Preliminary phytochemical and antibacterial screening of *Sesuvium portulacastrum* in the United Arab Emirates

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ABSTRACT

Background: The present study describes the phytochemical profile and antimicrobial activity of *Sesuvium portulacastrum*. **Materials and Methods:** Three extracts of *S. portulacastrum* obtained by extraction in aqueous, ethanolic and dichloromethane solvents, respectively, were compared for their antimicrobial activity and ethanolic extract further subjected to gas chromatography-mass spectrometry (GC-MS) analysis to find out the nature of the compounds responsible for the antimicrobial activity. The antibacterial activities were assessed by measuring the diameter of the inhibition zones, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values. **Results:** Compared to the aqueous and dichloromethane extract, the ethanolic extract showed better antimicrobial activity against *Staphylococcus aureus* and *E. coli*, indicating its potential application related to noscomial infections. GC-MS results revealed 22, 23-Dihydrostigmasterol, Benzoic acid, 3,4,5-trihydroxy-(Gallic acid), (2R,3R)-(-)-Epicatechin and Capsaicin in the ethanolic extract to be the molecules responsible for the antimicrobial activity of *S. portulacastrum*. **Conclusion:** To the best of our knowledge, this is the first report on analysis of antimicrobial components from *S. portulacastrum* in United Arab Emirates (UAE), and our results confer the utility of this plant extract in developing a novel broad spectrum antimicrobial agent.

Key words: Antibacterial, gas chromatography-mass spectrometry, phytochemical, *Sesuvium portulacastrum*

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INTRODUCTION

Sesuvium portulacastrum (S. portulacastrum) Aizoaceae is commonly known as Sea Purslane. It is a frequent pioneer species in the backshore zone of coastal beaches, where sand movement is influenced by prevalent winds near the born crest.[1] *S. portulacastrum* plant is distributed throughout the world since it is used as an ornamental plant.[2] Sipahimalani *et al.*, demonstrated the incorporation of mevalonic acid and cholesterol into ecdysone and ecdysterone and conversion of ecdysone into ecdysterone in the plant, *Sesuvium portulacastrum L.*[3] *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.[4] Kämpfer *et al.*, stated a gram-staining-positive coccus, belonging to genus *Salinicoccus*, was isolated from the rhizosphere of *Sesuvium portulacastrum.*[5] Magwa *et al.*, used hydrodistillation to extract the essential oil from the fresh leaves of *S. portulacastrum*, and the essential oil exhibited antibacterial, antifungal and antioxidant activity.[6] Chandrasekaran *et al.*, *expressed* the fatty acid methyl esters (FAME extract) from *S. portulacastrum* can be used in traditional medicine as a potential antimicrobial agent.[7] Nabikhan *et al.*, showed the effect of extracts from tissue culture-derived callus and leaf of the saltmarsh plant, *S. portulacastrum* L. on synthesis of antimicrobial silver nanoparticles using $AgNO_3$ as a substrate.[8] The aim of this study is to investigate the antibacterial activity for different plant extracts using aqueous, ethanolic and dichloromethane solvents and screening of the ethanolic extract through GC/MS. To the best of our knowledge, this is the first report on the analysis of antimicrobial components from *S. portulacastrum* in UAE.

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MATERIALS AND METHODS

Collection and validation of samples

The leaves and stems of *S. portulacastrum* were collected from the gardens of Ras Al Khaimah Medical and Health Sciences University (RAKMHSU) in July 2010. Plants were cross-identified by their vernacular names and later validated at the Faculty of Food and Agriculture, United Arab Emirates University, Al Ain - UAE by Dr. Adil Ismail El Awad.

Processing of samples of leaves and stems

Two hundred and fifty grams of withered leaves and stems of *S. portulacastrum* were splashed with tap water to eliminate dust. They were left in the shade to dry for 15-20 days. The dried material was sliced into small fragments and ground to fine powder using mortar and pestle. The powder passed through a sieve of pore size 0.5 mm, which was extracted at room temperature thrice with ethanol, distilled water and dichloromethane for 48 h on an orbital shaker to make the extracts.[9,10] Finally, the extracts were concentrated using a rota-evaporator (R 215 Buchi Instrument, Switzerland) at a reduced pressure and at < 40°C.

Phytochemical analysis

The presence of phytochemicals in the three extracts such as alkaloids, saponins, tannins (5% ferric chloride), terpenoids (2, 4-dintrophenyl hydrazine) and steroids (Liebermann-Burchard test) were evaluated according to the methods described by Edeoga *et al.*[11]

Preparation of media and nutrient agar

Media for assessing the antimicrobial activity was prepared by dissolving 8 g of nutrient broth (Merck, UK) in 1:l of freshly distilled or completely demineralized water. Agar was prepared by dissolving 5 g of Bacto-agar (Difco laboratories, UK) in 200 ml of distilled water with continuous stirring and heating until clear solution appears. Both the media and agar were sterilized by autoclaving at 121°C for 15 min. Then they were left to cool down at 50–55°C.

Staphylococcus aureus and *Escherichia coli* were obtained from clinical isolates and were supplied by microbiology department in *Ras Al Khaimah Medical and Health Sciences University, College Of medical Sciences*. The bacteria were grown in nutrient broth at 37°C and maintained on nutrient agar at 4°C.[12]

Determination of the antimicrobial activity

Standard methods were used to determine minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the whole extracts. Strains were cultured overnight at 37°C in nutrient broth. Concentrations of the whole extracts were 25, 50, and 100 μg/ml, respectively. In addition broth containing bacteria only without extract and broth containing the extract only without bacteria served as control. The test was carried out by weighing 25, 50, 100 μg/ ml, respectively of each extract dissolved or suspended in 1 ml of the solvent. Sterile agar media weighing 99 ml were added under aseptic conditions to 150 ml flat bottom sterile Petri dishes together with 1 ml of plant extract at different concentrations. These plates were allowed to solidify on a level surface. A loop full of inoculums suspensions (bacteria) was taken and streaked on a radial pattern on the agar containing the plant extracts, a triplicate agar plate was used in order to obtain accurate results, standard and control plates were treated in the same way without the plant extract (standard antibiotics were penicillin, vancomycin and cefotaxime). Results were taken from plates having the expected appearance of colonies; otherwise the plates were discarded. Subcultures of these were used to determine MBC. Two susceptibility endpoints were recorded for each isolate. The MIC is defined as the lowest concentration of compounds at which the microorganisms tested did not demonstrate the visible growth. The MBC is defined as the lowest concentration yielding negative subculture or only one colony.[13]

Determination of antimicrobial susceptibility using agar well diffusion assay

The extracts were tested for antimicrobial activity using the agar well diffusion method. Circular wells (6 mm in diameter) were cut in the agar culture media and filled with 25, 50, and 100 μg/ml extract.[14]

Gas chromatography/mass spectrometry (GC/MS)

GC-MS analysis was carried out on an Shimadzu 2010 QB gas chromatography with a MSD detector equipped with HP-5 fused silica capillary column (30m×0.25mm×25μm film thickness). The ethanolic plant extract was injected via an all- glass injector working with split mode, with the Heleium as the carrier gas with a flow rate of 1 ml/min. Temperature program: injected temp 200°C, ion source 200°C, interphase 200°C. Column temperature was raised to 45°C (3min hold at 45°C, 4°C/min), then gradually increased to 150°C (3min hold at 150°C, 4°C/min) then raised to 250°C and a 15 min hold. A split ratio of 1:5 was used. Identification of each individual constituent of the volatile compound was achieved by comparing the retention times with those of authentic compounds or the spectral data obtained from the Wiley Library and National Institute of Standards and Technologies library, and with data published in the literature.

Statistical analysis

Analysis of data was performed by using SPSS (version 18). Results are expressed as mean ± S.D. Statistical differences were determined by Student–Newman–Keul test for multiple comparisons after ANOVA (Freund, 1981).

RESULTS

Phytochemical analysis

Phytochemical screening of the ethanolic extract of the leaves and stems of *S. portulacastrum* showed the presence of steroids. While the aqueous extract was positive toward the presence of alkaloids, saponins, tannins and terpenoids. However, the dichloromethane extract was negative to all chemical tests done, as shown in Table 1. Presence of tested secondary metabolites in the leaves and stems of *S. portulacastrum* will be promising for further studies on the plant as a potential study area for other researchers. The phytoconstituents detected in the plant materials could be responsible for the antimicrobial activity though their exact mode of action which is poorly understood up till now.

Table 1: Phytochemical screening of ethanol, aqueous and dichloromethane extract of the leaves and stems of *S. portulacastrum*

Constituents	Aqueous	Ethanolic	Dichloromethane
Alkaloids • Dragendorff's test	+	-	-
Steroids • Libarman-Burchard's test	-	+	-
Terpenes • Salkowski test	+	-	-
Tannins • FeCl3 test • Gelatin test	+	-	-
Saponins • Frothing test	+	-	-

- = Negative (absent) + = Positive (present)

GC-MS study

The possible phytoconstituents were further confirmed through the use of GC-MS, which suggested the presence of the following phytochemicals: 22, 23-dihydrostigmasterol,[15] benzoic acid 3, 4, 5-trihydroxy-(gallic acid),[16] (2R, 3R)- (-)- epicatechin and capsaicin,[17,18] as shown in Table 2 and Figure 1.

Microbiological study

The ethanolic extract of the plant showed varying degree of antibacterial activities against the test bacterial species [Tables 4 and 5]. The antibacterial activities of the ethanol extract were compared with three standard antibiotics (penicillin G, cefotaxime and vancomycin) and showed a broad spectrum activity against gram negative and gram positive bacteria. The ethanolic extract of *S. portulacastrum* obtained MIC and MBC of 50 μg/ml against *Staphlyococcus aureus* and *E. coli*. The inhibition zone for the ethanolic extract was positive in different concentrations 25, 50 and 100 μg/ ml against *Staphlyococcus aureus* and *E. coli* as shown in Tables 4 and 5, Figure 2.

Table 2: Phytocomponents suggested in the ethanolic extract of leaves and stems of *S. portulacastrum* By GC-MS

RT	Name of compound	Molecular	MW	Peak area (%)	Reference
21.35	22,23-Dihydrostigmasterol	$C_{29}H_{50}O$	414.71	13.113	(15)
25.33	Benzoic acid, 3,4,5-trihydroxy-(Gallic acid)	$C_7H_6O_5$	170.12	5.8	(16)
27.95	Capsaicin	$C_{18}H_{27}NO_3$	305	4.32	(17)
28.04	(2R,3R)-(-)-Epicatechin	$C_{15}H_{14}O_6$	290.26	9.01	(18)

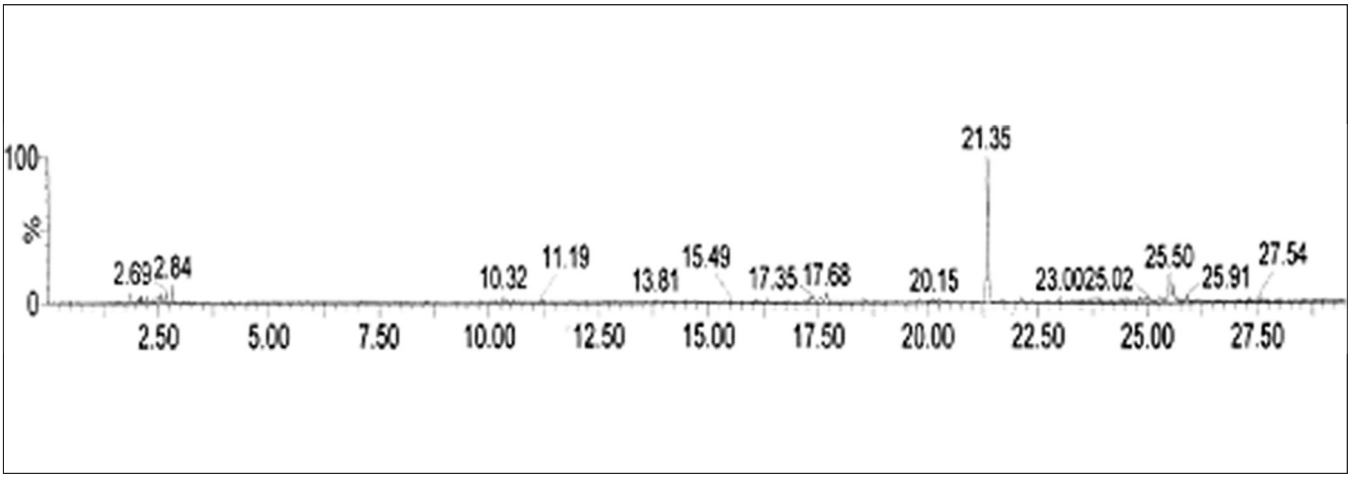

Figure 1: Chromatogram obtained from GC-MS with the ethanolic extracts of the leaves and stems of S. portulacastrum

DISCUSSION

The range of medicinal plants and herbs containing various phytochemicals with biological activity can be of therapeutic importance. Much of the protective effect of fruits and vegetables also has been attributed to the presence of phytochemicals. Different phytochemicals have been found to have a broad range of actions, which may help in protection and treatment against different diseases. In the present study, the leaves and stems of *S. portulacastrum* were subjected to phytochemical evaluation, where different solvent extracts used showed the detection of various phytochemical compounds through different chemical tests used based on their solubility. Phytochemical analysis with the use of GC- MS of the *S. portulacastrum* ethanolic extract revealed the presence of 22, 23-Dihydrostigmasterol, Benzoic acid 3, 4, 5-trihydroxy-, Epicatechin and Capsaicin. GC-MS is used for preliminary identification of main chemical components of the plant extract. All of these compounds have been shown to have antibacterial activity,[19] as shown in Table 3. 22, 23-Dihydrostigmasterol has been reported to have anti-inflammatory, antioxidant and neuroprotective activities.[20,21] Gallic acid has both analgesic and anti-inflammatory properties.[22,23] Tannins appear to have considerable cancer-prevention properties.[24] Alkaloid-containing plants have been used by humans for centuries for therapeutic and recreational purposes. They are known for their antimalarial, antimicrobial, cytotoxic and antiulcer properties.[25] Cytotoxic compounds are potentially interesting on their own or as lead compounds for the development of new anti-cancer drugs as well as drugs against parasites and viral infections. Through the use of GCMS, the *S. portulacastrum* containing these compounds may serve as a potential source of bioactive compounds in the prevention or cure of microbial and other disorders.

The current pioneering study suggests that this extract is a potent therapeutic agent. It paves the way for the development of several treatment regimens based on this extract. In addition, research is continuing to identify and purify the active compounds responsible for anti-bacterial activity.

Table 3: Phytocomponents and its biological activities obtained through the GC/MS study of *S. portulacastrum*

RT	Name of compound	Active biological activity**
21.35	22,23-Dihydrostigmasterol	
25.33	Benzoic acid, 3,4,5-trihydroxy- (Gallic acid)	Analgesic; AntiHIV; AntiMRSA; Antiadenovirus; Antiallergenic; Antiasthmatic; Antibronchitic; Anticancer; Antifibrinolytic;
27.95	Capsaicin	5-Lipoxygenase-Inhibitor; Anesthetic; Antioxidant; Antipsoriatic; Antiseptic; Antispasmodic, Antitumor (Lung); Antiulcer; Pesticide; Vasodilator;
28.04	(2R,3R)-(-)-Epicatechin	AntiHIV, Antihepatitic; Antihyperglycemic; Antiinflammatory; Antileukemic; Antimutagenic; Antioxidant, Antiviral; Hypocholesterolemic; Hypoglycemic

**reference: Dr. Duke's Phytochemical and Ethnobotanical Database[15]

Table 4: Minimum inhibitory concentration of the plant extracts against *Staphlyococcus aureus* and *E. coli*

Microorganism	Reference antibiotic	MIC (MBC) µg/ml		
		Aqueous	Dichloromethane	Ethanol
Staphylococcus aureus	a	ND	ND	50
Escherichia coli	b	ND	ND	50

A: penicillin G 5µg/ml, B: cefotaxime 10µg/ml, C: vancomycin 10 µg/ml[14] ND: no detected activity

Table 5: Effect of the plant extract on the growth of bacteria

Microorganism	Inhibition zone diameter IZD			
	Concentration	Aqueous	Dichloromethane	Ethanol
	25 µg/ml			
Escherichia coli			–	+
Staphylococcus aureus		–	–	+
	50 µg/ml			
Escherichia coli			–	+
Staphylococcus aureus		–	–	++
	100 µg/ml			
Escherichia coli			–	+++
Staphylococcus aureus		–	–	+++

+: 5mm IZD, ++: 10 IZD, +++: 15 IZD

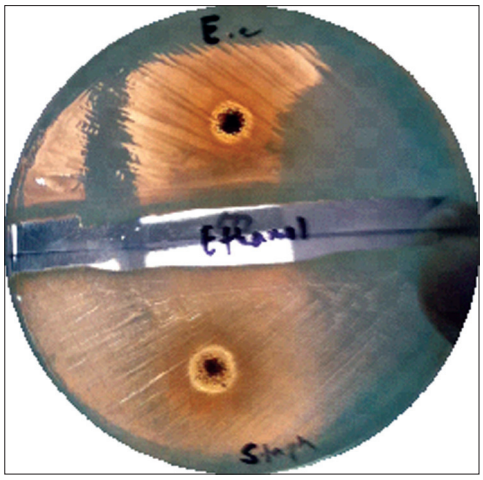

Figure 2: Determination of antimicrobial susceptibility using agar well diffusion assay for ethanolic extract 50 µg/ml against *Staphylococcus aureus* (staph) and *E. coli* (E.C.)

The ethanolic extract of the medicinal plant *S. portulacastrum* showed potential against the causative agents of nosocomial infections, and important pathogens associated with various gastrointestinal disorders leading to indigestion, dysentery, and diarrhea. Unfortunately, resistance to available antibiotics is on the rise and there are a limited number of antipseudomonal agents with reliable activity. Thus, the antibacterial activities of medicinal plant reported in the present study are noteworthy considering the importance of such microorganisms.

CONCLUSION

The present study confirmed the anti-bacterial potential of *S. portulacastrum*, with results comparable with those of standard compounds such as cefotaxime and vancomycin. These data further support the view that the leaves and stems of *S. portulacastrum* are promising sources of natural anti-bacterial, and could be seen as potential sources of useful drugs. Nonetheless, further *in vivo* studies and purification of the compounds responsible for anti-bacterial activity are needed with advanced instrumental analysis using nuclear magnetic resonance (NMR).

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