SHORT REPORT

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Rec. Nat. Prod. 6:1 (2012) 57-61

records of natural products

Secondary Metabolites from *Halostachys caspica* and Their Antimicrobial and Antioxidant Activities

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(Received December 24, 2010; Revised March 26, 2011; Accepted March 28 2011)

Abstract: Nine secondary metabolites have been isolated from the aerial parts of *Halostachys caspica* C. A. Mey. (Chenopodiaceae). By means of physicochemical and spectrometric analysis, they were identified as betaine (**1**), diphenylamine (**2**), benzyl-*O*-β-D-glucopyranoside (**3**), β-sitosterol (**4**), 4-hydroxy-3-methoxy benzoic acid (**5**), 4-hydroxy benzoic acid (**6**), 2-hydroxy benzoic acid (**7**), 4-hydroxy-3,5-dimethoxy benzoic acid (**8**), and 3,4-dihydroxy benzeneacrylic acid (**9**). All compounds were isolated from this plant species for the first time. They were screened to exhibit antimicrobial and antioxidant activities to some extent except for the compounds **1** and **3**. The results indicated that the isolated phenol acids and diphenylamine (**2**) could be the main bioactive components in the crude ethanol extract of *H. caspica*.

Keywords: Chenopodiaceae; *Halostachys caspica*; secondary metabolites; antimicrobial activity; antioxidant activity.

1. Plant Source

Halostachys caspica C. A. Mey. belongs to Chenopodiaceae family and is mainly distributed in the Provinces of Xinjiang and Gansu of Northwest China. It has been used as forage with a high yield and good nutrition in desert area [1].

The aerial parts of *H. caspica* were collected in August 2007 at Shihezi of Xinjiang Province of China, and was authenticated by Professor Ping Yan of Shihezi University of Xinjiang. A voucher specimen of this collection (BSMPMI-200708001) was deposited at the Herbarium of the Institute of Chinese Medicinal Materials, China Agricultural University. The plant materials were left to dry in the shade at room temperature to a constant weight.

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The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 08/ 05/2011 EISSN:1307-6167

2. Previous Studies

The lipids and flavonoids have been reported from *H. caspica* [2,3]. Our previous studies showed that the crude ethanol extract of the aerial parts of *H. caspica* exhibited an obvious antimicrobial activity [4], and seven flavonoids with antimicrobial and antioxidant activities have been isolated by bioassay-guided fractionation [3].

3. Present Study

The air-dried and powdered aerial parts (7.23 kg) of *H. caspica* were soaked three times in 95% ethanol (30 L) at room temperature for an interval of 10 days. After the combined filtrate was concentrated under vacuum at 50 °C, the brown residue (1640 g) was suspended in water. It was extracted with petroleum ether, then with EtOAc, and last with *n*-BuOH. The EtOAc extract (41.96 g) was subjected to silica gel column chromatography (CC) with a gradient of $CHCl_3$-MeOH (from 1:0 to 1:1, v/v) as an eluent, and six fractions (A, B, C, D, E and F) were collected according to TLC examining. Each fraction was further chromatographed repeatedly over silica gel, Sephadex LH-20 and reverse phase (RP-18) CC. Compounds **2** (20 mg) was separated from fraction A; **4** (12 mg) from fraction B; **1** (9 mg), **3** (17 mg), and **8** (17 mg) from fraction C; **5** (21 mg), **6** (14 mg), **7** (36 mg), and **9** (13 mg) from fraction D. After comparing the physicochemical and spectrometric data of the compounds (**1**-**9**) with those reported in literatures, they were known compounds and confirmed as betaine (**1**) [5], diphenylamine (**2**) [6], benzyl-*O*-β-D-glucopyranoside (**3**) [7], β-sitosterol (**4**) [8], 4-hydroxy-3-methoxy benzoic acid (**5**) [9], 4-hydroxy benzoic acid (**6**) [10], 2-hydroxy benzoic acid (**7**) [11], 4-hydroxy-3,5-dimethoxy benzoic acid (**8**) [12], and 3,4-dihydroxy benzeneacrylic acid (**9**) [13,14], which structures are shown in Figure 1.

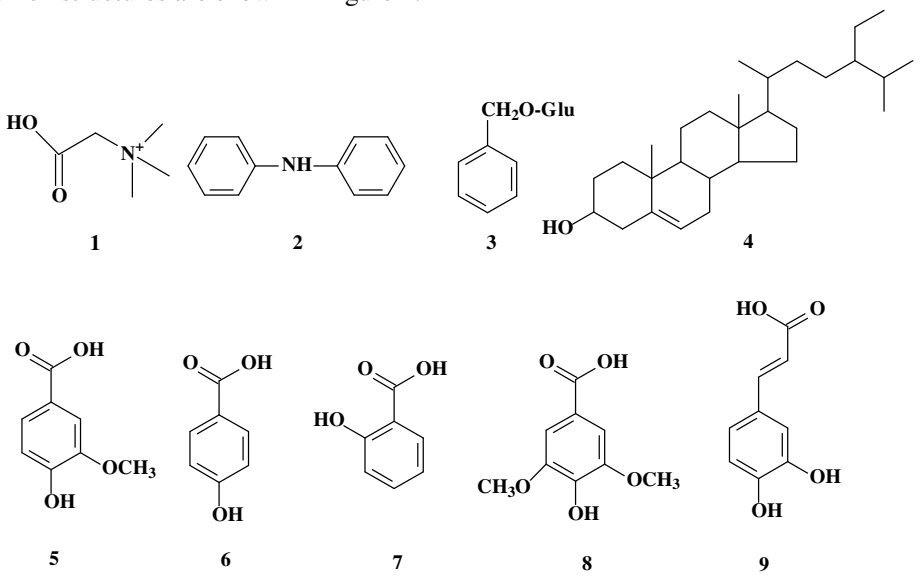

Figure 1. Chemical structures of the compounds **1**-**9**

Four Gram-negative (*Agrobacterium tumefaciens* ATCC 11158, *Escherichia coli* ATCC 29425, *Pseudomonas lachrymans* ATCC 11921 and *Xanthomonas vesicatoria* ATCC 11633) and three Gram-positive (*Bacillus subtilis* ATCC 11562, *Staphylococcus aureus* ATCC 6538 and *Staphylococcus haemolyticus* ATCC 29970) bacteria were selected for antibacterial activity assay by using the chromogenic reagent 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) [15]. The spore germination assay by using rice blast fungus *Magnaporthe oryzae* (strain P131) was employed to detect the antifungal activity of the compounds [16].

Table 1. MIC values of the compounds from *H. caspica* on test microorganisms.

Test Microorganism	MIC (μg/mL)									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	CK^+
A. tumefaciens	nd	200	nd	200	200	200	100	100	100	20
E. coli	nd	200	nd	200	100	200	100	200	50	20
P. lachrymans	nd	200	400	200	100	100	200	100	50	20
X. vesicatoria	400	200	nd	100	100	100	100	100	50	20
B. subtilis	nd	100	nd	200	200	200	200	100	100	10
S. aureus	nd	200	nd	200	100	200	200	200	100	100
S. haemolyticus	200	200	nd	400	100	200	100	100	100	20
M. oryzae	400	250	400	400	400	250	250	250	200	100

Note: The positive controls (CK^+) on bacteria and *M. oryzae* were streptomycin sulfate and carbendazim, respectively. The 'nd' means not detected.

Table 2. IC_{50} values of the compounds from *H. caspica* on test microorganisms.

Test Microorganism	IC_{50} (μg/mL)									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	CK^+
A. tumefaciens	nd	112.41	nd	106.62	83.23	127.07	37.80	40.35	66.82	8.34
E. coli	nd	180.63	nd	115.42	36.80	89.96	49.72	101.56	22.76	10.47
P. lachrymans	nd	106.56	230.39	96.62	89.27	76.22	135.61	79.84	30.19	9.01
X. vesicatoria	222.83	104.02	nd	73.27	63.10	67.52	72.64	82.32	32.33	11.62
B. subtilis	nd	62.59	nd	122.75	109.93	149.67	109.95	83.97	52.59	4.98
S. aureus	nd	112.41	nd	97.96	46.40	109.59	83.37	131.21	39.65	78.60
S. haemolyticus	113.25	89.93	nd	226.97	33.37	133.33	63.89	43.99	50.40	7.75
M. oryzae	266.79	153.87	303.26	206.57	157.57	167.54	90.17	170.88	90.32	38.45

Note: The same as Table 1.

The MIC and IC_{50} values of the compounds are summarized in Tables 1 and 2, respectively. 3,4-Dihydroxy benzeneacrylic acid (**9**) was the most active compound with the IC_{50} values ranging from 22.76 μg/mL to 66.82 μg/mL on the test bacteria, and 90.32 μg/mL on the spore germination of *Magnaporthe oryzae*, respectively. 4-Hydroxy-3-methoxy benzoic acid (**5**) was screened to show strong antimicrobial activity on *Escherichia coli*, *Staphylococcus haemolyticus* and *Staphylococcus aureus* with the IC_{50} values as 36.80 μg/mL, 33.37 μg/mL and 46.40 μg/mL, respectively. The other phenol acids (i.e. **6**, **7**, and **8**) also showed a broad antimicrobial spectrum of activity. For two alkaloids, diphenylamine (**2**) was more active than betaine (**1**) that just showed a limited antimicrobial spectrum of activity.

Both the radical scavenging on DPPH reduction and β-carotene-linoleic acid bleaching assays were employed to evaluate antioxidant activity of the compounds [17]. The IC_{50} values of the compounds are summarized in Table 3. By using radical scavenging assay, 2-hydroxy benzoic acid (**7**) and 3,4-dihydroxy benzeneacrylic acid (**9**) were the most active compounds with IC_{50} values of 35.66 μg/mL and 46.62 μg/mL, respectively. By using β-carotene-linoleic acid bleaching assay, diphenylamine (**2**), 2-hydroxy benzoic acid (**7**), hydroxy-3,5-dimethoxy benzoic acid (**8**), and 3,4-dihydroxy benzeneacrylic acid (**9**) were the most active compounds with IC_{50} values of 10.99 μg/mL, 36.21 μg/mL, 29.03 μg/mL, and 26.23 μg/mL, respectively.

In general, we first reported nine secondary metabolites mainly including five phenol acids and two alkaloids from *H. caspica* to exhibit antimicrobial and antioxidant activities to some extent. They could be the main bioactive components in the crude ethanol extract of *H. caspica*. The results provided additional data for future development and utilization of *H. caspica*.

Table 3. Antioxidant activity of the compounds from *H. caspica*.

Assay	IC_{50} (μg/mL)									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	CK^+
DPPH inhibition	537.66	274.65	601.59	186.92	116.82	63.67	35.66	110.59	46.62	18.80
β-Carotene bleaching	nd	10.99	72.31	225.37	68.75	128.88	36.21	29.03	26.23	31.46

Note: The positive control (CK^+) was BHT. The 'nd' means not detected.

Acknowledgments

This work was co-financed by the grants from the Specialized Research Fund for the Doctoral Program of Higher Education of China (20100008110037), the Hi-Tech R&D Program of China (2011AA10A202), and the Special Fund for Agro-Scientific Research in the Public Interest (200903052).

Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/RNP

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