Investigating the chemical Composition and the antimicrobial activity of the essential oil and Crude Extracts of *Sedum Microcarpum* (Sm.) schönl Growing Wild in Jordan

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ABSTRACT

Background: Several *Sedum* species, such as *S. album* L., S. acre L., *S. palaestinum* Bieb, and *S. pallidum* are used in traditional medicine to heal wounds, hemorrhoids, constipation, food fungi, and as laxative and diuretic agents. **Materials and Methods:** In this study, the antimicrobial activities of the hexane, n-butanol and aqueous methanol extracts as well as the essential oil from Jordanian *sedum microcarpum* (Sm.) schönl were investigated and Analysis of the oil constituents by using Gas chromatography-Mass spectrometer (GC/MS). **Conclusion:** The hexane extract showed no antibacterial activity against seven strains. The butanol and aqueous methanol extracts were active against the Gram-positive bacteria *Staphylococcus epidermidis*, *Bacillus cereus* and the Gram negative bacteria including *Enterococcus faecalis*, *Escherichia coli, Klebsiella pneumonia* and *Serratia marcescens*. Phytochemical screening of the active extracts revealed the presence of a mixture of quinones, tannins, alkaloids and flavonoids. The essential oil showed moderate activities against the tested strains. Analysis of the oil using Gas chromatography-Mass spectrometer (GC/MS) revealed a complex mixture of aldehydes, mono- and sesquiterpenes in addition to alkaloids. Oxygenated monoterpenes dominated the different classes of compounds detected (36.39%). The major volatile constituents from *S. microcarpum* included Myrtenol (21.7%), Caryophyllene oxide (8.2%) and z-1, 6-dioldihydromyrcene (5.87%).

Keywords: Antibacterial activity, Crassulaceae, Essential oil, GC-MS, Phytochemical screening, *Sedum microcarpum*.

INTRODUCTION

Sedum L. is one of the largest genera in the family Crassulaceae, comprising about 500 species distributed mainly in the Mediterranean region. The genus includes annual succulent herbs and shrubs inhabiting mostly open, sunny and arid habitats, and in some cases moist habitats. They are distinctive in having acuminate fleshy leaves, fleshy stems and yellow, yellowish or creamy to white tiny flowers.[1,2] Several *Sedum* species, such as *S. album* L., S. acre L., *S. palaestinum* Bieb, and *S. pallidum* are used in traditional medicine to heal wounds, hemorrhoids, constipation, food fungi, and as laxative and diuretic agents.[3–6]

Previous phytochemical screening of in the genus *Sedum* have shown that the genus is an excellent source of a variety of secondary metabolites including condensed tannins, alkaloids, flavonoids, hydroquinone, arbutin, free sugars, sedoheptose, wax esters, cyanogenic compounds, and triterpenoids.[7–16] However, little is known about the volatile constituents of *Sedum* and their biological activities and, to our knowledge, only two species

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(*Sedum pallidum* var. *bithynicum* and *S. spurium*) growing wild in Turkey have so far been examined for the chemical composition of their essential oils.[6] It has been found that the oil from *S. pallidum* was dominated by caryophyllene oxide (12.8%), *n*-nonanal (9.4%), α-bisabolol (6.8%), β-sesquiphellandrene (4.5%), and β-bisabolene (3.7%), whereas that from *S. spurium* was dominated by hexahydrofarnesyl acetone (15.7%), (*Z*)-phytol (10.2%), δ-cadinene (4.7%), *allo*aromadendrene (3.8%), and geranyl acetone (3.6%).[6] In Jordan, the genus is represented by nine naturally-occurring species. These include *S. laconicum* Boiss. and Heldr., *S. nicaeense* All., *S. microcarpum* (Sm.) Schönl., *S. caespitosum* Cav., *S. hispanicum* L., *S. rubens* L., *S. Pallidum* Bieb., *S. litoreum,* and *S. palaestinum* Boiss.[1]

Sedum microcarpum (Telmissa microcarpa; small-fruited stonecrop) (Sm.) Schönl. is a species of basically the Mediterranean Woodlands and Shrublands, Semi-steppe shrublands, and Shrub-steppes. This species is fairly abundant in the North West part of Jordan and extending, although rarely, to central regions.[1] It is an annual succulent herb growing largely in moist shallow cavities of rock outcrops, and distinguished by its showy red succulent leaves and branches, tiny white flowers, and one-seeded non-dehiscent nut-like fruit.[1,17] In this study, the chemical composition of the hydro-distilled essential oil of *S. microcarpum* was analyzed using GC/MS analysis techniques. Moreover, phytochemical screening of the crude extracts was performed to determine the identity of the major secondary metabolites. The antibacterial activities of these extracts were also evaluated.

MATERIALS AND METHODS

Plant material and fractionations

Whole plants of *S. microcarpum* were collected on February 2010 from natural populations around the city of Irbid, North of Jordan. The plant was identified using regional floristic literature.[18]

Sample treatment

The ground, whole air dried plant material (1.5 kg) was defatted by soaking in petroleum ether at room temperature (7 days) and then repeatedly soaked in ethanol at room temperature (50 L, 5 times, 7 days each). The residue obtained upon removal of ethanol under reduced pressure (107 g) was partitioned between chloroform and water. The dried chloroform layer was further partitioned between 10% aqueous methanol and hexane furnishing the aqueous methanol and hexane extracts. The polar organic compounds were extracted from water using n-butanol.

Isolation of the essential oil

Three fresh *S. microcarpum* samples (600 g powder) were subjected to hydro-distillations for 3.0 h using a Clevenger-type apparatus, yielding *ca.* 0.067% (w/w), pale yellow fragrant oils. Oils were then separately dried over anhydrous sodium sulfate and immediately stored in hexane at 4°C until use for further analysis by gas chromatography/mass spectrometry (GC/MS).

Qualitative analysis

The presence of the secondary metabolites of the tannins, flavonoids, anthocyanins, saponins, coumarines, quinones, anthraquinones and alkaloids was examined according to the methods of Kokate[19] and Harborne.[20] Detection of these phytochemicals was based on the visual observation of color change or formation of a precipitate after the addition of specific reagents. Table 1 summarizes the data of the various tests performed on the butanol and aqueous methanol extracts of *S. microcarpum* from Jordanian origin.

GC-FID analysis

Quantitative analysis of the essential oil was carried out using a Hewlett Packard HP-8590 gas chromatograph equipped with a split-splitless injector (split ratio, 1:50) and an FID detector. An OPTIMA-5 fused silica capillary column (30 m × 0.25 mm, 0.25 μm film thickness) was used. The oil was analyzed under a linear temperature program applied at 3°C/min starting from 60°C through 246°C. Temperatures of the injector and detector were maintained at 250°C and 300°C, respectively. Concentrations of oil ingredients were determined using their relative area percentages obtained from GC chromatogram.

GC-MS analysis

The chemical analysis of the essential oil was carried out using GC-MS (Varian chrompack CP-3800 GC/MS/MS-200 (Saturn, Netherlands). The chromatographic conditions were as follows: column oven program,

Table 1. Phytochemicals detected in extracts of *S. microcarpum.*

Compounds groups	Butanol	Aqueous methanol
Quinones	+	+
Anthocyanins	–	–
Anthraquinones	–	–
Flavonoids	+	+
Tannins	+	+
Alkaloids	+	+
Saponins	–	–
Coumarins	–	–

Key: (+) major class detected; (–): Class not detected

60ºC (1 min, isothermal) to 246ºC (3 min, isothermal) at 3ºC/min; the injector and detector temperatures were 250 and 300ºC, respectively. Helium was the carrier gas (flow rate 0.90 mL/min). An HP-5 MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thicknesses) was used. The actual temperatures in MS source reached 180ºC and the ionization voltage was 70 eV. A hydrocarbon mixture of *n*-alkanes (C_8 – C_{20}) was analyzed separately by GC/MS under same chromatographic conditions using the same HP-5 column.

Compound identification

Experimental retention indices of all the components were determined by Kovats method[21] using *n*-alkane (C_8 – C_{20}) mixture as a standard. Compound identification was achieved by comparing the experimentally obtained RI with those listed in literature,[22] co-injection of individual authentic components including pinenes (α and β), linalool, borneol, *n*-nonane, *n*-decanal, *n*-tetradecane, *n*-pentadecane, *n*-heptadecane, *n*-nanodecane, and by matching the mass spectral data with those held in the National Institute of Standards and Technology (NIST) and Wiley libraries of mass spectra and literature comparison.[22–24]

Antimicrobial study

In vitro antimicrobial activity of essential oil and hexane, butanol and aqueous methanol fractions from *S. microcarpum* were examined against seven different bacterial isolates (obtained from the Department of Biological Sciences, Yarmouk University, Jordan) using two different methods, namely the agar disc diffusion and the agar well diffusion methods. The seven bacterial isolates investigated included three *Gram*-positive bacteria *Bacillus cereus* (ATCC 11778), *Staphylococcus epidermidis* (ATCC 12228), and *Enterococcus faecalis* (ATCC 29212) and four *Gram*-negative bacteria *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 10031), *Serratia marcescens* (ATCC 27117) and *Proteus vulgaris* (ATCC 29905). Bacterial strains were cultured overnight at 37ºC in Trypton Soy broth (TSA).

Antibacterial activity was tested according to Parekh *et al.* (2005) and Vuddhakul *et al.* (2007)[25,26] with a slight modification. The molten Mueller Hinton agar was inoculated with the inoculums (1×10^8 CFU/ml) and poured into Petri plates. For agar disc diffusion method, the disc (0.7 cm) was saturated with 50 μg/mL of the test extract, dried, and introduced on the upper layer of the seeded agar plate. For agar well diffusion method, six equidistant wells (7 mm in diameter) were cut from the agar using a cork-borer. The test extract (50 μg/mL) was introduced into the well. The plates were incubated overnight at 37ºC. Microbial growth was determined by measuring the diameter of the inhibition zone. For each bacterial strain, controls were maintained and pure solvents were used. At the end of the period, all plates were examined for any zones of growth inhibition and the diameters of these zones were measured in millimeters. All tests were performed in duplicate and the results were shown as mean values.

A dilution agar method was used to determine the minimum inhibitory concentrations (MIC). Stock solutions were obtained by dissolving extracts in dimethylsulfoxide (DMSO 1%). Serial dilutions were made to obtain concentrations ranging from 0 to 1000 μg mL^{-1} for each of the essential oil, hexane, butanol and aqueous methanol fractions. Each mixture was added to Mueller–Hinton agar.[27,28] The Petri dishes contained a sterile solution of DMSO and the culture medium, respectively, after incubation at 37ºC for 24 h. The experiments were performed in triplicate.

RESULTS AND DISCUSSION

Table 1 shows the presence of various types of secondary metabolites in *S. microcarpum*, including quinones, flavonoids, tannins and alkaloids. Anthocyanins, anthraquinones, saponins, and coumarins were not detected in both the butanol and aqueous methanol fractions. Hydrodistillation of the fresh plant material yielded 40.0 mg of pale yellow oil, with an output of 0.067% (w/w). The general chemical profile of the essential oil, the percentage content, the retention indices of the constituents and the chemical class distributions of the essential oil components of the plant are summarized in Table 2. The compounds were separated into nine classes and were dominated by oxygenated monoterpenoids (36.39%). The remaining classes included aliphatic monoterpenes (1.64%), sesquiterpenes (8.70%), sesquiterpenoids (19.80%), alkaloids (2.18%), aldheydes (8.94%), alcohols (6.87%), alkanes (10.25%), and others (2.41%). The major compounds found in the essential oil of *S. microcarpum* were Myrtenol (21.7%), Caryophyllene oxide (8.2%), (Z)-1,6-diol dihydromyrcene (5.87%) decanol (4.91%), n-heptadecane (4.59%) and n-decanal(4.44%). Yayli et al. (2010) examined the constituents of essential oil from *S. pallidum* var. *bithynicum* and *S. spurium*. They found that the oil from *S. pallidum* was dominated by caryophyllene oxide (12.8%), *n*-nonanal (9.4%), α-bisabolol (6.8%), β-sesquiphellandrene (4.5%), and β-bisabolene (3.7%), whereas that from *S. spurium* was dominated by hexahydrofarnesyl acetone (15.7%), (Z)-phytol (10.2%), δ-cadinene (4.7%), *allo*aromadendrene (3.8%), and geranyl acetone (3.6%).[6] In this study, components of the essential oil from *S. microcarpum* were slightly different (Table 2). Results

Table 2. Identified components in the essential oils of *S. microcarpum* from Jordan, (values given are average of three independent measurements).

No.	RI[b]	Compound[a]	% Area	Identification method[c]
1-	804	Hexanal	1.25	MS, RI
2-	836	Furfural	0.39	MS, RI, Co
3-	861	3z-Hexenol	0.73	MS, RI
4-	907	n-Nonane	0.53	MS, RI, Co
5-	941	α-Pinene	0.45	MS, RI, Co
6-	981	β-Pinene	1.19	MS, RI,
7-	997	2-Pentyl furan	0.16	MS, RI,
8-	1073	*trans*-Linalool oxide	0.18	MS, RI,
9-	1090	*cis*- Linalool oxide	1.29	MS, RI,
10-	1103	Linalool	0.15	MS, RI, Co
11-	1108	n-Nonanal	1.43	MS, RI, Co
12-	1134	Terpineol	1.94	MS, RI,
13-	1150	Myrcenone	0.71	MS, RI,
14-	1202	Myrtenol	21.7	MS, RI, Co
15-	1204	n-Decanal	4.44	MS, RI,
16-	1208	Borneol	0.46	MS, RI, Co
17-	1231	Methyl -3,6-octadecanoate	0.11	MS, RI,
18-	1236	Nerol	0.21	MS, RI,
19-	1254	Geraniol	1.34	MS, RI,
20-	1265	Linalyl acetate	1.15	MS, RI,
21-	1274	Decanol	4.91	MS, RI,
22-	1298	n-pentyl pyrole	2.18	MS, RI,
23-	1305	4α-methyl-1,2,3,4α,5,6,7-octahydronaphthalene	0.40	MS, RI,
24-	1316	*p*-Guaiacol	1.43	MS, RI,
25-	1352	Thymol acetate	1.20	MS, RI,
26-	1360	Eugenol	0.61	MS, RI,
27-	1392	Vanillin	0.58	MS, RI, Co
28-	1397	n-Tetradecane[b]	1.83	MS, RI,
29-	1443	*trans*-β-5,6-epoxide ionone	3.44	MS, RI,
30-	1460	Alloaromadendrene	2.95	MS, RI,
31-	1462	Dehydroaromadendrane	1.52	MS, RI,
32-	1487	Germacrene D	0.12	MS, RI,
33-	1500	n-Pentadecane	0.36	MS, RI,
34-	1517	Menthyl pentanoate	0.19	MS, RI,
35-	1541	α-Calacorene	0.67	MS, RI,
36-	1558	Elemicin	0.10	MS, RI,
37-	1563	Ledol	0.40	MS, RI,
38-	1578	Caryophyllene oxide	8.20	MS, RI,
39-	1600	Pentadecanal	0.85	MS, RI,
40-	1619	epi-Cedrol	1.91	MS, RI,
41-	1622	Z-1, 6-diol-Dihydromyrcene	5.87	MS, RI,
42-	1664	Dihydro Eudesmol	0.38	MS, RI,
43-	1674	Caryophyllenol-II	2.58	MS, RI,
44-	1680	Khusinol	2.43	MS, RI,
45-	1687	α-Bisabalol	0.90	MS, RI,
46-	1687	8-cerden-13-ol	0.18	MS, RI,
47-	1694	*tans*-α-Bergamotal	0.14	MS, RI,
48-	1700	n-Heptadecane	4.59	MS, RI, Co
49-	1718	Curcuman-15-ol	0.13	MS, RI,
50-	1718	2Z,6Z-Farnesol	0.17	MS, RI,
51-	1772	n-Pentadecanol	1.23	MS, RI,
52-	1820	Acorone	0.25	MS, RI,
53-	1843	6,10,14-trimethyl-2-Pentadecanone	1.76	MS, RI,
54-	1900	n-Nanodecane	2.54	MS, RI, Co
55-	1916	Farnesyl acetone	0.37	MS, RI,

(Continued)

No.	RI[b]	Compound[a]	% Area	Identification method[c]
		Total identified	97.18	
		Monoterpenes hydrocarbon	1.64	
		Oxygenated monoterpenoids	36.39	
		Sesquiterpene hydrocarbons	8.70	
		Oxygenated Sesquiterpenoids	19.80	
		Alkaloids	2.18	
		Aldehydes	8.94	
		Alcohols	6.87	
		Alkanes	10.25	
		Others	2.41	

[a]Compounds are listed in order of their elution from a HP-5 column. [b]RI, retention index on HP-5MS column, experimentally determined using homologous series of C_8–C_{20} alkanes. [c]Identification methods: MS, by comparison of the mass spectrum with those of the computer mass library Wiely7Nist; RI, by comparison of RI with those reported in literature; Co, co-injection with authentic compound.

indicate that the essential oil of *S. microcarpum* shares some different components with other species of *Sedum* (*S. pallidum* var. *bithynicum* and *S. spurium*) which can be explained by the environmental factors.

The antimicrobial activities of the hexane, butanol and aqueous methanol fractions of *S. microcarpum* were assayed in vitro by the agar dilution method[25,26] against seven bacterial strains. The bacterial species were chosen as representatives of two major groups of microorganisms: *Gram*-positive bacteria and *Gram*-negative bacteria. The results are presented in Tables 3 and 4. No inhibitory effects of the hexane fraction extract against the seven bacterial strains were found. However, moderate antibacterial activity was shown by butanol and aqueous methanol extracts against *Staphylococcus epidermidis, Enterococcus faecalis*, *Escherichia coli, Klebsiella pneumoniae* and *Serratia marcescens*, but no activity against *Proteus vulgaris* and *Bacillus cereus* was observed. The antibacterial activity of the essential oil from *S. microcarpum* was also evaluated on the seven bacterial species. Moderate activities against all tested organisms compared to

Table 3. Antimicrobial activity of extracts and essential oil from *S. microcarpum*.

Microorganisms	Inhibition Zone (mm)					
	Hexane extract 1000 µg/mL	Butanol extract 1000 µg/mL	Aq-MeOH extract 1000 µg/mL	Essential oil 1000 µg/mL	Amoxicillin 100 µg/mL	Cephalexin 100 µg/mL
E. coli (ATCC 25922)	–	12	10	14	32	35
S. marcescens (ATCC 27117)	–	10	8	12	20	22
K. pneumoniae (ATCC 10031)	–	10	9	17	18	21
P. vulgaris (ATCC 29905)	–	–	–	10	18	23
B. cereus (ATCC 11778)	–	–	–	23	22	29
E. faecalis (ATCC 29212)	–	11	19	–	24	33
S. epidermidis (ATCC 12228)	–	13	21	26	30	35

Table 4. Minimal inhibitory concentration (MIC) (µg mL^{-1}) of extracts and essential oil from *S. microcarpum*.

Microorganisms	(MIC) (µg mL^{-1})			
	Hexane extract	Butanol extract	Aq-MeOH extract	Essential oil
E. coli (ATCC 25922)	–	625	650	500
S. marcescens (ATCC 27117)	–	700	750	650
K. pneumoniae (ATCC 10031)	–	700	750	350
P. vulgaris (ATCC 29905)	–	–	–	700
B. cereus (ATCC11778)	–	–	–	250
E. faecalis (ATCC 29212)	–	600	500	–
S. epidermidis (ATCC 12228)	–	625	350	200

reference antibiotics Amoxicillin and Cephalexin were observed.

The MIC values for the butanol, aqueous methanol, and essential oil extracts from *S. microcarpum* against all tested bacterial strains were determined and the results are shown in Table 4. Values ranged from 600–700 µg/mL for the butanol fraction, 350–750 µg/ml for the aqueous methanol fraction, and 200–700 µg/mL for the essential oil fraction. These data indicate that essential oil possesses striking inhibitory effects compared to butanol and aqueous methanol extracts. The MIC values obtained in this study support findings of the agar diffusion method. The MIC values of the aqueous methanolic and essential oil extracts exhibited the best inhibitory results against *Staphylococcus epidermidis* ATCC 12228, *Bacillus cereus* ATCC11778 and *Klebsiella pneumoniae* ATCC 10031.

CONCLUSIONS

The present study provides an evidence supporting the different chemical composition of the essnetial oil from different *Sedum* species, due to most likely climatic and genetic constraints. *S. microcarpum* extracts possess a broad spectrum of activity against a panel of bacterial strains responsible for the most common diseases. Further research is needed to determine the identity of the antibacterial compounds from this species and also to determine their spectrum of efficacy. However, the present study of *in vitro* antimicrobial evaluation of *Sedum microcarpum* extracts forms a primary platform for further phytochemical and pharmacological studies.

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