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Isolation of New Constituents from Whole Plant of *Salsola imbricata Forssk.* of Saudi Origin

Rami K. Suleiman,* Saviour A. Umoren, Wissam Iali, and Bassam El Ali

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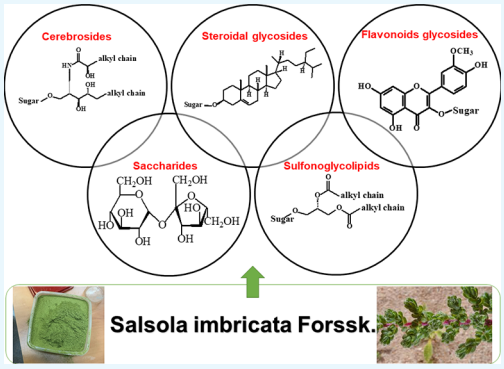
ABSTRACT: This work describes the first report of the known glycosidic constituents *β*-sitosterol-3-*O*-*β*-D-glucoside-6′-palmitate (**1**), *β*-sitosterol-3-*O*-*β*-D-glucoside (**2**), momor-cerebroside I (**3**), phytolacca cerebroside (**4**), 1,2-di-*O*-palmitoyl-3-*O*-(6-sulfoquinovopyranosyl)-glycerol (**5**), isorhamnetin-3-robinobioside (**6**), and isorhamnetin-3-rutinoside (**7**) from the plant *Salsola imbricata Forssk.* grown in the eastern region of Saudi Arabia. The structures of the isolated compounds were elucidated from extensive 1D and 2D nuclear magnetic resonance (NMR), gas chromatography−mass spectrometry (GC−MS), liquid chromatography-mass spectrometry (LC−MS), and chemical analyses. Compound **1** is reported for the first time from the Amaranthaceae family. In addition to the isolated and identified fatty alcohols, compounds **3**, **4**, **5**, and **6** are also reported for the first time from the genus *Salsola*. The findings of this study suggest a contribution of the isolated compounds to the various biological activities reported for this plant.



1. INTRODUCTION

Plants have been employed in traditional medicine in various forms since antiquity and continue to provide the medicinal world with therapeutic molecules for which no synthetic alternative exists in some circumstances.[1] They have also been shown to have potential applications in areas such as pollution, taxonomy, nutrition, and corrosion prevention.[2] Plants belonging to the genus *Salsola* (the largest genera in the Amaranthaceae family, formerly known as Chenopodiaceae) are common in the dry regions of the Middle East, Africa, and Europe. Numerous species of this genus are still employed in traditional medicine.[3,4] *Salsola imbricata Forssk.* (syn. *Chenopodium baryosmum*, *Salsola foetida*, *Caroxylon imbricata*, and *Salsola baryosma*) is a stout, hoary, pale, excessively branched, and wildly growing shrub in Saudi Arabia. It is known as "Harm" in Arabic and used as camel food.[5,6] Various medical and biological activities of this plant have been documented in literature including as oral contraceptives, anti-inflammatory, diuretic, antidiabetic, antioxidant, tyrosinase inhibitory, and central nervous system (CNS) depressant activities.[3,5,6] The plant proved also to be vermifuge, and its ashes are used to treat itch.[7] Earlier phytochemical studies on this plant led to the isolation of biphenylpropanoids,[3] triterpenes and triterpene saponins,[5,8] flavonoids and flavonoid glycosides,[6] coumarins and coumarin glycosides,[7] and phenolic compounds.[6,9,10] Despite the presence of the aforementioned constituents in this plant, the Saudi origin class has received little attention in the literature.[3] Given this and in light of the many classes of chemical constituents previously reported from this plant, we conducted a phytochemical assessment of the entire *S. imbricate Forssk.* plant from Saudi Arabia in this work. Different organic solvents were used to extract the plant in the hopes of isolating a novel class of compounds.

2. RESULTS AND DISCUSSION

The phytochemical investigation of the chloroform residue of the alcoholic extract of the plant *S. imbricate Forssk.* resulted in the isolation of constituents reported here for the first time from this plant. The newly isolated compounds were carefully characterized and identified as indicated in the discussion below.

The early fraction of the column chromatography (CC) of the chloroform extract of our studied plant produced a white fatty solid which was identified by ^{1}H and ^{13}C NMR as a mixture of fatty alcohols (Figures S1 and S2). The two spectra showed characteristic highly intense peaks at $\delta_{H/C}$ 1.25/29.4, respectively, which corresponds to the long fatty chain part of the molecules. Moreover, the alcoholic functionality of the compounds was confirmed by the triplet peak at $\delta_{H/C}$ 3.64/63.1 in the ^{1}H and ^{13}C NMR spectra, respectively. These results are in agreement with the literature data.[11] The identification of the fatty alcohols in the solid of F1 was achieved by GC−MS analyses (Figure S3), which confirmed the presence of fatty alcohols of C-20−C-28 carbon atoms in this fraction. The GC−

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MS data proved also that the fatty alcohols, namely 1-eicosanol, 1-heptacosanol, and 1-octacosanol, are the main components of this fatty mixture. These fatty alcohols are reported here for the first time from the genus *Salsola*.

Compounds **1** and **2** were precipitated after washing the fractions F2 and F4, respectively, with methanol. The two compounds exhibited the same visualized pink color on TLC but interestingly with different retardation (R_f) factors (Figure S4). Furthermore, compound **1** was completely soluble in chloroform, while **2** was fully soluble in a methanol/chloroform mixture. The ^{1}H and ^{13}C NMR spectra of the two compounds (Figures S5−S8) exhibited similar characteristic peaks in the downfield region except for the additional peak that corresponds to an ester carbonyl at δ_C 174.4 in the ^{13}C NMR spectrum of **1** (Figure S6). The presence of the extra fatty acid moiety in **1** in comparison to **2** was evidenced by the presence of peaks corresponding to the long CH_2 chain in the upfield region of the ^{1}H and ^{13}C NMR spectra (Figures S5 and S6). The steroidal skeleton of **1** and **2** was confirmed by the presence of peaks in the range δ_H 0.67−0.94 in their ^{1}H NMR spectra (Figures S5 and S7, respectively) that corresponds to the methyl groups. The signal corresponding to the olefinic carbons (C-5 and C-6) in **1** and **2** appeared at δ_C (140.3 and 122.1) and (140.9 and 121.7) in their ^{13}C NMR spectra (Figures S6 and S8), respectively. The two spectra showed also the presence of six signals for the sugar moiety, in which the peak of the anomeric carbon resonates at δ_C 101.2. The chemical shift values of the sugar moiety in **1** and **2** were comparable except for the C-6′, which is shifted downfield in **1** (δ_C 63.5) as compared to **2** (δ_C 61.5), confirming the presence of the ester linkage at this carbon.[12] The identity of the fatty acid linkage in **1** was determined to be a palmitic acid based on the GC−MS analysis of its acid-catalyzed methanolysis product (Figure S9). Moreover, this result was further supported by the ESI-MS analysis (Figure S9) that gave an *m/z* at 838 [M + Na]$^+$, corresponding to the molecular formula of $C_{51}H_{91}O_7$. Hence, compound **1** is identified as *β*-sitosterol-3-*O*-*β*-D-glucoside-6′-palmitate and **2** as *β*-sitosterol-3-*O*-*β*-D-glucoside (daucosterol)Figure 1. The NMR data of compounds **1**[13] and

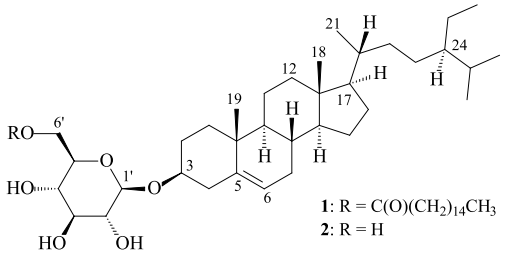

Figure 1. Chemical structures of compounds **1** and **2**.

2[14] are in agreement with those reported in the literature. This is the first report of the isolation of **2** from this plant, while **1** is isolated here for the first time from the genus *Salsola*.

The addition of methanol: chloroform mixture (1:1) to F5 led to the precipitation of pure white solids identified by NMR, MS, and chemical analyses as a mixture of the glycolipids **3** and **4**, whereas the isolation of another glycolipid **5** was achieved from fraction F6 following the same purification procedure. The chemical structures of the three glycolipids are depicted in Figure 2.

The ^{1}H, ^{13}C, and DEPT-135 NMR spectra of F5-solid (Figures S11−S13, respectively) support the presence of a

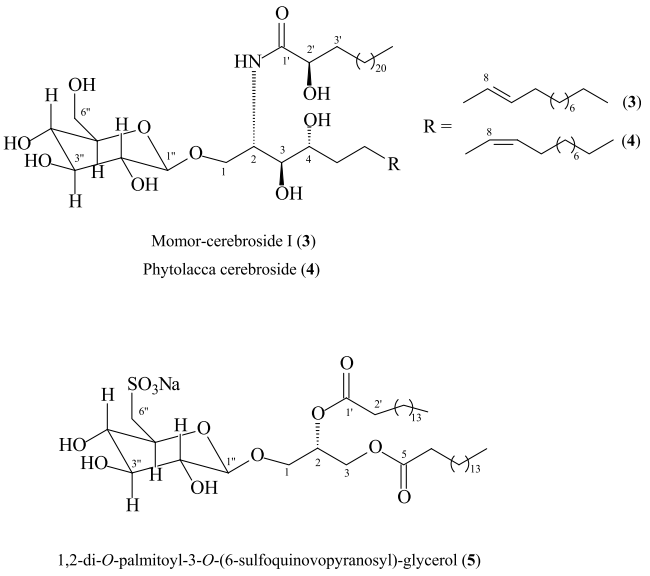

Figure 2. Chemical structures of **3**, **4**, and **5**.

glycosphingolipids skeleton that consists of sugar, an amide linkage, and long-chain aliphatic moieties (Table 1 and Figure

Table 1. ^{13}C and ^{1}H NMR Data for Compounds 3, 4, and 5

	3 and **4** (CD_3OD)		**5** (DMSO-d_6)	
position	^{13}C	^{1}H	^{13}C	^{1}H
1a	68.5	4.06 (dd, *J* = 11.2, 4.1 Hz)	63.1	3.78 (m)
1b		3.81 (m)		4.35 (m)
2	50.2	4.26 (m)	70.2	5.14 (m)
3	74.1	3.62 (t, *J* = 6.0 Hz)	65.1	4.81 (d, *J* = 4.5 Hz), 4.70 (d, *J* = 6.2 Hz)
4	71.5	3.53 (m)		
5			172.9	
8 (*Z*)	129.4	5.38 (dd, *J* = 5.4, 5.8 Hz)	22.6 to 33.9 (CH_2)	1.23 to 2.25 (m)
9 (*Z*)	129.5			
8 (*E*)	130.0	5.44 (dt, *J* = 14.8, 2.3 Hz)		
9 (*E*)	130.1			
CH_3	13.1	0.93 (t, *J* = 7.5 Hz)	14.4	0.84 (t, *J* = 7.2 Hz)
1′	175.7		173.0	
2′	71.5	4.02 (dd, *J* = 11.4, 3.8 Hz)		
glucose				
1″	103.4	4.30 (d, *J* = 7.9 Hz)	98.7	4.57 (d, *J* = 3.4 Hz)
2″	73.6	3.19 (dd, *J* = 8.9, 1.2 Hz)	72.1	3.19 (m)
3″	76.4	3.38 (m)	73.4	3.39 (m)
4″	76.6	3.30 (m)	74.6	2.91 (m)
5″	70.1	3.29 (m)	69.0	3.90 (dd, *J* = 5.7, 4.6 Hz)
6a″	61.2	3.67 (m)	54.8	2.96 (m)
6″b		3.88 (m)		2.56 (m)

2). ^{1}H NMR spectrum of the solid (in CD_3OD; Figure S11) displayed a triplet signal at δ_H 0.93 (*J* = 7.5 Hz) and several multiplet signals in the region δ_H 1.31−2.07 indicating the presence of long aliphatic chains in this compound.[15] The two AB doublets at δ_H 4.02 (*J* = 11.4, 3.8 Hz) and δ_H 4.06 (*J* = 11.2, 4.1 Hz) reveal the presence of hydroxyl functionality on the aliphatic chain. The two signals at δ_H 5.38 (dd, *J* = 5.4, 5.8 Hz)

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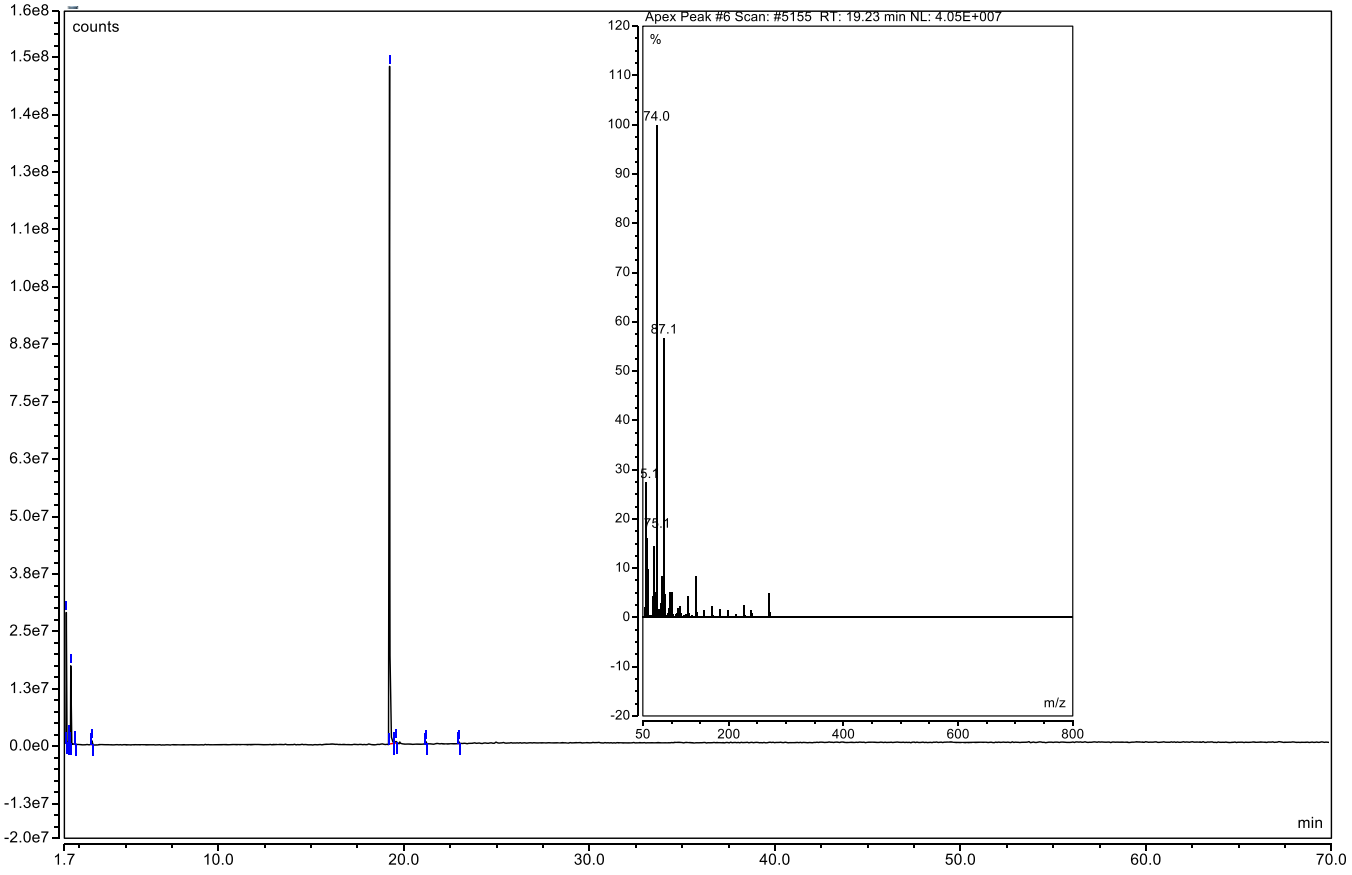

Figure 3. GC−MS chromatogram of the methanolysis product of **5** along with its respective MS data.

and δ_H 5.44 (dt, J = 14.8, 2.3 Hz) suggest the presence of two olefinic bonds of Z and E configurations, respectively, in the chemical structure of the glycosphingolipids that was confirmed by the vicinal coupling constant.[16] The ^{13}C NMR spectrum of glycosphingolipids (Figure S12) provides more structural details by showing the presence of peaks corresponding to the carbon atoms of the various structural components constituting the glycosphingolipids. The classification of the type of each carbon atom was achieved based on the results of the DEPT-135 NMR experiment (Figure S13). The Z and E geometries of the alkenyl double bonds in the glycosphingolipids can as well be verified by noticing the chemical shift of the allylic carbons since the allylic carbon signals of Z and E isomers were observed at δ_C 26.9/27.0 and δ_C 32.3/32.4, respectively, considering that the chemical shift of carbons next to the *cis* double bond resonances are usually more upfield than the *trans* ones.[15] However, the higher intensity of the carbon peaks of the E double bond compared to the one of Z suggests the presence of a mixture of E/Z isomers of a certain glycosphingolipid. The peaks at δ_C 103.4, 76.6, 76.4, 73.6, 70.1, and 61.2 are characteristic signals of a glucose sugar moiety in the glycosphingolipid mixture of **3** and **4**. The β-configuration of the glucose moiety was assigned based on the J-coupling value (7.9 Hz) of the doublet signal resonating at δ_H 4.30 in the ^{1}H NMR spectrum (Figure S11) of the glycosphingolipids. The analysis of the correlation signals in the 2D COSY, HSQC, and HMBC NMR spectra of the purified solid of F5 (shown in Figures S14−S16) confirmed the linkage of the three-component units of compounds **3** and **4** to be glycosphingonine-like compounds. The protons of the olefinic bonds correlate clearly with its adjacent methylene protons in the COSY spectrum (Figure S14). The 2D spectrum shows also a ^{1}H−^{1}H correlation between protons on C-2 and C-3. In the HSQC spectrum (Figure S15), the anomeric proton at δ_H 4.30 (d, J = 7.9 Hz) correlated to the carbon signal at δ_C 103.4 (C-1″), δ_H 3.81 and δ_H 4.06 correlated to C-1 (δ_C 68.5), δ_H 4.26 correlated to C-2 (δ_C 50.2), δ_H 3.62 correlated to C-3 (δ_C 74.1), and δ_H 4.02/3.56 correlated to C-2′ (δ_C 71.5) and C-4 (δ_C 71.5), respectively. The HMBC spectrum of glycosphingolipid mixture of **3** and **4** (Figure S16) showed that the carbon signal at δ_C 175.7 (C-1′) correlated with the proton signals at δ_H 4.26 (H-2) and 4.02 (H-2′). The proton signal at δ_H 4.26 (H-2) gave cross-peaks with the carbon signals at δ_C 74.1 (C-3) and 68.5 (C-1). In addition, the latter also correlated with the proton signal at δ_H 4.28 (C-1″). The proton signal δ_H 4.02 (H-2′) correlated with the methylene carbon signal at δ_C 34.4 (C-3′). The proton signal at δ_H 3.62 (H-3) correlates with C-4 at 71.5 (C-4). The proton signal at δ_H 4.26 (H-2) and the carbon signals at δ_C 68.5 (C-1), 50.2 (C-2), 74.1 (C-3), 175.7 (C-1′), and 71.5 (C-2′) of **3** and **4** were in good agreement with those reported for glycosphingonines having the 2S,3S,4R,2′R configuration.[16,17] Comparison of the NMR chemical shifts of the newly isolated cerebrosides with those previously reported by several researchers in the literature was proved to be a successful methodology for assigning the configuration of cerebrosides and many other phytochemicals.[15−18] No HMBC correlation was observed between H-3 and the olefinic carbons, which suggests that the E/Z alkenyl double bond is located internally in the lipid chain.[17−19] The location of the double bonds and the lengths of the two aliphatic chains of **3** and **4** were identified by the GC−MS analysis of the methanolysis products as well as the ESI-MS characterization. The methanolysis of **3** and **4** (Figure S17) afforded various oleic acid esters by GC−MS analysis. The MS

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spectra of these esters showed peaks of molecular weight data that correspond to the fragment ions resulting from the cleavages of a double bond located at C8. The use of ESI-MS data to identify the location of the double bond in cerebrosides can be less informative as more than one fragmentation pattern can be attributed to a single fragment ion. This gives, in our opinion, less validity to this approach. Despite this, some peaks in the ESI-MS spectrum (Figure S18) of compounds **3** and **4** can be utilized to support the proposed location of the double bond. As an example, the peak at m/z 474 can be attributed to the [M−glucose−hydroxyl functionalities−$C_{11}H_{22}$]$^+$ fragment. The presence of the 2-hydroxylignocerate lipid moiety was confirmed by the fragment at m/z 479 [M−long-chain ester]$^+$, while the peak at m/z 163 corresponds to the glucopyranosyl fragment.

Based on the above analysis, we propose the F5-solid to contain a mixture of **3** and **4** of the chemical structure identified as 1-*O*-[β-D-glucopyranosyl-(2S,3S,4R,8E/Z)-2-[2′-hydroxy-lignoceroylamino]-8-octadecene-l,3,4-triol, respectively. The NMR data for **3** and **4** are listed in Table 1 and showed excellent agreement with the published values.[20] This is the first report of compounds **3** and **4** from the genus *Salsola*.

It is worthy to mention that various biological activities have been reported for sphingolipids such as plant growth stimulatory action, apoptosis, pathogenic defense, anti-inflammatory effects, cytotoxicity, improvement of the barrier function of the skin,[25] cancer-protective action, antimicrobial and proangiogenic action, and antiviral or Ca^{2+} ATPase activity.[19,21]

The ^{1}H, ^{13}C, and DEPT-135 NMR spectra of **5** (DMSO-d_6; Figures S19−21, respectively) demonstrated signals that correspond to similar structural units (glycerol, fatty acid, and sugar) that are existing in **3** and **4**. The presence of the lipid chain moiety can be easily confirmed by the multiplet signals in the region of δ_H 0.84−1.48 in the ^{1}H NMR spectrum (Figure S19) of **5**. Its ^{13}C NMR spectrum (Figure S20) showed the lipid signals at δ_C 14.4 ($\underline{C}H_3$) and from δ_C 22.6 to 33.9 (CH_2), in which the most intense lipid signal is noted at δ_C 29.5. The two carbonyl signals at δ_C 172.9 (C-5) and 173.0 (C-1′) can be assigned to the two fatty acyl moieties. The signals at δ_C 63.1 (C-3), 65.1 (C-1), and 70.2 (C-2) correspond to the glycerol unit in **5**, in which two consecutive carbon atoms are attached to the two fatty acid moieties, while the third one is attached to C-1″ of the sugar unit. The ^{1}H and ^{13}C NMR regions of the sugar unit gave rise to a signal at $\delta_{H/C}$ 4.57/98.7; the small observed $J_{1,2}$ coupling of the anomeric proton (3.4 Hz) revealed an α-glycosidic configuration for the sugar unit. Interestingly, an inverted DEPT signal at δ_C 54.8 (Figure S21) that is correlated to the proton signals at δ_H 2.56 and 2.96 suggests the presence of a methylene carbon attached directly to a sulfur atom. This characteristic signal in addition to the chemical shifts of other carbon atoms confirmed that compound **5** is a sulfonoglycolipid of the sulfonoquinovosyldiacylglyceride structure. The fatty acid composition of **5** was determined by the GC−MS analysis of its acid-catalyzed methanolysis products. The GC−MS chromatogram (Figure 3) depicts the presence of a single peak identified by its MS data as the methyl ester derivative of palmitic acid. The very minor peaks shown in the chromatogram correspond to the palmitic acid and its acyl chloride derivative. The nature of the lipid chain in **5** was further supported by the ESI-MS analysis (Figure S22), which demonstrated a peak at m/z 794, corresponding to the [M-Na]$^+$ and the chemical formula $C_{41}H_{77}NaO_{12}S$.

Based on the above discussion, compound **5** is identified as 1,2-di-*O*-palmitoyl-3-*O*-(6-sulfoquinovopyranosyl)-glycerol (Figure 2), and its NMR data (Table 1) agree well with the literature data.[22−25] The compound **5** is isolated here for the first time from the genus *Salsola*. It is noteworthy to mention that sulfonoglycolipids have shown specific inhibitory biological activities against DNA polymerase, certain types of viruses, telomerase, angiogenesis, and inflammation/proliferation.[26] Their anticancer properties suggest that these compounds could be used to prevent human cancer disorders and as functional foodstuffs with cytotoxic properties.[27] This prompted us to investigate the biological activities of compounds **3**, **4**, and **5**, and the results will be communicated later in a future paper.

Compounds **6** and **7** were isolated as a mixture from fraction F7 and gave a dark yellow spot on a TLC plate when sprayed with the anisaldehyde TLC-visualization reagent. The mixture nature of F7-solid was arrived at after detailed analysis of the ^{1}H, ^{13}C, DEPT-135, and 2D NMR data (Figures S23−S28) of the pale-yellow solid. In particular, the ^{13}C NMR spectrum (Figure S24) depicted the presence of two adjacent signals of very close chemical shifts in all regions of the spectrum. This indicated the presence of two compounds of close chemical structures in this solid mixture. The two compounds were also confirmed to have a flavonoid skeleton based on the analysis of the NMR spectra of the solid mixture. The ^{1}H and ^{13}C NMR spectra of the flavonoid mixture (Figures S23 and S24, respectively) showed a methoxy group signal resonating at $\delta_{H/C}$ 3.97/55.6, which correlated with the carbon resonance at δ_C 147.3 (C-3′) in the HMBC spectrum (Figure S28). The ^{1}H NMR spectrum showed two singlets at δ_H 6.23 and 6.43, which are in agreement with the carbon signals at δ_C 98.8 and 93.6 in the HSQC spectrum (Figure S27). This suggests that the positions of these two protons are located at C-6 and C-8 of the 5,7-dihydroxysubstituted A-ring of the flavonoid framework.[6] A doublet of doublet at δ_H 7.63 (dd, J = 8.0, 4.0 Hz) and two doublets at δ_H 6.92 (J = 8.0 Hz) and 7.96 (J = 4.0 Hz) were assigned as H-6′, H-5′, and H-2′, respectively, of the B-ring. The position of these signals was determined based on their HMBC correlations in which H-6′ is associated with C-2′ (δ_C 113.1), C-4′ (δ_C 149.5), and C-2 (δ_C 157.5). The assignment of the location of the proton in ring B was further confirmed by noticing the correlation between H-6′ and H-5′ in the COSY spectrum (Figure S26). The presence of a rhamnosyl sugar unit in the flavonoids was confirmed by the doublet signals at δ_H 1.12/1.19 (J = 5.7 Hz) and δ_H 4.55 (J = 1.0 Hz), corresponding to the methyl and anomeric protons (α-configuration) of this sugar, whereas the doublets at δ_H 5.21 (J = 7.8 Hz) and 5.23 (J = 7.5 Hz) can be attributed to the presence of a galactose or glucose sugar moiety (β-configuration) in the flavonoid compounds.[27] The position of glycosidation, as well as the sequential arrangement of the saccharide unit in the flavonoid structure, was determined using the COSY and HMBC 2D NMR data. A correlation between the anomeric proton at δ_H 5.21/5.23 (H-1″) and the carbon signal at δ_C 134.0 (C-3) in the HMBC spectrum indicates a glycosidic linkage of the galactose/glucose unit at C-3 (ring B). Moreover, the rhamnosyl anomeric proton at δ_H 4.55 (H-1‴) correlated with the galactosyl/glucosyl C-6″ atom at δ_C 67.1/66.0, indicating a robinobiosyl/rutinosyl moiety. The position of glycosidation at C-3 of ring B was further supported by the long-range coupling in the COSY spectrum between the methoxy group protons at δ_H 3.97 and the anomeric proton of the galactose/glucose unit at δ_H 5.21/5.23 (H-1″).[28]

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The separation of compounds **6** and **7** using the conventional TLC was not possible. Hence, the mixture was subjected to the analytical LC−MS analysis using the chromatographic conditions described in the Materials and Methods section. The obtained chromatogram of this mixture (depicted in Figure S29) revealed the presence of two separated peaks but of similar MS data in which the peak at *m/z* 625 corresponds to the molecular ion $[M + 1]^+$, while the peaks at *m/z* 479 and 317 emanated from the sequential loss of rhamnose and galactose/glucose sugar moieties, respectively. It is worthy to mention that the direct ESI-MS analysis of the flavonoid mixture (Figure S30) shows a major peak at *m/z* 1270 corresponding to $[M_6 + M_7 + Na]^+$ and a peak at *m/z* 647 ascribed to the $[M + Na]^+$ of **6** or **7**. From all these data, the chemical structures of **6** and **7** were concluded to be [3-(6-*O*-*α*-L-rhamnopyranosyl-*β*-D-galactopyranosyloxy)-3′-methoxy-4′,5,7-trihydroxyflavone] (isorhamnetin-3-robinobioside) and [3-[[6-*O*-(6-deoxy-*α*-L-mannopyranosyl)-*β*-D-glucopyranosyl]oxy]-5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-4*H*-1-benzopyran-4-one] (isorhamnetin-3-rutinoside), respectively (Figure 4). The NMR data of the two compounds

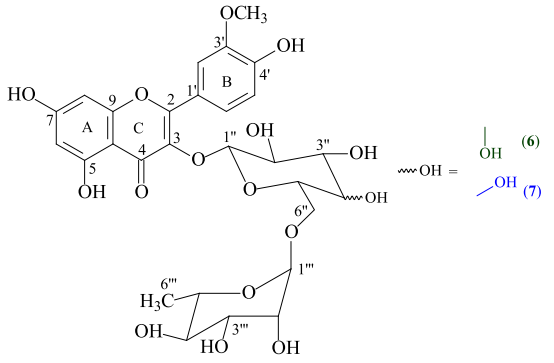

Figure 4. Chemical structures of **6** and **7**.

are in good agreement with those reported in the literature.[28,29] Compound **7** has been reported previously from the genus *Salsola*;[30] however, this is the first report of compound **6** from this genus. Compound **6** is reported to exhibit antiproliferative and antioxidative activities.[31,32] The two isorhamnetin derivatives that were isolated previously from the leaves of our studied plant have also shown distinct anti-inflammatory activity.[6]

In the course of purification of the flavonoid mixture, a white crystalline material was precipitated and identified by the ^{1}H NMR analysis (Figure S31) as the natural sugar sucrose.[33] Furthermore, around 10 g of a salt mixture was also precipitated during the extraction process of the plant (Figure S32). This agrees with the reports that highlighted the role of the plants of the genus *Salsola* in helping the restoration and reclamation of degraded salty areas and saline soils.[4,34]

3. MATERIALS AND METHODS

3.1. Chemicals. All chemicals involved in the extraction and the column chromatography (CC) separation were of analytical grade (Sigma-Aldrich Company, USA) and used without any further purification. Doubly distilled water and solvents of HPLC grade were used for the LC−MS analysis. The column chromatography (CC) was performed with silica gel having 60 Å as pore size and a particle size range of 0.040−0.063 mm, while the thin layer chromatography (TLC) analyses were conducted on precoated Merck Kieselgel 60 F254 plate (0.25 ram), and spots were detected using the anisaldehyde solution-detecting reagent.

3.2. Plant Material. The whole part of the *S. imbricata Forssk.* plant was collected (20 kg) from the KFUPM campus, city of Dhahran, Saudi Arabia, and kept drying at room temperature and open to air for 1 week. The dried plant was powdered prior to extraction.

3.3. Extraction and Isolation. The air-dried and powdered whole part of *S. imbricata Forssk.* (7 kg) was extracted in parallel and at room temperature with three different solvents: methanol (1.5 kg, 7.5 L), butanol (1.5 kg, 7.5 L), and 96% ethanol (4.0 kg, 15 L). The crude extracts of the three solvents were evaporated to dryness under reduced pressure, combined using chloroform as a solvent, and dried under a fume hood to give 998 g of a greenish residue. The residue was partitioned between 10% aqueous methanol and *n*-hexane (1:1), and the two layers were evaporated in vacuo. The aq. methanol residue was dissolved in chloroform and extracted with water (500 mL × 3), and the two layers were evaporated in a fume hood to afford 59.7 and 339.2 g of chloroform and water extracts, respectively. The chloroform extract was separated on a silica gel 60 column using ethyl acetate as eluent and increasing polarity with methanol (70 fractions, 250 mL each) to give 7 groups of fractions (F1−F7) according to their TLC behavior. F1 precipitated a fatty residue (160 mg) upon washing with methanol, which was proven to contain a mixture of fatty alcohols upon analysis by NMR and GC−MS. The addition of methanol to F2 and F4 precipitated the compounds **1** (20 mg) and **2** (175 mg), respectively, whereas F3 did not show any major spot on TLC; hence, it was not investigated any further. The mixture of compounds **3** and **4** (45 mg) as well as compound **5** (39 mg) was purified by washing the fractions F5 and F6 with a $CHCl_3/CH_3OH$ (1:1) mixture. F7 showed a UV-active spot, which exhibited a dark yellow color on TLC upon spraying with the anisaldehyde solution. Further purification of this fraction on TLC using methanol:dichloromethane (1:3) as a solvent system yielded 20 mg of a yellowish solid, which is a mixture of the compounds **6** and **7** that are confirmed by NMR and LC−MS analyses. A white material was also obtained from this fraction and identified as the natural sugar sucrose.

3.4. NMR Spectroscopy. 1D and 2D NMR spectra were recorded in deuterated chloroform ($CDCl_3$), methanol (CD_3OD), and dimethyl sulfoxide (DMSO-d_6) on a Bruker Avance 400 MHz spectrometer (Bruker Biospin GmbH, Rheinstetten, Germany) operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. The chemical shifts were recorded in parts per million (δ) downfield of tetramethylsilane (TMS, internal standard). The DEPT-135 experiments were conducted using a transfer pulse of 135^0 to produce positive signals for CH_3 and CH and negative ones for CH_2. The homonuclear correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC), and heteronuclear multiple bond correlation (HMBC) 2D-NMR experiments were conducted using the conventional Bruker pulse sequence. The NMR experiments and data were recorded using Bruker TopSpin 4.1.1 software. Some selected parameters for the conducted NMR experiments in our study are available in Supporting Information Table S1.

3.5. GC−MS and Mass Spectrometry Parameters. The GC−MS analysis of the volatile fractions and the derivatives of the non-volatile compounds were achieved using an ISQ 7000

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Single Quadrupole GC−MS apparatus equipped with a TraceGOLD TG-5MS GC (5% phenyl and 95% dimethyl polysiloxane) capillary column (30 m × 0.25 mm i.d.; 0.25 μm film). The experimental parameters of the GC−MS instrument are similar to our previous published work.[35]

The mass spectrometric data were obtained using an ESI-MS mass spectrometer with an LCQ FLEET system from Thermo Scientific Company (USA) with a mass scanning range of 0−2000. Nitrogen was used as nebulizing gas, and helium (grade 5.8) was used as carrier gas. The sample was dissolved in HPLC-grade acetonitrile and introduced by direct infusion with a syringe pump employing a 250 mL syringe at a constant flow rate of 3 mL min^{-1}.

3.6. LC−MS Parameters. The LC−MS analysis of compounds **6** and **7** was achieved using an Agilent LCMS-6120B coupled with a diode-array detector (DAD) and using a Poroshell 120 EC-C18 (4.6 × 150 mm, 4 μm) column with a flow rate of 1.0 mL/min and an injection volume of 20 μL of the sample. The temperature of the column oven was kept at 25 °C. An isocratic separation method was used holding a mobile phase consisting of 0.3% formic acid in water (solution A, 86%) and acetonitrile with 0.3% formic acid (solution B, 14%) for 60 min. The following parameters were used for the mass spectrometer: positive ion mode; peak width, 0.100 min; cycle time, 1.06 s/cycle; drying gas flow, 12.0 L/min; heated capillary temp., 350 °C; capillary voltage, 3000 V; nebulizer pressure, 35 psi.[36]

3.7. Acid Hydrolysis of Compounds 1, 3, and 4. Compounds **1**, **3**, **4**, and **5** (ca. 5 mg) were heated at 70 °C with 10% HCl in MeOH (5 mL each) for 18 h. The reaction mixture was extracted with *n*-hexane, the *n*-hexane layer was concentrated in vacuo, and the residue was subjected to GC−MS analysis.[37]

4. CONCLUSIONS

The chemical composition of the whole part of the *S. imbricata Forssk.* plant conducted in this work proved that this plant is very rich in various classes of glycosidic constituents (glycosteriods, glycolipids, triterpenoid saponins, and flavonoid glycosides). The isolated glycosidic compounds as well as the fatty alcohols have never been reported previously from this plant. The varieties of the chemical classes of the constituents reported here support the success of the followed methodology by considering solvents of different polarities during the extraction procedure of the crude powdered plant. In view of the isolated compounds in this study, the testing of this plant in various biological applications is suggested. Moreover, the investigation of the corrosion-inhibition properties of the isolated compounds in this work is part of an ongoing study by the authors.

■ ASSOCIATED CONTENT

Supporting Information

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Selected parameters for the conducted NMR experiments; ^{1}H NMR spectrum for F1-solid; ^{13}C NMR spectrum for F1-solid; GC−MS chromatogram for F1-solid; TLC behavior for **1** & **2**; ^{1}H NMR spectrum for **1**; ^{13}C NMR spectrum for **1**; ^{1}H NMR spectrum for **2**; ^{13}C NMR spectrum for **2**; GC−MS chromatogram for the methanolysis product of **1**; ESI-MS spectrum for **1**; ^{1}H NMR spectrum for **3** and **4**; ^{13}C NMR spectrum for **3** and **4**; DEPT-135 NMR spectrum for **3** and **4**; ^{1}H-^{1}H COSY NMR spectrum for **3** and **4**; ^{1}H-^{13}C HMQC NMR spectrum for **3** and **4**; ^{1}H-^{13}C HMBC NMR spectrum for **3** and **4**; GC−MS Chromatogram for the methanolysis product of **3** and **4**; ESI-MS spectrum for **3** and **4**; ^{1}H NMR spectrum for **5**; ^{13}C NMR spectrum for **5**; DEPT-135 NMR spectrum for **5**; ESI-MS spectrum for **5**; ^{1}H NMR spectrum for **6** and **7**; ^{13}C NMR spectrum for **6** and **7**; DEPT-135 NMR spectrum for **6** and **7**; ^{1}H−^{1}H COSY NMR spectrum for **6** and **7**; ^{1}H-^{13}C HMQC NMR spectrum for **6** and **7**; ^{1}H−^{13}C HMBC NMR spectrum for **6** and **7**; LC−MS chromatogram for **6** and **7**; ESI-MS spectrum for **6** and **7**; ^{13}C NMR spectrum for sucrose; photo-digital image of the salt mixture precipitated during plant extraction (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Rami K. Suleiman − *Interdisciplinary Research Center for Advanced Materials, King Fahd University of Petroleum & Minerals (KFUPM), Dhahran 31261, Saudi Arabia;* orcid.org/0000-0002-6776-9266; Email: ramismob@kfupm.edu.sa; Fax: +966 13 860 8317

Authors

Saviour A. Umoren − *Interdisciplinary Research Center for Advanced Materials, King Fahd University of Petroleum & Minerals (KFUPM), Dhahran 31261, Saudi Arabia;* orcid.org/0000-0002-8564-4897

Wissam Iali − *Chemistry Department, King Fahd University of Petroleum & Minerals (KFUPM), Dhahran 31261, Saudi Arabia; Center for Refining & Advanced Chemicals, King Fahd University of Petroleum & Minerals (KFUPM), Dhahran 31261, Saudi Arabia;* orcid.org/0000-0002-9428-2023

Bassam El Ali − *Chemistry Department, King Fahd University of Petroleum & Minerals (KFUPM), Dhahran 31261, Saudi Arabia; Center for Refining & Advanced Chemicals, King Fahd University of Petroleum & Minerals (KFUPM), Dhahran 31261, Saudi Arabia;* orcid.org/0000-0003-4005-6386

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.2c02332

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Notes

The authors declare no competing financial interest.

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