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

Amad Al-Azzawi, Alyaa Alguboori¹, Mahmoud Y. Hachim², Najat M³, Al Shaimaa A², Maryam Sad³

Ras Al Khaimah Medical and Health Sciences University, College of Pharmaceutical Sciences, AlMamoura, Ras Al Khaimah, ¹Ras Al Khaimah Medical and Health Sciences University, Almamoura, Ras Al Khaimah, ²Medical Microbiology and Immunology Department, Ras Al Khaimah College of Medical Sciences, ³Ninth Semester Student, Ras Al Khaimah College of Pharmaceutical Sciences, Ras Al Khaimah Medical and Health Sciences University, Ras Al Khaimah, United Arab Emirates

Submitted: 12-10-2011 Revised: 07-01-2012 Published: 11-10-2012

**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*

**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] Kämpfe 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 AgNO₃ 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.
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, US) 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 to 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.[4]

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.

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, benzoic acid 3, 4, 5-trihydroxy-(gallic acid), (2R, 3R)-(-)-epicatechin and capsaicin 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 *Staphylococcus aureus* and *E. coli*. The inhibition zone for the ethanolic extract was positive in different concentrations 25, 50 and 100 µg/ml against *Staphylococcus aureus* and *E. coli* as shown in Tables 4 and 5, Figure 2.

| 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)

| 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_{41} | 414.71 | 13.113 | (15) |
| 25.33 | Benzoic acid, 3,4,5-trihydroxy-(Gallic acid) | C_{7}H_{6}O_{5} | 170.12 | 5.8 | (16) |
| 27.95 | Capsaicin | C_{18}H_{27}N_{3}O | 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 antulcer 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 GC-MS, 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 | Analgesic; Anti-HIV; Anti-MRSA; Anti-Adenovirus; Anti-allergic; Antithrombic; Anticancer; Antifibrinolytic; |
| 25.33 | Benzoic acid, 3, 4, 5-trihydroxy-(Gallic acid) | 5-Lipoxygenase-Inhibitor; Antinociceptive; Antioxidant; Antipsoriatic; Antiseptic; Antispasmodic, Antitumor (Lung); Antiluetic; Pesticide; Vasodilator; Anti-HIV, Antihepatic; Antihyperglycemic; Antiinflammatory; Antileukemic; Antimitogenic; Antioxidant, Antiviral; Antiulcer; Hypocholesterolemic; Hypoglycemic |
| 27.95 | Capsaicin | CNS depressant; Analgetic; Anti-HIV; Anti-MRSA; Anti-Adenovirus; Antiallergenic; Antiasthmatic; Antibronghital; 
| | | Capsaicin | Epicatechin | Anti-HIV, Antihepatitic; Antihyperglycemic; Antiinflammatory; Antileukemic; Antimitogenic; Antioxidant, Antiviral; Hypocholesterolemic; Hypoglycemic |
| 28.04 | (2R,3R)-(−)-Epicatechin | Anti-HIV, Antihepatitic; Antihyperglycemic; Antiinflammatory; Antileukemic; Antimitogenic; Antioxidant, Antiviral; Hypocholesterolemic; Hypoglycemic |

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

| Table 4: Minimum inhibitory concentration of the plant extracts against *Staphylococcus 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** | **Concentration** | **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
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 vitro* studies and purification of the compounds responsible for anti-bacterial activity are needed with advanced instrumental analysis using nuclear magnetic resonance (NMR).

**ACKNOWLEDGMENT**

Vice Chancellor of Ras Al Khaimah Medical and Health Sciences University, Dean of College of Pharmaceutical Sciences, Ras Al Khaimah Medical and Health Sciences University. Dean of Food and Agriculture, UAE University Al-Ain, United Arab Emirates.

**REFERENCES**

1. Robert IL, Frank W. The biological flora of Coastal and Wetlands *Sesuvium portulacastrum* L. J Coast Res 1997;13:96-104.
2. Rabhi M, Giuntini D, Castagna A, Remorini D, Baldan B, Smaoui A, *et al*. *Sesuvium portulacastrum* maintains adequate gas exchange, pigment composition, and thylakoid proteins under moderate and high salinity. J Plant Physiol 2010;16:1336-41.
3. Sipahimalani AT, Banerji A, Chada MS. Biosynthesis and interconversion of phytocedysones in *Sesuvium portulacastrum* L. J Chem Soc Chem Commun 1972;5:692-3.
4. Lokhande VH, Srivistava S, Patade VY, Dwivedi S, Tripathi RD, Nikam TD, *et al*. Investigation of arsenic accumulation and tolerance potential of *Sesuvium portulacastrum* (L.). Chemosphere 2011;4:529-34.
5. Kämpfer P, Arun AB, Busse HJ, Young CC, Lai WA, Rekha PD, *et al*. *Salinicoccus sesuivi* sp. nov., isolated from the rhizosphere of *Sesuvium portulacastrum*. Int J Syst Evol Microbiol 2011;61:2348-52.
6. Michael LM, Mazuru G, Nyasha G, Godfred H. Chemical composition and biological activities of essential oil from the leaves of *Sesuvium portulacastrum*. J Ethnopharmacol 2006;103:85-9.
7. Chandrasekaran M, Senthilkumar A, Venkatesalu V. Antibacterial and antifungal efficacy of fatty acid methyl esters from the leaves of *Sesuvium portulacastrum* L. Eur Rev Med Pharmacol Sci 2011;7:775-80.
8. Nabikhan LA, Kandasamy K, Raj A, Alkunhi NM. Synthesis of antimicrobial silver nanoparticles by callus and leaf extracts from saltmarsh plant, *Sesuvium portulacastrum*. Colloids Surf B Biointerfaces 2010;2:488-93.
9. Gülçin I, Kireççi E, Akkemik E, Topal F, Hisar O. Antioxidant and antimicrobial activities of an aquatic plant: Duckweed (*Lemma minor* L.). Turk J Biol 2010;34:175-88.
10. Tohma HS, Gülçin I. Antioxidant and radical scavenging activity of aerial parts and roots of Turkish liquorice (*Glycyrrhiza glabra* L.). Int J Food Properties 2010;13:657-71.
11. Edeogal HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr J Biotechnol 2005;7:685-8.
12. Senthilkumar CS, Kumar MS, Pandian MR. *In Vitro* Antibacterial activity of crude leaf extracts from tecoma stans (L.) Juss. et Kunth, Coleus Forskohlii and Pogostemon Patchouli against human pathogenic bacteria. Int J Pharm Tech Res 2010;2:438-42.
13. Pessini GL, Dias Filho BP, Nakamura CV, Cortez DA. Antibacterial activity of extracts and neolignans from Piper regnellii (Miq.) C.DC. var. pallescenes (C. DC.). Yunck. Mem Inst Oswaldo Cruz 2003;98:1115-20.
14. Cutler RR, Wilson P. Antibacterial activity of a new, stable aqueous extract of allicin against methicillin-resistant *Staphylococcus aureus*. Br J Biomed Sci 2004;5:243-7.
15. Hui C, Mei JJ, Hong XW. Essential oil from marchantia convoluta: Extraction and components. J Chin Chem Soc 2007;52:1.
16. Molnár-Perl I, Horváth K, Bartha R. GC-MS quantitation of benzoic acid and aralkyl carboxylic acids as their trimethylsilyl derivatives: In model solution I. Chromatographia 1998;48:101-10.
17. Maria IM, Constantin M, Delia NB, Cornelia AG, Constantin P. Application of TLC and GC-MS to the detection of capsaicin from hot peppers (*Capsicum annuum*). J Planar Chromatogr - Modern TLC 2004;17:147-8.
18. Jennifer LD, Devanand LL, Phil S, Andrew LW. Analysis of (1)-catechin, (2)-epicatechin and their 39- and 49-O-methylated analogs, A comparison of sensitive methods. J Chromatogr B 1999;726:277-83.
19. Roy1 P, Amdekar S, Kumar A, Singh V. Preliminary study of the
Al-Azzawi, et al.: Phytochemical and antibacterial of *Sesuvium portulacastrum* in the UAE

20. Hui C, Mei JJ, Hong XW. Essential oil from *marchantia convoluta*: Extraction and components. J Chil Chem Soc 2007;52:1088-91.

21. Prager N, Bickett K, French N, Marcovici G. The naturally occurring 5-alpha reductase inhibits Beta Sitosterol and Saw Palmetto are effective in treating androgenic alopecia. J Altern Complement Med 2002;2:143-52.

22. Kroes BH, van den Berg AJ, Quarles van Ufford HC, van Dijk H, Labadie RP. Anti-inflammatory activity of gallic acid. Planta Med 1992;6:499-504.

23. Sawant L, Pandita N, Prabhakar B. Determination of gallic acid in *Phyllanthus emblica* Linn. dried fruit powder by HPTLC. J Pharm Bioallied Sci 2010;2:105-8.

24. Li H, Wang Z, Liu Y. Review in the studies on tannins activity of cancer prevention and anticancer. Zhong Yao Cai 2003;6:444-8.

25. De Sousa Falcão H, Leite JA, Barbosa-Filho JM, de Athayde-Filho PF, de Oliveira Chaves MC, Moura MD, et al. Gastric and duodenal antiulcer activity of alkaloids: A review. Molecules 2008;13:3198-223.

Cite this article as: Al-Azzawi A, Alguboori A, Hachim MY, Najat M, Al Shaimaa A, Sad M. Preliminary phytochemical and antibacterial screening of *Sesuvium portulacastrum* in the United Arab Emirates. Phcog Res 2012;4:219-24.

Source of Support: Nil, Conflict of Interest: No.