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Assessment of Phytochemical Analysis and Antibacterial Potential of Selected Halophytic Plants from Thoothukudi, Tamilnadu, India

M. Muthu Sheeba[1*], G. Adaikala Raj [2]and D. Sarnya[3]

[1*]Assistant Professor of Botany, Department of Botany, Kamaraj College, Thoothukudi-628003.
[2]Assistant Professor of Botany, Department of Rural Development Science, Arul Anandar College (Autonomous), Karumathur, Madurai-625514.
[3]Assistant Professor of Botany, Department of Botany, Immaculate College for Women, Chinnakanganankupam, Cudalore-607 006.

***Corresponding Author:** Dr. M. Muthu Sheeba
*****Assistant Professorof Botany, Kamaraj College, Thoothukudi-628003.Tamil Nadu, India.Mobile: +91 9500402878

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Halophytic plants, Antibacterial activity, Disc Diffusion, MIC, MBC, FT-IR.

ABSTRACT:
The current study aimed to investigate the phytochemical contents and antibacterial activities of four halophytic plants, namely, *Salicornia brachiata* Roxb., *Suaeda maritima* (L.) Dumon. and *Sesuvium portulacastrum* (L.) was against *Bacillus subtilis*,*Streptococcus pyogenes, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, P. vulgaris, Pseudomonas aeruginosa, Salmonella typhimurium, Shigella flexneri* and *Vibrio cholerae.* The extent of inhibitory zone, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined. The ethanol extract of *Suaeda maritima* showed the highest antibacterial activity against all the bacterial strains tested than the other extracts. The mean zones of inhibition produced by the extracts in agar diffusion assays against the tested bacterial strains ranged from 7.0 to 20.5 mm. The MIC was between 62.5 µg/ml and 500 µg/ml, while the MBC were between 125 µg/ml and 1000 µg/ml. The phytochemical analysis of ethanolic extracts of *S. brachiata, S. maritima* and *S. portulacastrum* had showed the presence of alkaloids, terpenoids, flavonoids, glycosides, tannins, phenolic compounds and steroids. The highest mean of zone inhibition (20.5 mm) was observed in ethanol extract of *S. maritima against B. subtilis*. Finally it can be concluded that the suggest that the ethanol extract of *S. maritima* can be used as an antibacterial substance for the treatment of bacterial infections

Introduction

Salinity is one of the leading problems in coastal areas due to the impact of natural and anthropogenic factors that strongly influence the distribution of plants in certain regions. Approximately 400 million hectares of land are currently affected by salinity, whereas 20% of the arable land in the world and half of the irrigated soils are exposed to increased salt concentrations in the substrate. Most plant species do not have the ability to tolerate high concentrations of salt in the substrate, and consequently they cannot grow on saline habitats. However, plants known as salt tolerant the halophytes have developed various mechanisms to cope with salinity stress (Dajić, 2006; Aslam et al., 2011).

Halophytes are plant species native to habitats with increased concentration of salt in soil. They can either be separated into inland, coastal, or near coastal species depending on the type of habitat they occupy, i.e., upon the proximity to the open sea. There are more than 2500 halophyte species with different mechanisms of adaptation to saline habitats. Some plant families such as Amaranthaceae, Plumbaginaceae, Poaceae, and Asteraceae contain a significant number of salt tolerant species, used as foods, medicinal plants, ornamentals, fodder, fuels, source of fiber, highly nutritious oilseeds, biomass, etc. (Lukovic et al., 2021; Stankovic et al., 2021). In rural areas, medicinal plants have a significant biological activity that has been shown to treat a wide range of illnesses and bacterial infections. Additionally, in both in vitro and in vivo conditions, their active metabolites exhibit antimicrobial, antiviral, antiproliferative, and antioxidant action (Dagar and Singh, 2007).

Journal of Chemical Health Risks

www.jchr.org

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Several studies have reported halophytes' chemistry and biological activities. It has been conclusively established that these plants are high-potential sources of various phenolic and flavonoid compounds and have markedly exhibited antioxidant, anticancer, and antimicrobial activities (Mohammed et al., 2021; Ali et al., 2019; Ikuta et al., 2022; Wagenlehner et al., 2022; Alnuqaydan and Rah, 2022; Mohammed et al., 2021). The phenolics and flavonoids are considered chemical constituents exhibiting antimicrobial and antibiofilm properties, which make them attractive candidates for new drug discovery for treating infections (Alhomaidan, et al., 2021). To this effect, various studies have been carried out on the antioxidant, antibacterial, and antibiofilm activities of different halophytic plants and their phenolics and flavonoid phytochemicals extracted from halophytic plant sources(Mohammed et al., 2021; Ali et al., 2019; Ikuta et al., 2022; Wagenlehner et al., 2022; Alnuqaydan and Rah, 2022; Mohammed et al., 2021). This attention to the antimicrobial agent's discovery is embedded in global concern for the high mortality rate caused by various infections (Alhomaidan, et al., 2021; Ahmed et al., 2019). Where in antibiotic resistance is a significant barrier to effective microbial control globally (Giaouris et al., 2020).

The antimicrobial potential of several halophytes was reviewed by Giordano et al. (2021) reported that the the availability of certain phytoconstituents such as: phenols and fatty acids, with potential antimicrobial activity, in these salt-tolerant plants.*S. portulacastrum* has been utilize for the treatment of epilepsy, conjunctivitis, dermatitis, haematuria, leprosy and purgative, toothache and also as antimicrobial agent. Extract of this plant and the essential oil from the fresh leaves of *S. portulacastrum* showed antibacterial, antifungal as well as antioxidant activity. The ethanolic extract of the medicinal plant *S. portulacastrum* showed potential against the causative agents and pathogens related to various gastrointestinal disorders leading to indigestion, dysentery, and diarrhoea. Moreover the ethanolic extract of the medicinal plant *S. portulacastrum* showed potential against the causative agents of nosocomial infections, *S. aureus* and *E. coli.* Several species of *Salicornia* possess antibacterial and antihypertensive properties and are quoted in folk medicine for relief of toothache and chronic rheumatism (Rizk, 1986), constipation, obesity, diabetes and cancer.(Deepa et. al., 2013).Phytochemical studies on plant of *S. maritima* showed the presence of alkaloid, flavonoid, sterols, phenolic compounds, and tannins (Singh et.al., 2013). As it is essential to find out antibacterial activity from natural products to save the humans, the present study was made to phytochemical analysis and screens certain salt marsh halophytic species for antibacterial activity.

Materials and methods

Collection sample

Halophytes of *Salicornia brachiata* Roxb., *Suaeda maritima* (L.) Dumon. and *Sesuvium portulacastrum* (L.) were collected from Tuticorin at (Lat. 8.7563°N; Long. 78. 1791° E) Tuticorin District, the Gulf of Mannar Marine biosphere, Tamilnadu, India. The collections were made during November, 2022. The halophytes were identified by K. Sivakumar, Professor, Department of Plant science, and Madurai Kamaraj University.

Preparation of crude extracts

The halophytic plants samples were handpicked during low tide and manually cleaned from sand, epiphytes and animal waste. Then the samples were rinsed with seawater to remove associated debris, planktons and loosely attached microorganisms. Morphologically distinct thallus of halophytes was placed separately in new polystyrene bags and were kept in an ice box containing slush ice and transported to laboratory. Further, the material was washed thoroughly with tap water to remove the salt on the surface of the samples and the water was drained off from the halophytes and spread on the blotting paper to remove the excess water.

The shade dried samples were again cleaned with sterile distilled water to remove the remaining salt on the surface of the samples to avoid pumping of the solvents during the extraction process. The halophytes samples were shade dried followed by oven drying at 50 °C for an hour and milled in an electrical blender.Five hundred grams of finely ground halophytes powder material were packed in Whatman filter paper. The powdered samples were extracted with different organic solvents in a Soxhlet apparatus for 72 hours with ethanol. The extracts were concentrated to solvents free by evaporation in a rotary vacuum evaporator (Heidolph, Germany) at a temperature less than 40 °C. The crude extracts thus obtained were kept at 4 °C for further analysis.

Phytochemical screening

The ethanol extracts of *S, brachiata,S. maritima* and *S. portulacastrum* were subjected to qualitative phytochemical studies. Phytochemicals like alkaloids, terpenoids, flavonoids, glycosides, anthraquinones tannins, phenolic compounds, steroids and catechin were carried out according to the method (Harborne, 1973; Trease and Evans, 1983).

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(4s), 387-395 | ISSN:2251-6727

Collection of bacterial strains

The Standard Bacterial strains *viz., Bacillus subtilis*(MTCC 441), *Streptococcus pyogenes* (MTCC 442), *Escherichia coli* (MTCC 443), *Klebsiella pneumoniae* (MTCC 109), *Pseudomonas aeruginosa* (MTCC 741), *Proteus mirabilis* (MTCC 425), *Proteus vulgaris* (MTCC 426), *Salmonella typhimurium* (MTCC 98), *Shigella flexneri* (MTCC 1457) and *Vibrio cholerae* (MTCC 3906) were procured from Microbial Type Culture Collection (MTCC), Chandigarh. *In vitro* antibacterial activity was determined by using Mueller Hinton Agar (MHA) and Mueller Hinton Broth (MHB) was obtained from Himedia, Mumbai.

Disc Diffusion Method

The antibacterial activity of crude extracts of *S. brachiata,S. maritima* and *S. portulacastrum*was determined by disc diffusion method according to Bauer et al. (1966) with modifications. Petri dishes were prepared by pouring 20 ml of Mueller Hinton Agar. Then the plates were allowed to solidify and used in susceptibility test. The standardized inoculum using bacterial suspensions containing 10^8 colony forming units (CFU) per mL, were swabbed on the top of the solidified media and allowed to dry for 10 minutes. The extracts was dissolved in 10 per cent Dimethyl sulfoxide (DMSO) and under aseptic conditions sterile discs were impregnated with 20 µl of three different concentrations of the crude extracts (500, 250 and 125 µg/disc). The discs with extracts were placed on the surface of the medium with sterile forceps and gently pressed to ensure contact with inoculated agar surface. Ciprofloxacin (10 µg/disc) was used as positive antibacterial control and 10 per cent DMSO was used as blind control in all the assays. Finally, the inoculated plates were incubated at 37 °C for 24 h for all the bacterial strains tested. The zones of inhibitions were observed and measured in millimeters. The assay in this experiment was repeated three times.

Microdilution broth assay

Determination of theMinimum Inhibitory Concentration (MIC)

The MIC was determined for the crude extracts of *S. brachiata, S. maritima* and *S.portulacastrum* were determined in MHB by using a modified reaszurin microtitre plate assay was carried out according to methods of Sarker *et al.*(2007). 50 µl of Sterile MHB were transferred in to each well of a sterile 96-well micro titer plate. The halophytic plant extracts were dissolved in 10 per cent DMSO to obtain 2000 µg/ml stock solutions. A volume of 50 µl of crude extracts stock solution was added into the first well. After fine mixing of the crude extracts and 50 µl of the broth solution was transferred to the second well and in this way, the serial dilution procedure was continued to a twofold dilution to obtain concentrations like 1000 to 15.625 µg/mL of the ethanolic extracts in each well. To each well, 10 µL of resazurin indicator solution was added. (The resazurin solution was prepared by dissolving a 270 mg tablet in 40 mL of sterile distilled water. A vortex mixer was used to ensure that it was a well-dissolved and homogenous solution). Finally, 10 µl of bacterial suspension was added to each well to achieve a concentration of approximately 5×10^5 CFU/mL. Each plate had a set of controls: a column with all solutions with the exception of the crude extracts; a column with all solutions with the exception of the bacterial solution adding 10 µl of MHB instead and a column with 10 % DMSO solution as a negative control. The plates were incubated at 37 °C for 24 h for all the bacterial strains tested. The colour change was then assessed visually. The growth was indicated by colour changes from purple to pink (or colorless). In this study, the MIC was the lowest concentration ofcrude extracts that inhibited the growth of the organisms values by visual reading.

Determination of the Minimum Bactericidal Concentration (MBC)

The MBC of the extracts were determined by plating a loop ful of samples from each MIC assay well with growth inhibition into freshly prepared Mueller Hinton Agar. The plates were incubated at 37 ℃ for 24 h for all the bacterial strains tested. The MBC were recorded as the lowest concentration of the extract that did not permit any visible bacterial growth after the period of incubation.

Fourier transform infrared spectrum

FT-IR spectrum was recorded in AVATAR-330 FT-IR spectrophotometer (Thermo Nicolet). The active principles were mixed with KBr and pellet technique was adopted to record the spectra.

Statistical analysis

The results are expressed as the mean ± SD. All statistical analyses were performed using SPSS version 16.0 statistical software (SPSS Inc., Chicago, IL, USA). Student's t-test was performed to determine any significant difference between ethanol solvents extracts for *in vitro* antibacterial assays.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(4s), 387-395 | ISSN:2251-6727

Results and Discussion

Phytochemical investigation

The phytochemical analysis of ethanolic extracts of *S. brachiata,S. maritima* and *S. portulacastrum* had showed the presence of alkaloids, terpenoids, flavonoids, glycosides, tannins, phenolic compounds and steroids are presented in Table.1. Anthraquinones and catechin are not present in all the extracts tested. Tannins were absent in all the plant extracts tested except *S. maritime* extracts. Terpenoids and saponins were present in all the extracts except *S. portulacastrum* extracts. Glycosides were absent in *S. brachiata* ethanol extracts.

Table 1. Phytochemical analysis of ethanolic extracts of Halophytes plants

S.no	Secondary metabolites	*S. maritima*	*S. brachiata*	*S. portulacastrum*
1	Alkaloids	+	+	+
2	Anthraquinones	_	_	-
3	Catechin	_	_	_
4	Flavonoids	+	+	+
5	Glycosides	+	_	+
6	Phenolic group	+	+	+
7	Saponins	+	+	_
8	Tannins	+	_	_
9	Terpenoids	+	+	_

- = Absence, + = weak, ++ = medium, +++ = strong

Antibacterial potential

The antibacterial activity of ethanolic extracts of *S. brachiata*, *S. maritima* and *S. portulacastrum* were studied against bacterial strains. All the extracts of *S. maritima* possessed significant antibacterial activity against all the bacterial strains tested when compared to the available antibiotics tested. The mean values are presented in (Table 2 to 4). Ethanol extracts were assayed against the test bacteria by agar diffusion assays, the mean zones of inhibition obtained were between 7.0 and 20.5 mm.Ciprofloxacin (10 µg/disc) antibacterial positive control produced mean zones of inhibition ranged from 7.0 to 20.5 mm. The blind control (10 % DMSO) did not produce any zone of inhibition for all the bacterial strains tested.The MIC values of the different extracts of *Suaeda maritima* ranged from 62.5 to 500 µg/ml, while the MBC values were between 125 and 1000 µg/ml.

Table 2. Antimicrobial activity of ethanol extract of *Suaeda maritima*

S.No.	Microorganisms	Mean zone of inhibition[a] (mm)[b]			Ciprofloxacin (10 µg/disc)	MIC (µg/ml)	MBC/ MFC (µg/ml)
		Concentration of the disc (µg/disc)					
		500	250	125			
1.	*Bacillus subtilis*	19.5 ± 0.8	15.3 ± 0.5	11.0 ± 0.5	31.5 ± 0.3	62.5	125
2.	*Sterptococcus pyogenes*	18.0 ± 0.4	14.8 ± 0.5	10.0 ± 0.6	34.3 ± 0.5	62.5	125
4.	*Staphylococcus aureus*	16.0 ± 0.8	13.3 ± 0.5	10.0 ± 0.2	29.0 ± 0.8	125	62. 5
5.	*Pseudomonas aeruginosa*	13.5 ± 0.7	12.5 ± 0.3	9.0 ± 0.4	30.0 ± 0.8	250	500
6.	*Klebsiella pneumoniae*	14.5 ± 0.7	12.0 ± 0.4	9.8 ± 0.3	32.5 ± 0.7	250	500
7.	*Proteus mirabilis*	14.0 ± 0.4	11.3 ± 0.3	9.8 ± 0.3	30.3 ± 0.7	250	500
8.	*Proteus vulgaris*	13.3 ± 0.3	11.8 ± 0.8	9.0 ± 0.8	27.5 ± 0.5	250	500

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(4s), 387-395 | ISSN:2251-6727

9.	*Salmonella typhimurium*	14.8 ± 0.9	11.0 ± 0.8	9.4 ± 0.8	29.8 ± 0.6	500	1000
10.	*Shigella flexneri*	12.5 ± 0.3	10.3 ± 1.0	7.5 ± 0.8	28.3 ± 0.4	500	1000
11.	*Vibrio cholerae*	12.0 ± 0.3	10.0 ± 0.8	7.1 ± 0.8	30.3 ± 0.2	500	1000

a– diameter of zone of inhibition (mm) including disc diameter of 6 mm; b-mean of four assays; ± standard deviation; Cip – Ciprofloxacin antibacterial drug.

Table 3. Antibacterial activity of ethanol extract of *Sesuvium portulacastrum*

S.No.	Microorganisms	Mean zone of inhibition[a] (mm)[b]			Ciprofloxacin (10 µg/disc)	MIC (µg/ml)	MBC/ MFC (µg/ml)
		Concentration of the disc (µg/disc)					
		500	250	125			
1.	*Bacillus subtilis*	20.5 ± 0.6	15.1 ± 0.5	11.0 ± 0.5	29.5 ± 0.3	62.5	125
2.	*Sterptococcus pyogenes*	17.0 ± 0.3	13.0 ± 0.5	10.0 ± 0.8	30.3 ± 0.5	62.5	125
4.	*Staphylococcus aureus*	16.0 ± 0.8	13.1 ± 0.5	10.0 ± 0.7	28.0 ± 0.4	125	62.5
5.	*Pseudomonas aeruginosa*	13.0 ± 0.7	12.0 ± 0.3	9.0 ± 0.4	29.0 ± 0.6	250	500
6.	*Klebsiella pneumoniae*	14.5 ± 0.3	11.0 ± 0.4	9.8 ± 0.2	30.5 ± 0.7	250	500
7.	*Proteus mirabilis*	14.0 ± 0.2	11.1 ± 0.3	9.8 ± 0.3	30.3 ± 0.7	250	500
8.	*Proteus vulgaris*	12.3 ± 0.3	11.0 ± 0.8	9.1 ± 0.6	27.5 ± 0.2	250	500
9.	*Salmonella typhimurium*	14.3 ± 0.7	11.2 ± 0.8	9.0 ± 0.5	28.8 ± 0.6	500	1000
10.	*Shigella flexneri*	12.7 ± 0.3	10.3 ± 1.0	7.3 ± 0.8	27.3 ± 0.9	500	1000
11.	*Vibrio cholerae*	12.3 ± 0.3	10.0 ± 0.8	7.1 ± 0.7	28.3 ± 0.5	500	1000

a– diameter of zone of inhibition (mm) including disc diameter of 6 mm; b-mean of four assays; ± standard deviation; Cip – Ciprofloxacin antibacterial drug.

Table 4. Antibacterial activity of ethanol extract of *Salicornia brachiata*

S.No.	Microorganisms	Mean zone of inhibition[a] (mm)[b]			Ciprofloxacin (10 µg/disc)	MIC (µg/ml)	MBC/ MFC (µg/ml)
		Concentration of the disc (µg/disc)					
		500	250	125			
1.	*Bacillus subtilis*	15.5 ± 0.7	11.1 ± 0.3	10.0 ± 0.2	28.5 ± 0.4	125	62. 5
2.	*Sterptococcus pyogenes*	14.0 ± 0.3	10.0 ± 0.5	9.8 ± 0.9	29.3 ± 0.6	125	62. 5
4.	*Staphylococcus aureus*	14.6 ± 0.4	11.1 ± 0.7	10.0 ± 0.7	30.0 ± 0.9	125	62. 5
5.	*Pseudomonas aeruginosa*	13.0 ± 0.7	10.0 ± 0.3	9.0 ± 0.6	29.0 ± 0.6	250	500
6.	*Klebsiella pneumoniae*	12.5 ± 0.3	9.8 ± 0.4	9.8 ± 0.2	31.5 ± 0.8	250	500

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(4s), 387-395 | ISSN:2251-6727

7.	*Proteus mirabilis*	12.0 ± 0.2	9.1 ± 0.3	9.8 ± 0.3	30.3 ± 0.7	250	500
8.	*Proteus vulgaris*	12.3 ± 0.3	10.0 ± 0.8	9.1 ± 0.6	27.5 ± 0.2	250	500
9.	*Salmonella typhimurium*	12.5 ± 0.8	9.2 ± 0.8	8.5 ± 0.5	28.8 ± 0.6	500	1000
10.	*Shigella flexneri*	12.3 ± 0.4	10.0 ± 1.0	7.1 ± 0.3	28.6 ± 0.8	500	1000
11.	*Vibrio cholerae*	12.1 ± 0.6	10.2 ± 0.7	7.0 ± 0.5	29.5 ± 0.5	500	1000

a–diameter of zone of inhibition (mm) including disc diameter of 6 mm; b-mean of four assays; ± standard deviation; Cip – Ciprofloxacin antibacterial drug.

FT-IR spectrum

FT-IR spectrum analysis was used to characterize the functional group of active constituents based on the peak value in the infrared region in Fig. 1. FT-IR spectrum of the extracted compound was recorded in the spectral range of 4000-400 cm^{-1} and the bands of compound indicated at 3852, 3741, 3308, 2962, 2066, 1718, 1657, 1541, 1456, 1384, 1308, 1170, 1124, 651 and 584 cm^{-1} respectively.

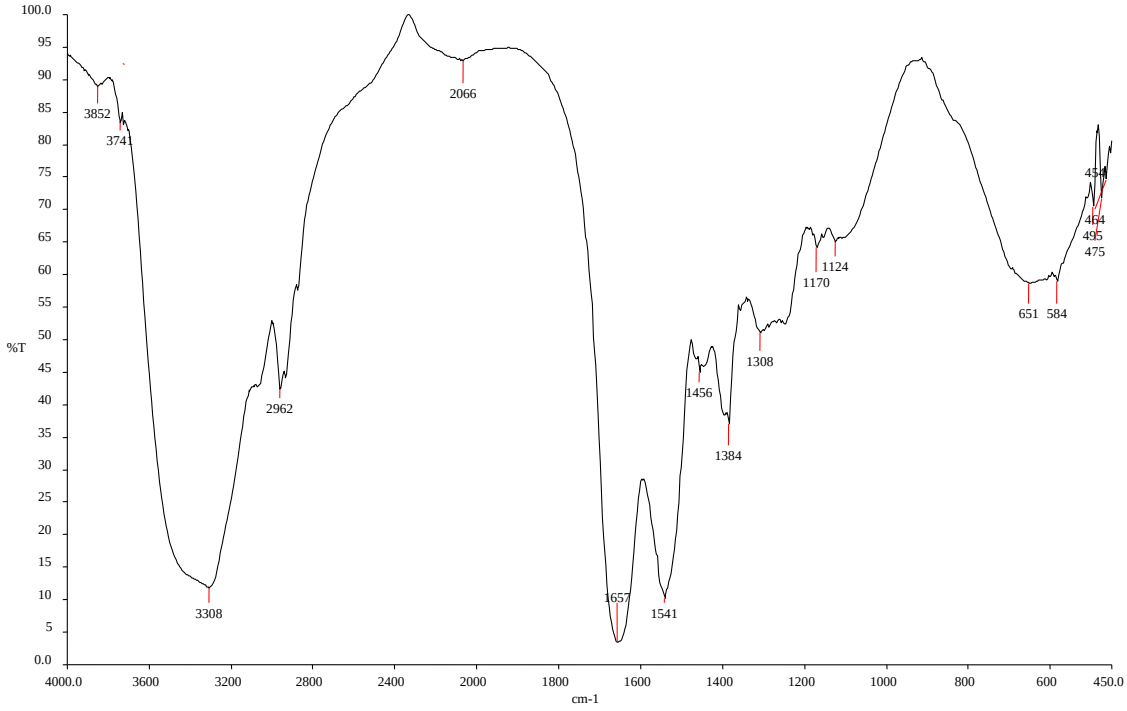

Fig.1. FT-IR spectrum analysis of ethanolic extracts of *Suaeda maritima*

The phytochemical analysis of ethanolic extracts of *Salicornia brachiata*,*Suaeda maritima* and *Sesuvium portulacastrum*had showed the presence of alkaloids, terpenoids, flavonoids, glycosides, tannins, phenolic compounds and steroids.In general, phenolic compounds possess specific physical, chemical and biological activities that make them useful as drugs. Phenolics were also responsible for the antimicrobial, anti-inflammatory, anti-feedant, anti-viral, anticancer and vasodilatory actions (Rievere et al., 2009). Phenolic compounds may affect growth and metabolism of bacteria. They could have an activating or inhibiting effect on microbial growth according to their constitution and concentration (Reguant et al., 2000). Flavonoids are naturally occurring phenols which possess numerous biological activities including anti-microbial, antiinflammatory, antiallegic, antithrombotic and vasoprotective effects (Ogunleye,et al 2003).Terpenoids have been found to be useful in the prevention and therapy of several diseases, including

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(4s), 387-395 | ISSN:2251-6727

cancer. Terpenoids are also known to possess antimicrobial, antifungal, antiparasitic, antiviral, anti-allergenic, antispasmodic, antihyperglycemic, antiinflammatory and immunomodulatory properties (Mandal et. al., 2005: Manjunatha, 2006).

The presence of phenolic compounds in the plants indicates that these plants may be anti-microbial agent. This agreed with the findings of Ofokansi et al. (2005).Tannins have general antimicrobial and antioxidant activities (Rievere et. al., 2009). Current reports show that tannins may have potential value such as cytotoxic and antineoplastic agents (Aguinaldo et. al., 2005).Anthraquinone derivatives have been used since centuries for medical applications, for example, as laxatives and antimicrobial and antiinflammatory agents. Current therapeutic indications include constipation, arthritis, multiple sclerosis, and cancer.(Rabi et al., 2009, Wagner et al., 2003).

In the present study, salt marsh halophytes possessed moderate antibacterial activity. Some of the salt marsh halophytes were reported for their antimicrobial activity by many researchers. The methanol extracts of *Sesuvium portulacastrum* showed antibacterial activity against some marine fouling bacteria *viz.,Pseudomonas* sp., *Vibrio* sp., *Staphylococcus* sp., *Flavobacterium* sp. and *Arthrobacterium* sp. (Prabha Devi et al., 1997). The 70 per cent ethanol extracts of leaves of *Sesuvium portulacastrum* and shoots of *Salicornia brachiata* possessed antiviral activity against hepatitis Bvirus (Premanathan, 1999) and shoots of *Salicornia brachiata* possessedactivity against encephalomycarditis virus (Premanathan et al., 1994).The essential oil from *Sesuvium portulacastrum* exhibited antibacterial, antifungal and antioxidant activities (Magwa *et al.*, 2006).

The aqueous extracts of bark of *Rhizophora mangle* exhibited antibacterial activity (Melchor et al., 2001) and also exerted a remarkable protective effect on gastric ulcers induced by ethanol-hydrochloric acid (Sánchez Perera et al., 2001). The topical action of the aqueous extract of *Rhizophoramangle* in accelerating wound healing has been explained by several mechanisms, such as coating the wound, forming complexes with proteins of microorganism's cell wall, chelating free radicals and reactive oxygen species, stimulating the contraction of the wound and increasing the formation of new capillaries andfibroblasts (Fernandez et al., 2002). Chandrasekaran et al. (2006) reported that the methanol and aqueous extracts of *Rhizophora lamarkii* showed antibacterial activity against 10 isolates of MRSA.

The antibacterial activity of ethanolic extracts of *S. brachiata,S. maritima* and *S. portulacastrum*were studied against bacterial strains tested.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 Prasanna Lakshmi and Narasimha Rao (2013) reported *in vitro* antibacterial activity of leaf of *S. portulacastrum* petroleum ether, benzene, ethyl acetate, methanol, and ethanol plant extracts against some gram positive bacteria *Bacillus subtilis, Bacillus megaterium* and *Lactobacillus acidophilus* and 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*. 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, the gram positive bacteria were more susceptible than the gram negative bacteria. The greater resistance of gram negative bacteria to plant extracts has been documented previously for seeds of *Syzygium jambolanum* (Chandrasekaran and Venkatesalu, 2004a)and bark of *Cassia siamea* (Chandrasekaran and Venkatesalu, 2004b) *Caulerpa chemnitzia* (Adaikala Raj et al., 2015). The gram positive bacteria should be more susceptible since they have only an outer peptidoglycan layer which is not an effective permeability barrier (Scherrer and Gerhardt, 1971). The resistance of gram negative bacteria towards antibacterial substances is related to lipopolysaccharides in their outer membrane (Sawer et al., 1997; Gao et al., 1997). The reason for different sensitivity between gram positive and gram negative bacteria could be ascribed to the morphological differences between these microorganisms (Arias et al., 2004). The majority of plant derived antimicrobial compounds generally have higher MICs than bacterial or fungal produced antibiotics, thus limiting their therapeutic potential (Gibbons, 2004).

Conclusion

Finally it can be concluded that the present study indicated that *S. brachiata, S. maritima* and *S. Portulacastrum* were found to have antibacterial action against *B. subtilis, S. pyogenes, E. coli, K. pneumoniae, P.mirabilis, P. vulgaris P. aeruginosa, S. typhimurium, S. flexneri* and *V. cholerae* in this investigation. The demonstration of action against Gram positive bacteria indicates a broad spectrum of activity and can thus be utilized to source antibiotic compounds for medication development that can be employed to control various bacterial illnesses. *S. maritime* in to its activity against a broader variety of bacteria, identification and purification of its chemical

Journal of Chemical Health Risks

www.jchr.org

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constituents, and toxicity studies of plant extracts should be conducted in order to produce novel medications for human consumption.

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