogenes*, *B. Saccharomyces cerevisiae* and *C. Enterobacter cloacae*

Table 1: Antimicrobial activity of *Suaeda maritima* leaf extract in four different solvents- hexane, chloroform, methanol and water

Microorganisms	100mg/ml				300mg/ml				500 mg/ml				Stan dard
	H	C	M	W	H	C	M	W	H	C	M	W	
Bacillus subtilis GP	14.6 ±0.6	11.3± 0.6	12.0± 1.0	14.0± 1.0	13.7± 0.6	17.7± 1.2	15.7± 0.6	19.7± 0.6	14.6± 0.6	19.0± 1.0	17.7 ±1.2	22.7± 1.2	32
Bacillus megaterium GP	10.0 ±1.0	11.7± 1.2	13.7± 1.2	16.7± 1.2	11.7± 1.2	15.3± 1.2	17.7± 1.2	21.3± 1.5	12.7± 1.2	13.7± 0.6	19.0 ±1.0	26.7± 1.5	28
Lactobacillus acidophilus GP	13.7 ±0.6	13.0± 1.0	-	15.7± 0.6	14.6± 0.6	16.3± 0.6	15.3± 1.6	18.7± 0.6	21.3± 1.5	18.3± 1.6	16.7 ±1.2	21.3± 1.5	30
Escherichia coli GN	14.0 ±1.0	14.6± 0.6	-	19.0± 1.0	17.7± 1.2	18.0± 1.0	-	20.3± 1.5	19.0± 1.0	19.7± 0.6	-	25.3± 1.5	34
Enterobacter aerogenes GN	15.3 ±1.2	15.7± 0.6	13.3± 0.6	15.7± 0.6	17.3± 1.6	17.7± 1.2	15.7± 0.6	20.3± 1.5	21 ±1.0	22.3± 1.6	18.7 ±0.6	22.7± 1.2	31
Enterobacter cloacae GN	13.7 ±0.6	10.0± 1.0	-	16.7± 1.2	19.0± 1.0	15.3± 1.2	-	18.3± 1.6	22.7± 1.2	15.3± 1.2	-	22.3± 1.6	32
Klebsiella pneumonia GN	12.7 ±1.2	-	12.7± 1.2	14.6± 0.6	17.7± 1.2	-	17.7± 1.2	18.7± 0.6	22.3± 1.6	-	21.3 ±1.5	22.7± 1.2	31
Candida albicans FS	12.0 ±1.0	11.7± 1.2	12.0± 1.0	16.3± 0.6	15.7± 0.6	14.6± 0.6	15.7± 0.6	17.7± 1.2	17.7± 1.2	20.3± 1.5	19.0 ±1.0	19.0± 1.0	35
Mucar recemosus FS	12.7 ±1.2	-	16.7± 1.2	16.7± 1.2	16.7± 1.2	-	17.0± 1.0	19.0± 1.0	21.3± 1.5	-	22.7 ±1.2	22.3± 1.6	32
Rhizoctonia solani FS	13.3 ±0.6	14.6± 0.6	13.3± 0.6	15.7± 0.6	14.6± 0.6	15.7± 0.6	15.3± 1.2	18.3± 1.6	16.7± 1.2	18.7± 0.6	17.7 ±1.2	22.7± 1.2	34
Rhizopus stolonifer FS	17.7 ±1.2	14.0± 1.0	13.7± 0.6	16.7± 1.2	18.7± 0.6	17.7± 1.2	16.7± 1.2	17.3± 1.6	24.3± 1.5	24.0± 1.0	19.0 ±1.0	20.3± 1.5	30
Saccharomyces cerevisiae FS	13.7 ±0.6	15.3± 1.2	13.7± 1.2	20.3± 1.5	17.7± 1.2	19.0± 1.0	16.7± 1.2	22.3± 1.6	22.7± 1.2	22.7± 1.2	21.3 ±1.5	24.3± 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

Table 2: Antimicrobial activities of *Suaeda maritima* stem extract in four different solvents- hexane, chloroform, methanol and water

Microorganisms	100mg/ml				300mg/ml				500 mg/ml				Stan dard
	H	C	M	W	H	C	M	W	H	C	M	W	
Bacillus subtilis GP	15.3± 1.2	16.3± 0.6	-	18.3± 1.6	16.7± 1.2	18.0± 1.0	18.3± 1.6	18.7± 0.6	22.3± 1.6	20.7± 0.6	21±1. 0	21.0± 1.5	32
Bacillus megaterium GP	-	13.7± 0.6	17.0 ±1.0	19.0± 1.0	17.0± 1.0	15.7± 0.6	17.7± 1.2	21.3± 1.5	18.7± 0.6	20 ±1.0	20.3± 1.5	23.7± 1.2	28
Lactobacillus acidophilus GP	-	16.7± 1.2	-	17.7± 1.2	16.7± 1.2	18.3± 1.6	18.0± 1.0	19.7± 0.6	22.7± 1.2	19.7± 0.6	21.3± 1.5	22.7± 1.2	30
Escherichia coli GN	14.0± 1.0	13.3± 0.6	-	18.3± 1.6	-	16.6± 0.6	16.7± 1.2	20.3± 1.5	21.3± 1.5	18.3± 1.6	18.7± 0.6	24.3± 1.5	34
Enterobacter aerogenes GN	-	-	15.7 ±0.6	18.0± 1.0	17.7± 1.2	15.3± 1.2	17.0± 1.0	19.7± 0.6	22.3± 1.6	19.0± 1.0	18.7± 0.6	25.3± 1.5	31
Enterobacter cloacae GN	15.7± 0.6	12.7± 1.2	-	15.7± 0.6	16.3± 0.6	17.0± 1.0	16.7± 1.2	17.0± 1.0	18.7± 0.6	22.7± 1.2	19.0± 1.0	18.7± 0.6	32
Klebsiella pneumonia GN	-	14.6± 0.6	-	16.7± 1.2	18.7± 0.6	15.3± 1.2	16.3± 0.6	18.3± 1.6	18.7± 0.6	20.3± 1.5	19.7± 0.6	21 ±1.0	31
Candida albicans FS	-	13.3± 0.6	-	18.7± 0.6	16.0± 1.0	15.3± 1.2	-	20 ±1.0	19.7± 1.2	18.7± 0.6	16.7± 1.2	22.3± 1.6	35
Mucar recemosus FS	-	-	-	18.7± 0.6	-	-	13.7± 1.2	20.3± 1.5	16.7± 1.2	16.3± 0.6	15.7± 0.6	21.3± 1.5	32
Rhizoctonia solani FS	-	14.6± 0.6	-	15.7± 0.6	-	14.6± 0.6	13.7± 0.6	18.7± 0.6	18.3± 1.6	17.0± 1.0	18.3± 1.6	20.7± 0.6	34
Rhizopus stolonifer FS	-	-	-	16.7± 1.2	-	15.0± 1.0	-	9.0 ±1.0	20.7± 0.6	21.3± 1.5	-	22.3± 1.6	30
Saccharomyces cerevisiae FS	-	13.7± 0.6	14.0 ±1.0	19.0± 1.0	15.3± 1.2	15.7± 0.6	14.6± 0.6	21 ±1.0	17.0± 1.0	17.7± 1.2	16.3± 0.6	22.7± 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

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