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Chemical Composition of Desert Willow (*Salix psammophila*) Grown in the Kubuqi Desert, Inner Mongolia, China: Bark Extracts Associated with Environmental Adaptability

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Supporting Information

ABSTRACT: Bark of desert willow (*Salix psammophila,* Spsa) grown in Inner Mongolia was successively extracted with *n*-hexane, diethyl ether, acetone, methanol, and hot water to examine chemical components associated with its environmental adaptability to desert conditions. The yield of *n*-hexane extract (5.0% based on dry bark), mainly composed of wax, was higher than those of acetone and methanol extracts (3.7% and 4.2%, respectively), whereas the yields of *n*-hexane extract (1.4%) from willow bark grown in humid areas were much lower than those of acetone (17.4% and 19.9%) and methanol (12.5% and 14.0%) extracts. Unlike other willow bark samples, Spsa bark contained a certain amount of sugar alcohols. In particular, we identified arabinitol (0.21%), which has not previously been reported as a major component of extracts of willow bark. The high content of wax and sugar alcohol would be associated with the ability of Spsa to survive in desert conditions. Accumulation of wax on the outer bark surface would reduce water loss, while sugar alcohols might improve freezing tolerance.

KEYWORDS: *Salix psammophila, bark extracts, environmental tolerance, sugar alcohols, wax*

INTRODUCTION

Willow is a highly diversified genus of trees, consisting of more than 350−400 species.[1] In general, willow can be found in humid environments in subarctic and temperate areas. However, some willows grow in areas with high environmental stress such as tundra and arid lands.[2,3] Desert willow (*Salix psammophila*) grows in northwestern China and has a high tolerance to environmental stresses such as severe drought and large temperature changes of the ground surface from −30 to 60 °C.[4,5] Desert willow has a well-developed root system, which can fix wind-blown sand on the ground surface.[6,7] Therefore, desert willow has been used for desert greening in the Kubuqi Desert because of their favorable environmental adaptability and adequacy as an afforestation plant.[8] As with most other willow genera, desert willow grows quickly even in arid lands, with an average height of 4.8 m after the first 3 years. Harvestable biomass can also reach 5.2 t/ha in 3 years; however, the growth rate of desert willow decreases and the tree dies approximately 3−5 years after planting without management.[8,9] Therefore, it is necessary to harvest the parts of desert willow above ground every 3−5 years to allow it to regenerate by sprouting for sustainable management with high productivity.[4,8] Harvested willow has traditionally been used as a fuel, animal fodder, and raw material of willow crafts. Recently, value-added industrial materials such as fiber boards and wood pulp have been produced from desert willow, and other potential uses such as production of bioethanol have also been reported.[10−12]

In some cases, plants grown under stress accumulate stress resistance components such as specific physiologically active substances.[13,14] In fact, the bark and roots of desert willow have been used in China as herbal medicines with antipyretic and antiphlogistic effects, which indicates that desert willow may contain specific chemicals with physiological activity.[4,5] Although a number of reports have been published about the chemical components of the willow genus, few have discussed those specifically from desert willow.[15] Therefore, in this manuscript, we determine the chemical components of bark extracts of desert willow, especially those associated with environmental adaptability for survival in a desert.

MATERIALS AND METHODS

Materials. Three willow species were used in this research. Two-year-old desert willow (*Salix psammophila*, Spsa) was harvested from the Kubuqi Desert, Inner Mongolia, China, and the willows Onoe (*Salix sachalinensis*, Ssac) and Ezonokinu (*Salix pet-susu*, Spet), which are two of the most common willows found near rivers in Japan, were harvested from Shimokawa, Hokkaido, Japan. The willow samples were manually fractionated into bark and xylem, and both fractions

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were separately powderized to less than 2 mm using a cutter mill. All chemicals used in this research were research grade and purchased from Wako Pure Chemicals, Japan.

Chemical Composition of Desert Willow. The contents of ethanol/benzene (1:2 v/v) extracts and Klason lignin were determined in accordance with TAPPI standards T 204 cm-07 and T 222 om-11, respectively. Klason lignin content was not corrected for acid-soluble lignin because the gram UV absorbance of the bark samples was not evaluated. Holocellulose content was measured by the Wise method.[16] The delignification process was repeated four times for all samples. The contents of flavan-3-ols and total phenols were determined by vanillin−HCl assay and Folin−Ciocalteu method for 70% acetone extracts, respectively.[17,18] Chemical composition data shown are the average of two different runs.

Solvent Extractions. Powderized barks were successively extracted with *n*-hexane, diethyl ether, acetone, and methanol by Soxhlet extraction for 4 h for each solvent. Extracted sample (2 g) was boiled in water (400 mL) and then filtered through a filter crucible (pore size of 10−16 μm) to extract the water-soluble fraction. All extracts were separately evaporated and dried at 40 °C in a vacuum oven over P_2O_5 to determine the extracted yield. Yields of extracts are shown as the average of three different runs.

Analysis. Attenuated total reflection/Fourier transform infrared (ATR FT-IR) analysis of bark samples was performed using a Nicolet 6700 FT-IR (Thermo Fisher Scientific, Waltham, MA, USA) spectrometer equipped with a MIRacle single reflection ATR apparatus with a ZnSe window (PIKE Technologies, Madison, WI, USA). In FT-IR measurements, 256 scans were collected with a spectral resolution of 4.0 cm^{-1}. High-performance liquid chromatography (HPLC) analysis of phenolic extracts was performed using a Shimadzu Prominence HPLC instrument (Shimadzu, Japan). Gradient elution starting with 10 mM H_3PO_4 (97%)−MeOH/MeCN (1:1 v/v) (3%) was used in the C18 ODS column (CERI, L-column2 ODS 4.6 mm × 150 mm), and the content of MeOH/MeCN was increased linearly to 37.5% at 39 min in the HPLC run. Sugar alcohols extracted with water were quantified by an HPLC instrument equipped with a DECADE II pulsed electrochemical detector (ANTEC Leyden, The Netherlands). In this HPLC run, a DIONEX MA-1 column (4 mm × 250 mm) was used with 0.48 M NaOH as the eluent. The major components in the acetone and MeOH extracts were isolated by preparative HPLC using a semipreparative column (CERI, L-column2 ODS 20 mm × 250 mm) and column chromatography with Sephadex LH-20 gel, respectively, for structural analysis. All NMR spectra were recorded using a α-500 NMR spectrometer (JEOL, Japan). Fast atom bombardment mass spectrometry (FAB-MS) was conducted on a HX-110A spectrometer (JEOL, Japan) using glycerol as a matrix. Optical rotations were measured on a P-1020 polarimeter (Jasco, Japan) at room temperature (~25 °C).

RESULTS AND DISCUSSION

Chemical Composition of Willow Samples. Results of the compositional analysis of bark and xylem of Spsa are listed in Table 1. The contents of ethanol/benzene extract, holocellulose, and Klason lignin in xylem of Spsa were 3.1%, 79.1%, and 20.4%, respectively, which are comparable to those for Ssac (2.4%, 81.5%, and 20.6%, respectively) and Spet (2.5%, 83.3%, and 20.3%, respectively).[19] The holocellulose content of Spsa bark was similar to those of Ssac and Spet. In contrast, the content of ethanol/benzene extract in Spsa bark (10.9%) was somewhat lower, and the Klason lignin content (31.7%) was higher than the equivalent values for bark of Ssac (13.6% and 23.8%, respectively) and Spet (13.6% and 26.5%, respectively). Lignin would have various functions in plant such as light and drought tolerance components.[20] However, the Klason lignin of bark samples does not represent actual lignin content because condensed tannin and other compounds such as sulfuric acid insoluble suberin are included in the Klason lignin content.[21] Therefore, we did not discuss the differences in the lignin content of willows in this manuscript. By contrast, the quantitative difference in the ethanol/benzene extracts might indicate that the chemical composition of Spsa bark extract differs from those of Ssac and Spet. It has been reported that the bark of willows grown in humid areas including Ssac and Spet contain various phenolic glycosides, phenolic compounds including condensed tannin, and sugar esters as major components.[22−25] To compare the contents of these polar components in Spsa bark with those in other willow samples, bark samples were also extracted with 70% acetone. The yield of 70% acetone extracts of Spsa bark (12.2%) was much lower than those for Ssac (27.5%) and Spet (30.0%) (Table 2). In general, aqueous acetone or methanol can be used to extract polyphenols, glycosides, and other polar compounds from lignocelluloses. Therefore, the low yield of the 70% acetone extract of Spsa bark indicated that this sample contained lower contents of condensed tannins and/or glycosides than the other samples. To confirm this, the contents of flavan-3-ols of the bark samples were measured by the vanillin−HCl assay using the 70% acetone extracts. The content flavan-3-ols of Spsa bark (1.7%) was much lower than those in bark from Ssac (12.6%) and Spet (15.2%). The vanillin−HCl assay preferentially detects flavan-3-ols containing a phloroglucinol substructure, such as condensed tannin.[26] Therefore, the low value obtained for the vanillin−HCl assay indicates that Spsa bark has a low content of condensed tannin. Interestingly, the content of flavan-3-ols of Spsa bark was almost the same as that for total phenols determined by the Folin−Ciocalteu method (1.6%). This result indicates that the contents of phenolic compounds other than flavan-3-ols are very low in Spsa bark, although some polyphenols with high molecular weight cannot be extracted with 70% acetone.[27,28] Therefore, the chemical composition of Spsa bark differs both qualitatively and quantitatively from those of Ssac and Spet. Tannin is known as an insect and animal antifeedant.[29−31] The low content of condensed tannin

Table 1. Chemical Composition of Bark and Xylem of Desert Willow (*Salix psammophila*)[a]

sample	extract[b]	holocellulose	Klason lignin
xylem	3.1	79.1	20.4
bark	10.9	58.1	31.7

[a]All values are shown in % based on dry samples. Holocellulose might contain small amount of lignin residues. [b]Ethanol/benzene (1/2 v/v) extracts.

Table 2. Yields of 70% Acetone Extracts, Flavan-3-ols, Total Phenol Contents, and Successive Extracts of Willow Bark Samples[a]

	S. psammophila	*S. sachalinensis*	*S. pet-susu*
70% acetone	12.2	27.5	30.0
flavan-3-ols	1.7	12.6	15.2
total phenol	1.6	10.3	11.3
n-hexane	5.0 ± 0.0	1.4 ± 0.0	1.4 ± 0.0
diethyl ether	0.9 ± 0.0	0.5 ± 0.1	0.4 ± 0.1
acetone	3.7 ± 0.2	19.9 ± 0.0	17.4 ± 0.1
methanol	4.2 ± 0.1	14.0 ± 0.0	12.5 ± 0.5
hot water	7.1 ± 0.2	8.9 ± 0.4	9.4 ± 0.6

[a]All values are shown in % based on dry samples.

in Spsa bark might be one reason why Spsa has been used as a fodder for livestock.

Successive Solvent Extraction of Willow Bark Samples. To examine the bark extracts in more detail, Spsa bark was extracted successively with various solvents. The extracted yields are also listed in Table 2, along with the equivalent data for bark from Ssac and Spet. The total extracted yield for Spsa (20.9%) was almost half of those for Ssac (44.7%) and Spet (41.1%), although the quantitative difference in ethanol/benzene extract between Spsa and Spet was 10.9%/13.6%. Ethanol/benzene is a common solvent mixture used to remove extracts from various lignocellulosic samples and is generally capable of extracting a wide range of chemicals. However, polar polymeric compounds such as condensed tannin and oligosaccharides are not extracted efficiently by this mixture.[32] Therefore, one reason for the high extraction yield from successive extraction would be the higher extraction efficiency of individual solvents for selected components. As mentioned above, the yields of 70% acetone extracts including condensed tannin were much lower for Spsa bark than those for Ssac and Spet. Therefore, the low yield for the total extraction of Spsa might be caused by the low content of condensed tannin in Spsa bark.

The extracted yields for Ssac and Spet bark with nonpolar solvents *n*-hexane and diethyl ether were lower than those with polar solvents acetone and methanol. The extracted yields for Ssac and Spet bark were almost the same for all solvents. In contrast, in the case of Spsa bark, the yield of *n*-hexane extracts was higher than those for acetone and methanol extracts. A ^{13}C NMR spectrum of the *n*-hexane extracts of Spsa bark contained an intense signal at 30 ppm and some small signals at higher magnetic field (Figure 1A). DEPT (distortionless enhancement by polarization transfer) analysis of this fraction allowed the intense signal to be assigned to methylene carbon (Supporting Information, Figure S1). This indicates that the major component in the *n*-hexane extract of Spsa bark is wax. A ^{13}C NMR spectrum of the diethyl ether extract (Figure 1B) gave a profile similar to that for the *n*-hexane extract, although the yield for the diethyl ether extract was low. The sum of the yields of *n*-hexane and diethyl ether extract was 5.9%, which was close to 30% of the total amount extracted. To estimate the location of wax accumulated in Spsa bark, the outer and inner regions of Spsa back were investigated by ATR FT-IR analysis. The FT-IR spectra of the outer bark contained characteristic bands at 2917 and 2849 cm^{-1} with weak/intermediate bands at 1464 and 719 cm^{-1}, which could be assigned to alkyl groups (Figure 2A). In contrast, clear bands were not detected at the corresponding wavenumbers in the FT-IR spectrum of the inner region of Spsa bark (Figure 2B). These FT-IR results indicate that wax accumulated on the outer region of Spsa bark. Other bands were detected at 1736 and 1165 cm^{-1} in the spectrum of outer bark, which were consistent with nonconjugated ester and/or carbonyl, and ether groups, respectively. A broad band observed at 1613 cm^{-1} in the spectrum of the inner bark sample was attributed to alkenes. The intensity of the broad OH stretching band was weaker for the outer bark sample than for the inner one. This indicates that the content of polar hydroxyl groups in the outer bark sample was lower than that of the inner bark. The water content of a stem of green Spsa is reported to be ~35%, which is lower than those for Ssac and Spet (50−70%).[19,33] The accumulation of wax as well as the low content of polar functional groups on the outer bark surface of Spsa would improve its drought tolerance by minimizing water loss through transpiration from the surface of the stem in arid environments.

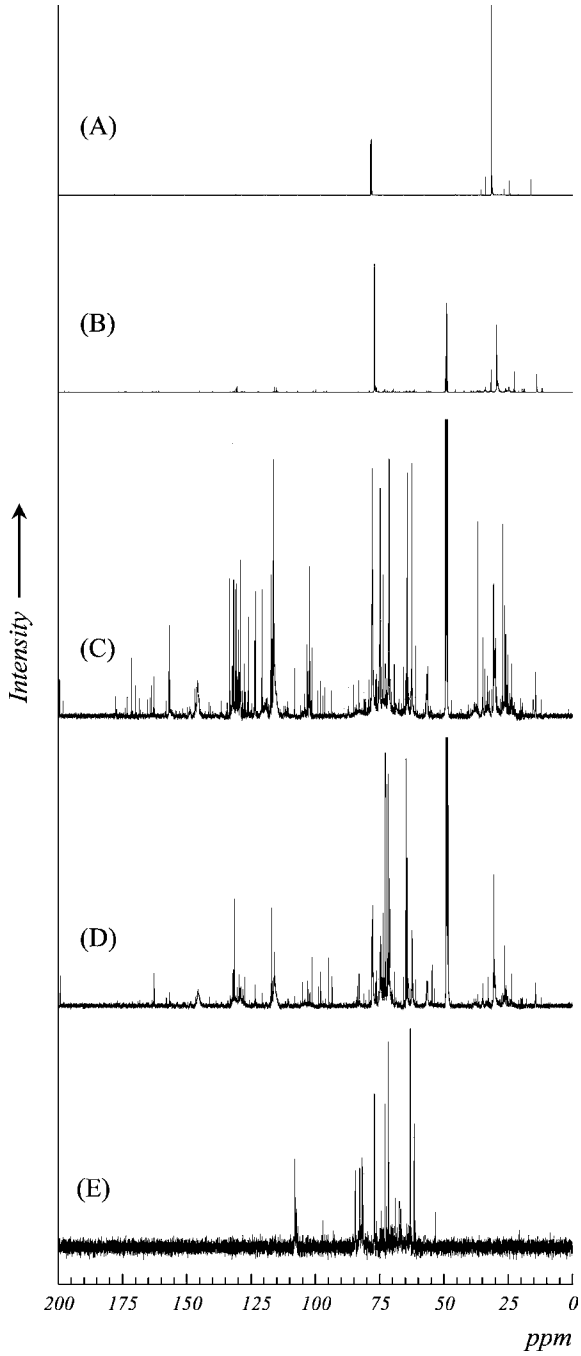

Figure 1. ^{13}C NMR spectra for extracts from desert willow bark: (A) *n*-hexane extract (NMR solvent = $CDCl_3$); (B) diethy ether extract ($CDCl_3$/MeOH-d_4 = 9:1); (C) acetone extract (MeOH-d_4); (D) methanol extract (MeOH-d_4); (E) hot water extract (D_2O).

Identification of Compounds in Acetone and Methanol Extracts. NMR spectra of the acetone and methanol extracts suggested that both extracts contained sugars (60−105 ppm) and aromatic (110−150 ppm) compounds (Figure 1C and Figure 1D). The ^{13}C NMR spectra revealed that the acetone extract was rich in aromatic structures, while the methanol extract contained more sugars than the acetone one. However, some signals appeared at the same chemical shift in

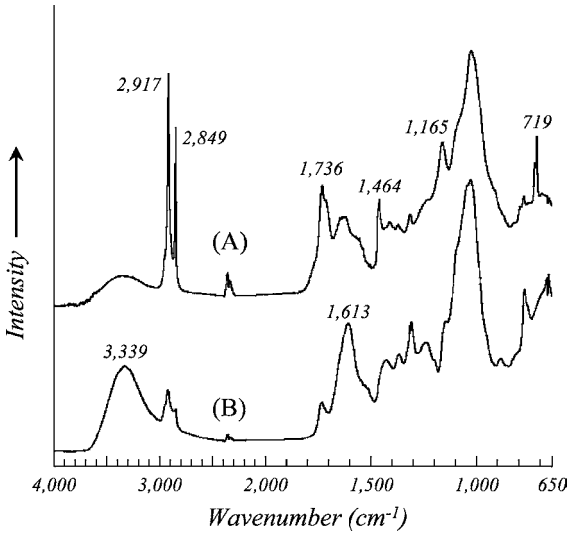

Figure 2. ATR FT-IR spectra of desert willow bark: (A) outer bark; (B) inner bark.

both spectra, which indicated that similar compounds were present in both fractions.

HPLC analysis of the acetone extracts (detected at UV_{270}) revealed that Spsa bark contained different compounds from those in Spet bark (Figure 3). This indicated that the phenolic compounds extracted from bark samples with acetone differed between Spsa and Spet. The three major peaks detected in each chromatogram (peaks a, b, and c in Figure 3A) were isolated by preparative HPLC and subjected to structural analysis. Compounds of (a) and (c) were identified as picein and its aglycone, *p*-hydroxyacetophenone, respectively. Picein and *p*-hydroxyacetophenone were also detected in methanol extracts by HPLC (Figure 3B). Therefore, to calculate the total contents of picein and *p*-hydroxyacetophenone in Spsa bark, 70% acetone extracts were subjected to quantitative HPLC analysis. On the basis of the peak intensity of picein and *p*-hydroxyacetophenone, their contents in dry bark were calculated to be 0.25 and 0.06%, respectively. Compound of (b) was identified as (+)-ampelopsin. Although the HPLC peak area of (+)-ampelopsin detected at UV_{270} was much lower than those of the other two compounds, its content was calculated to be 0.12% based on dry bark. (Spectral data of isolated compounds are listed in the Supporting Information.) The contents of extractable phenolic compounds in Spsa bark were low. However, the three compounds, picein, *p*-hydroxyacetophenone, and (+)-ampelopsin, detected in the acetone and methanol extracts of Spsa bark might be important phenolic compounds to prevent transmission of UV-B into the cambium layer under the high solar exposure in a desert environment.[34,35]

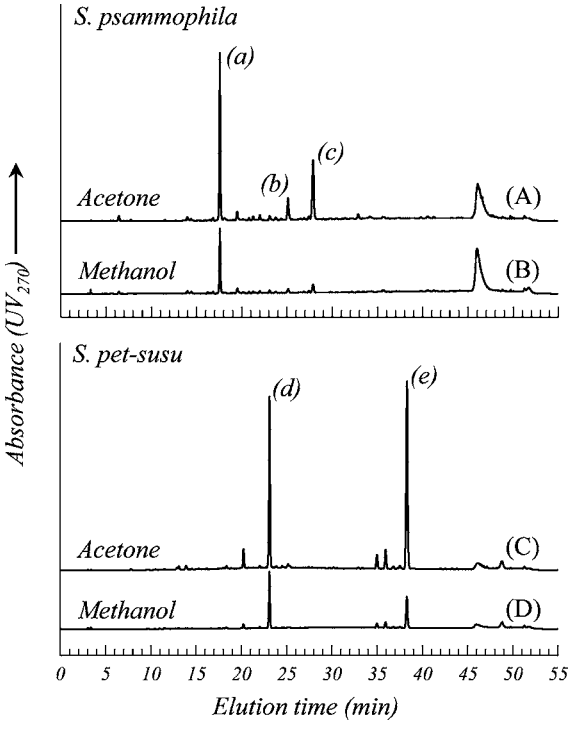

Figure 3. HPLC chromatograms for acetone and methanol extracts from willow bark samples: (a) picein; (b) (+)-ampelopsin; (c) *p*-hydroxyacetophenone; (d) 3-(4-hydroxyphenyl)-2-propenyl β-D-glucopyranoside; (e) 3-(4-methoxyphenyl)-2-propenyl β-D-glucopyranoside.

To isolate compounds other than UV_{270} detectable compounds, the methanol extract was separated using a Sephadex LH-20 column. By use of ethanol as eluent, arabinitol and picein were isolated as major components. Major signals in the ^{13}C NMR spectrum of the methanol extract agreed well with those of authentic picein and arabinitol. (Extended NMR spectra of acetone and methanol extracts are shown in the Supporting Information along with those of authentic compounds.) Therefore, the NMR results also indicate that picein and arabinitol could be the major components of the methanol extract of Spsa bark. Picein is reported to be one of the major phenol glycosides in the bark of various willows,[36,37] although it was not detected as a major component in the acetone and methanol extracts of Spet bark by HPLC analysis. Sugar alcohols can be found in leaf and bark of various woody plants. It was also reported that Corsican pine (*Pinus nigra* ssp. Laricio) contains arabinitol in cone (6% of ethanol extracts, which equal to 0.065% of dry sample) and wood (1%, 0.019%) parts.[38] However, arabinitol has not been reported as a major bark extract of willows. The presence of a certain amount of arabinitol in Spsa bark may be one of the unique chemical characteristics of Spsa. Therefore, Spsa bark was directly extracted with hot water to quantify its arabinitol content by HPLC analysis using a Dionex MA-1 column. In this HPLC analysis, along with some of minor peaks, two intense peaks were detected at 14.0 and 22.7 min, which were assigned to glycerol and arabinitol, respectively (Figure 4). The contents of glycerol and arabinitol calculated from the peak areas were 0.11% and 0.21%, respectively, based on dry bark. It has been reported that sugars and sugar alcohols can improve the freezing tolerance of plant cells, although it has not been confirmed that sugar alcohols can enhance freezing tolerance more than reducing sugars.[39,40] The contents of reducing sugars and sugar alcohols in the bark of various woody plants vary seasonally. However, seasonal variation of sugar alcohols in barks is smaller than that of reducing sugars,[40] which might relate to the metabolic cycle. Therefore, the existence of a substantial amount of sugar alcohols in Spsa bark might be related to the antifreezing properties of Spsa in the desert. That is, desert willow might stably accumulate sugar alcohols to enhance freezing tolerance to temperature variation in the desert. Here we examined some of the components in the bark of Spsa that are potentially related to its ability to survive in the

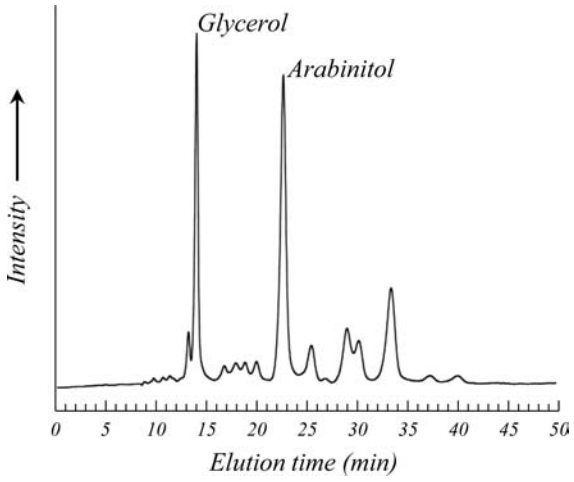

Figure 4. HPLC chromatogram for the hot water extract of desert willow.

desert. However, we did not determine the seasonal and/or climatic variation of these components. Further study is required to see how these chemicals affect the ability of Spsa to live in desert environments.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Chemical composition of bark and xylem of desert willow (*Salix psammophila*); yields of 70% acetone extracts, flavan-3-ols, and total phenol contents of willow barks; spectral data for the main compounds in an acetone extract of *Salix psammophila*; ^{13}C and DEPT spectra of *n*-hexane extract of *Salix psammophila* bark; ^{13}C NMR spectrum of methanol extract of *Salix psammophila* bark (0−210 ppm); ^{13}C NMR spectrum of methanol extract of *Salix psammophila* bark (60−80 ppm). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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