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Dammarane-type saponins from leaves of Ziziphus spina-christi

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1. Introduction

Ziziphus species (Rhamnaceae) occur in warm-temperate and sub-tropical regions around the world, and are commonly used in folk medicine for the treatment of various diseases, such as digestive disorders, weakness, liver complaints, obesity, urinary problems, diabetes, skin infections, fever, diarrhea, and insomnia (Abdel-Zaher et al., 2005), Ziziphus spina-christi (L.) Desf. (Christ's Thorn Jujube, Sedr in Farsi, and Konar in Arabic) is a wild evergreen tree characterized by spiny branches and small orange fruits that is native of subtropical eastern Africa, the Middle East, and the Indian subcontinent, but also widespread in northwestern Africa. In some countries of the Middle East, leaves of Z. spina-christi are harvested from the wild or from cultures to be mainly sold on local markets. The Iranian province of Khuzestan is known for the extensive cultivation of Z. spina-christi, and leaves are used in Iran as a natural detergent and shampoo due to the saponin content. Surprisingly, the phytochemistry of Z. spina-christi leaves is poorly studied, and only a limited number of polyphenols (Nawwar et al., 1984) and jujubogenin glycosides have been reported up to now (Mahran et al., 1996). More information is available regarding the

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ABSTRACT

Phytochemical profiling of *Ziziphus spina-christi* leaves led to the characterization of 10 dammarane-type saponins and 12 phenolic compounds. Isolation was achieved by gel chromatography on Sephadex LH20, open column chromatography on silica gel, and semi-preparative HPLC with PDA and ELSD detectors. Structural characterization was performed by extensive 1D and 2D NMR, mass spectrometry, and by GC-MS of sugar derivatives. A biosynthetic pathway leading to three previously undescribed sapogenins is proposed. The saponin profiles in *Z. spina-christi* leaves of four different origins were compared by means of HPLC-ESIMS.

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phytochemical composition of *Z. spina-christi* and *Z. jujuba* fruits. Pawlowska et al. (2009) identified numerous flavonoids in both species, and various phenolics, triterpenoids, sterols, cyclopeptide alkaloids, and amino acids have been reported from *Z. jujuba* (Gao et al., 2013). In view of a possible use of *Z. spina-christi* leaf extract as a cosmetic ingredient due to its surfactant properties, a phytochemical analysis of the leaves was performed with an emphasis on saponins. Finally, the saponin profiles in *Z. spina-christi* leaf samples of four different origins were compared by means of HPLC-ESIMS, in order to assess the variability of saponin patterns.

2. Results and discussion

Leaves of *Z. spina-christi* were submitted to sequential extraction with EtOAc and 35% (v/v) ethanol. After evaporation of the hydroalcoholic extract, the residue was suspended in water and partitioned with *n*-BuOH to remove sugars and other highly polar compounds. The *n*-BuOH layer was separated by gel filtration on Sephadex LH20, open column chromatography on silica gel, and semi-preparative HPLC to afford ten triterpene saponins **1**–**10**. Of these, compounds **8**–**10** contained new dammarane type aglycons. In addition, eleven phenolic compounds (**11**–**21**) were identified, including the new quercetin glycoside **11**. The HPLC profile of the *n*-BuOH layer, and peaks corresponding to compounds **1–21** are shown in Fig. 1.

Structure elucidation was achieved by means of extensive 1D and 2D NMR analysis and HRESIMS. In addition, sugar moieties in saponins **1**, **6**, and **9**, and in flavonoid **11** were identified by GC-MS

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Fig. 1. HPLC profile of the *n*-BuOH layer of Ziziphus spina-christi leaf extract recorded with PDA (254 nm), ELSD, and ESIMS (positive and negative ion mode). Peak numbers designate saponins 1–10 and polyphenols 11–21.

after acid hydrolysis followed by derivatization with L-cystein methyl ester and subsequent silylation, and comparison with derivatized reference sugars. In case of minor saponins **2–5**, **7**, **8** and **10**, we did not perform a hydrolysis due to the limited amount of material. We here assume the same absolute configuration of sugars as found in saponins **1**, **6**, and **9**, given the same nature of individual sugars, and the relatedness of sugars chains to those in **1**, **6** and **9**.

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Saponins **1** and **2** were identified as jujuboside B1 (Matsuda et al., 1999) and christinin A (Mahran et al., 1996) which had been previously reported from leaves of *Z. jujuba* and *Z. spina-christi*, respectively (Fig. 2).

The negative ion HRESIMS spectrum of **3** showed a signal at m/z969.5150 [M-H]⁻ corresponding to a molecular formula of C₄₉H₇₈O₁₉ (calcd for C₄₉H₇₇O₁₉: 969.5137). 1D and 2D NMR spectra of **3** were virtually superimposable to those of **2**. The only differences were in the presence of an oxygenated methine ($\delta_{\rm H}$ 4.51, br s) in **3** vs a methylene in **2**, and of an acetyl moiety (δ_{H} 2.05, s; δ_{C} 169.5) in **3**. The location of the oxygenated methine at C-22 was inferred from its COSY correlation with H-23, and corroborated through HMBC correlations with C-17, C-20, and C-21 (Fig. 3). An HMBC correlation of H-22 with the carboxyl group at δ 169.5 established the location of the acetyl moiety. The relative configuration of the triterpene skeleton corresponded to that reported for christinin A (Fig. 2). The ROESY contact of H-23/H-15a (Fig. 3) indicated a β orientation of H-23, while the α -orientation of the 22-acetoxy moiety was inferred from the small J_{HH} coupling of H-22/H-23 (br s), and corroborated by the absence of ROESY contacts of H-22/H-17. Thus, the structure of **3** was established as 22α -acetoxy christinin A (Fig. 2).

Saponins **4** and **5** were obtained as an inseparable mixture. Molecular formula of $C_{49}H_{78}O_{18}$ and $C_{50}H_{78}O_{20}$ were deduced from ions at m/z 953.5161 [M-H]⁻, (calcd for $C_{49}H_{77}O_{18}$: 953.5188) and 997.5072 [M-H]⁻, (calcd for $C_{50}H_{77}O_{20}$: 997.5086) in the HRESIMS spectra of **4** and **5**, respectively. Inspection of the NMR data revealed the presence of jujubogenin as aglycon in both saponins, and of a trisaccharidic moiety that closely resembled that of **2**. Additional signals were detected in the mixture of **4** and **5**, namely of a methylene group ($\delta_{\rm H}$ 3.50, $\delta_{\rm C}$ 70.7), a methyl group ($\delta_{\rm H}$ 1.99, $\delta_{\rm C}$ 21.6) (Fig. S8), and signals attributable to carboxylic groups in the DEPTq spectrum (δ 167.8 and δ 168.7) (Fig. S9). Hence, saponins were esterified with an acetyl and malonyl moiety, respectively. The signals corresponding to the CH₂-6 of the glucose moiety ($\delta_{\rm H}$ 4.31 and 4.09/ $\delta_{\rm C}$ 64.7) appeared downfield compared to those in **2** ($\delta_{\rm H}$ 3.65 and 3.45/ $\delta_{\rm C}$ 61.0), and thereby indicated attachment of the acyl moieties at this position. The saponins were thus identified as jujubogenin 3- β -O-(6-O-acetyl)- β -D-glucopyranosyl-(1 \rightarrow 3)-[α -Dfucopyranosyl-(1 \rightarrow 2)]- α -L-arabinopyranoside (**4**) and jujubogenin 3- β -O-(6-O-malonyl)- β -D-glucopyranosyl-(1 \rightarrow 3)-[α -D-fucopyranosyl-(1 \rightarrow 2)]- α -L-arabinopyranoside (**5**), and were named as christinin A1 and A2, respectively (Fig. 2).

Compound 6 had a molecular formula of C₄₈H₈₀O₁₈, as deduced from the signal at m/z 943.5363 (calcd for C₄₈H₇₉O₁₈: 943.5345) in the negative ion HRESIMS spectrum. ¹H and ¹³C NMR spectra showed the presence of six methyl singlets in the high field region (δ 0.74, 0.80, 0.83, 0.93, 1.02, and 1.15), of a 3-methyl-2-butenyl group (δ 1.58 and 1.65, H₃-26 and 27; δ 5.15, H-24; δ 2.13, H₂-23), and of two oxygenated quaternary carbons (δ 109.5, C-16; and δ 76.5, C-20). The NMR data of the aglycon were in good accord with those of lotogenin (Renault et al., 1997). In addition, three anomeric protons were detected in the ¹H NMR spectrum at δ 4.24 (d, J = 7.4 Hz), δ 5.34 (br s), and δ 5.03 (br s), with ¹³C resonances of their corresponding carbon atoms at δ 104.0, δ 98.9, and δ 101.0. By ¹H-¹H-COSY, 1D selective TOCSY, and 2D HSQC-TOCSY experiments the three sugars spin systems were assigned to one β -glucose and two units of α -rhamnose, respectively (Table 3). β -Glucose was identified by the J values of H-1' to H-5' that showed all-trans diaxial interactions. The α -rhamnose units were identified by the small J values of H-2"/H-2" and their vicinal protons (dd, ${}^{3}J_{H-2/H-}$ $_3 = 3.6$ Hz, and $^3J_{\text{H-2/H-1}} = \text{br s}$ (same for compound **11**), while the ¹³C-NMR shifts of C-3''/C-3''' (δ 71.2 and 70.9) and C-5''/C-5''' (δ 65.9 and 68.8) confirmed the α configuration at the anomeric carbons. Long range ${}^{3}J_{HC}$ HMBC correlations of H-1^{'''} (δ 5.03) of Rha II and C-4" (δ 78.4) of Rha I, and H-1" (δ 5.34) of Rha I with C-2' (δ 75.0) of

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Fig. 2. Structures of saponins 1–10 and flavonoid 11.

Glc established the trisaccharide chain. ROESY contacts of H-1^{'''} (δ 5.03) and H-4'' (δ 3.33), H-1'' (δ 5.34) and H-2' (δ 3.28) (Fig. 3) supported this assignment. Attachment of the trisaccharide moiety at C-3 of the aglycon via a β -oriented glycosidic linkage was inferred from the ³*J*_{HC} HMBC correlation between H-1' (δ 4.24) and C-3 (δ 88.3) of the aglycon, and by the *J*_{HH} coupling of H-3 (δ 3.03, dd, *J* = 11.4, and 4.0 Hz) which was indicative of its axial disposition in the chair-like conformation. The relative configuration of the

aglycon was identical to that reported for lotogenin, as indicated by key ROESY correlations (Fig. 3). The absolute configurations of glucose and rhamnose were determined as described for compound **1**, **6**, and **9**. Thus, compound **6** was established as lotogenin $3-\beta$ - $O-\alpha$ -L-rhamnopyranosyl- $(1 \rightarrow 4)-\alpha$ -L-rhamnopyranosyl- $(1 \rightarrow 2)-\beta$ -D-glucopyranoside, and was named as lotoside III (Fig. 2).

A molecular formula of C44H72O15 was assigned to compound 7

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Fig. 3. Key ¹H-¹H ROESY and HMBC correlations in compounds 3–10.

on the basis of a molecular ion at m/z 839.4849 in the negative ion HRESIMS spectrum (calcd for C44H71O15: 839.4871). Inspection of the NMR data indicated lotogenin as aglycon, and the presence of an acetyl moiety (δ_H, 1.98, s; δ_C 21.6, C-32; δ_C 169.9, C-31). Attachment of the acetyl group at C-15 was established by an HMBC correlation of H-15 (δ 5.06) to C-31 (δ 169.9), and confirmed by the downfield shift of H-15 compared to H-15 in **6** (δ 3.73). The two sugars moieties in 7 were identified by COSY and 1D selective TOCSY experiments as β -glucopyranoside and α -rhamnopyranoside (Figs. S18 and S22). Key HMBC correlations of H-1^{$\prime\prime$} (δ 5.24) and C-2^{\prime} (δ 76.6), H-1' (δ 4.23) and C-3 (δ 87.8) established the interglycosidic linkage and the attachment of the disaccharide to C-3 of lotogenin (Renault et al., 1997) (Fig. 3). Hence, the structure of 7 was established as 15-acetoxy-lotogenin $3-\beta$ -O- α -L-rhamnopyranosyl- $(1 \rightarrow 2)$ - β -D-glucopyranoside, and the compound was named as 15-acetoxy lotoside IV (Fig. 2).

Saponin **8** showed a molecular ion at m/z 943.5339 in the negative ion HRESIMS spectrum, corresponding to a molecular formula of C₄₈H₈₀O₁₈ (calcd for C₄₈H₇₉O₁₈: 943.5345). Chemical shifts of three anomeric protons at δ 4.25 (d, J = 7.4 Hz), δ 5.34 (br s), and δ 5.06 (br s) with corresponding carbons at δ 103.5, 98.7, and 100.5 were identical to those of the sugar moiety in **6**. Analysis of key HMBC and ROESY correlations confirmed the trisaccharide moiety as α -rhamnopyranosyl-(1 \rightarrow 4)- α -rhamnopyranosyl-(1 \rightarrow 2)- β -

glucopyranoside (Fig. 3). Additional resonances detected in the HSQC and HMBC spectra were eight methyls, seven methylenes, eight methines, and seven quaternary carbons including a carbonyl (δ 216.1) indicating a lotogenin-like aglycon (Renault et al., 1997). Compared to lotogenin, compound 8 lacked of the quaternary carbon attached to a hemiacetal group (δ 109.5). This was replaced by a carbonyl (δ 216.1), as confirmed by HMBC correlations of H-17/C-16 and H-15/C-16 (Fig. 3). Consequently, C-20 and C-22 were located on a side chain, and the spin system was identified as shown in Fig. 2. The relative configuration of the triterpene skeleton was corroborated by key ROESY correlations of H-17/H₃-30 and H-13/H-15 (Fig. 3), which established the relative configuration at C-17 and C-15. However, the relative configuration at C-20 could not be established due to the free rotation of the side chain, and limited amounts of material precluded derivatization. The aglycon was identified as 3β , 15α , 20, 25-tetraol-dammar-23(24)-ene-16-one, and this new dammarane-type triterpenoid was named sidrigenin. Thus, compound **8** was sidrigenin 3-O- α -L-rhamnopyranosyl-(1 \rightarrow 4)- α -Lrhamnopyranosyl- $(1 \rightarrow 2)$ - β -D-glucopyranoside (Fig. 2).

Compound **9** showed a molecular ion of m/z 929.5563 in the negative HRESIMS spectrum corresponding to a molecular formula of C₄₈H₈₂O₁₇ (calcd for C₄₈H₈₁O₁₇: 929.5552). The sugar moiety was identical to that of **8**, but the aglycon portion was slightly different. By means of COSY, HSQC, and HMBC experiments C-16 was

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identified as a methylene ($\delta_{\rm H}$ 1.82 and 1.24), while the methylene C-22 in compound **8** (δ_{C} 42.3) was replaced by a hydroxylated methine ($\delta_{\rm C}$ 75.3). The signal of the only olefinic proton in **9** (δ 5.21, br t, I = 7.0 Hz; H-24) was coupled with a methylene group (δ 2.08, ddd, J = 13.0, 7.0, 3.0 Hz; H-23a; δ 1.80, m; H-23b) due to the presence of a double bond at positions 24 and 25 (Table 2). The relative configuration of the tetracyclic portion of the aglycon was established by analysis of J couplings, and confirmed by ROESY data (Fig. 3). However, the relative configuration at the stereogenic carbons C-21 and C-22 could not be established by NMR due to the flexibility of the side chain. Formation of an acetonide (2,2dimethoxypropane and catalytic amount of *p*-toluenesulfonic acid in dry acetone at r.t. for 2 h) (Ihre et al., 1998) was attempted with the aglycon after acid hydrolysis, but failed due to decomposition of the aglycon during the reaction. The aglycon was thus established as 3β , 15α , 20, 22-tetraol-dammar-24(25)-ene and was named as konarigenin. Hence, **9** was konarigenin $3-O-\alpha$ -L-rhamnopyranosyl $(1 \rightarrow 4)-\alpha$ -L-rhamnopyranosyl- $(1 \rightarrow 2)-\beta$ -D-glucopyranoside (Fig. 2).

Compound **10** had a molecular formula of $C_{48}H_{78}O_{18}$ (*m/z* 941.5167 in the negative HRESIMS; calcd for C₄₈H₇₇O₁₈: 941.5188). ¹H and ¹³C-NMR chemical shifts of the trisaccharide moiety were superimposable to those of 6, 8, and 9 (Table 3, Table 4). The aglycon of **10** showed close similarity to lotogenin, but with differences in the fragment of C23-C27. The olefinic methine at C-24 of lotogenin was replaced in **10** by an oxygenated sp³ methine (δ 3.95, br s), and the 2-methylpropenyl in lotogenin was replaced in 10 by an isopropylene residue (δ 4.89, br s, and 4.71, br s; H-27a and H-27b; and δ 1.66, s, H₃-26). Furthermore, an epoxy bridge between C-20 and C-24 was in accord with the molecular formula and required degrees of hydrogen deficiency. The relative configuration was determined by ROESY experiments (Fig. 3). Key ROESY contacts of H₃-21 with H-17 and H-22 established the cofacial orientation of these groups. Due to the close chemical shifts of H-22 and H-24

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¹H and ¹³C spectroscopic data for the aglycon moieties of **3–6** (DMSO- d_6) (δ in ppm, J in Hz).

$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	No.	3		4		5		6		
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$		δ_H^a	δ _C ^b , type	$\delta_{H}{}^{a}$	$\delta_C{}^b$, type	$\delta_H{}^a$	$\delta_C{}^b$, type	$\delta_H{}^a$	δ_{C} , type	
088, m088,	1	1.58	38.1, CH ₂	1.58	38.1, CH ₂	1.58	38.1, CH ₂	1.58	39.0, CH ₂	
2 1.71, m 25, CH2 1.71, m 25, CH2 1.90 25, CH2 1.90 25, CH2 1.54 3 30.3, 86, OCH 30.3, 30.3, 86, OCH 30.3, 3		0.88, m		0.88, m		0.88, m		0.88, m		
154154154154154154154154154150add (12.0, 40)303, 68, 9, CH303, 303, 68, 9, CH303, C	2	1.71, m	25.5, CH ₂	1.71, m	25.5, CH ₂	1.71, m	25.5, CH ₂	1.90	26.1, CH ₂	
3303, (12.0.4.0)869, CH (d(12.0.4.0)303, (d(12.0.4.0)869, CH (d(12.0.4.0)303, (d(12.0.4.0)869, CH (d(12.0.4.0)303, C (d(12.0.4.0)883, CH (d(12.0.4.0)4-33.3, C-39.3, C-99.3, C-141-141-141141140-140, C140, C <td></td> <td>1.54</td> <td></td> <td>1.54</td> <td></td> <td>1.54</td> <td></td> <td>1.50</td> <td></td>		1.54		1.54		1.54		1.50		
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$ \begin{array}{cccccccccccccccccccccccccccccccccccc$		dd (12.0, 4.0)		dd (12.0, 4.0)		dd (12.0, 4.0)		dd (11.4, 4.0)		
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	4	_	39.3, C	-	39.3, C	-	39.3, C	-	38.8, C	
bit bi	5	0.71	55.1, CH	0.71	55.1, CH	0.71	55.1, CH	0.68,	56.1, CH	
6 1.46 1.2, CH2 1.46 1.2, CH2 1.47 1.39 1.39 7 1.47 35.1, CH2 1.47 35.1, CH2 1.47 35.1, CH2 1.47 35.1, CH2 1.37 1.37 1.37 1.37 1.37 1.37 1.37 1.35 1.35 9 0.83, 51.7, CH 0.83, 51.7, CH 0.83, 51.7, CH 0.83, 51.7, CH 0.65, C 9 0.83, 51.7, CH 0.83, 51.7, CH 0.83, 51.7, CH 0.65, C 10 - 364, C - 0.64, C - 0.64, C - 0.65, C 11.4 1.54 2.07, CH2 1.51 2.07, CH2 1.69 1.46 1.46 12.9 1.34 1.44 1.44 1.44 1.44 1.44 12.9 1.59 1.59 1.14 1.44 1.44 12.9 1.69 1.34, CH 1.44 1.44 1.44 1.4								br d (12.0)		
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	6	1.46	17.2, CH ₂	1.46	17.2, CH ₂	1.46	17.2, CH ₂	1.41	17.6, CH ₂	
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$		1.39		1.39		1.39		1.34		
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	7	1.47	35.1, CH ₂	1.47	35.1, CH ₂	1.47	35.1, CH ₂	1.47	35.5, CH ₂	
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$		1.37		1.37		1.37		1.35		
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	8	-	36.5, C	-	36.5, C	-	36.5, C	-	40.6, C	
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	9	0.83,	51.7, CH	0.83,	51.7, CH	0.83,	51.7, CH	1.22, m	50.5, CH	
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$		br d (12.0)		br d (12.0)		br d (12.0)				
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	10	-	36.4, C	-	36.4, C	-	36.4, C	-	36.5, C	
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	11	1.51	20.7, CH ₂	1.51	20.7, CH ₂	1.51	20.7, CH ₂	1.40	20.6, CH ₂	
		1.34		1.34		1.34		1.14		
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	12	1.69	27.3, CH ₂	1.69	27.3, CH ₂	1.69	27.3, CH ₂	1.68	25.6, CH ₂	
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$		1.59		1.59		1.59		1.14		
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	13	2.44, m	35.2, CH	2.44, m	35.2, CH	2.44, m	35.2, CH	1.93, m	34.9, CH	
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	14	-	52.9, C	-	52.9, C	-	52.9, C	-	52.2, C	
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	15a	1.94, d (8.2)	35.4, CH	1.94, d (8.2)	35.4, CH ₂	1.94,	35.4, CH ₂	3.73	75.6, CH	
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	15b	1.05		1.05		d (8.2)		-		
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$						1.05				
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	16	—	108.5, C	—	109.4, C	-	109.4, C	-	109.5, C	
br d (6.2) 18 1.03, s 18.0, CH ₃ 1.03, s 18.0, CH ₃ 1.03, s 18.0, CH ₃ 1.02, s 160, CH ₃ 19 0.79, s 15.7, CH ₃ 0.79, s 15.7, CH ₃ 0.79, s 15.7, CH ₃ 0.80, s 166, CH ₃ 20 - 68.8, C - 68.8, C - 68.8, C - 76.5, C 21 0.96, s 24.5, CH ₃ 1.03, s 29.3, CH ₃ 1.03, s 29.3, CH ₃ 1.15, s 26.9, CH ₃ 22 4.51, br s 73.3, CH 1.37 44.2, CH ₂ 1.37 44.2, CH ₂ 3.66 87.5, CH 23 4.73, 67.8, CH 4.59 67.2, CH 1.24 1.24 1.24 1.24 1.24 1.24 1.24 1.24 1.24 1.24 1.24 1.25, CH 1.5, br t (7.0) 122.5, CH 1.5, br d (8.0) 1.50, br d (8.0) 1.51, br d (8.0) <td< td=""><td>17</td><td>1.10,</td><td>48.3, CH</td><td>0.84</td><td>52.8, CH</td><td>0.84</td><td>52.8, CH</td><td>1.69, d (11.4)</td><td>61.9, CH</td></td<>	17	1.10,	48.3, CH	0.84	52.8, CH	0.84	52.8, CH	1.69, d (11.4)	61.9, CH	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$		br d (6.2)								
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	18	1.03, s	18.0, CH ₃	1.03, s	18.0, CH ₃	1.03, s	18.0, CH ₃	1.02, s	16.0, CH ₃	
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	19	0.79, s	15.7, CH ₃	0.79, s	15.7, CH ₃	0.79, s	15.7, CH ₃	0.80, s	16.6, CH ₃	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	20	—	68.8, C	—	68.8, C	-	68.8, C	-	76.5, C	
22 4.51, br s 73.3, CH 1.37 44.2, CH ₂ 1.37 44.2, CH ₂ 3.66 87.5, CH 23 4.73, 67.8, CH 1.24 1.24 1.24 1.24 1.24 23 4.73, 67.8, CH 4.59 67.2, CH 4.59 67.2, CH 2.13, m 27.2, CH ₂ br d (8.0) 121.0, CH 5.08, br d (8.0) 125.9, CH 5.05, CH 5.15, br t (7.0) 122.5, CH 25 - 135.2, C - 133.5, C - 133.5, C - 131.1, C 26 1.61, s 17.9, CH ₃ 1.61, s 17.9, CH ₃ 1.61, s 17.9, CH ₃ 1.65, s 25.0, CH ₃ 1.65, s 25.0, CH ₃ 1.65, br s 25.7, CH ₃ 27 1.65, s 25.0, CH ₃ 1.65, s 25.0, CH ₃ 1.65, s 25.0, CH ₃ 1.65, br s 25.7, CH ₃	21	0.96, s	24.5, CH ₃	1.03, s	29.3, CH ₃	1.03, s	29.3, CH ₃	1.15, s	26.9, CH ₃	
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	22	4.51, br s	73.3, CH	1.37	44.2, CH ₂	1.37	44.2, CH ₂	3.66	87.5, CH	
23 4.73, br.d. 67.8, CH 4.59 67.2, CH 4.59 67.2, CH 2.13, m 27.2, CH ₂ br.d. (8.0) br.d. (8.0) 121.0, CH 5.08, br.d. (8.0) 125.9, CH 5.08, br.d. (8.0) 125.9, CH 5.08, br.d. (8.0) 125.9, CH 5.08, br.d. (8.0) 24 4.99, d. (8.0) 121.0, CH 5.08, br.d. (8.0) 125.9, CH br.d. (8.0) br.d. (8.0) 25 - 135.2, C - 133.5, C - 133.5, C - 131.1, C 26 1.61, s 17.9, CH ₃ 1.61, s 17.9, CH ₃ 1.61, s 17.9, CH ₃ 1.58, br.s 17.8, CH ₃ 27 1.65, s 25.0, CH ₃ 1.65, s 25.0, CH ₃ 1.65, s 25.0, CH ₃ 1.65, br.s 25.7, CH ₃ 28 0.91, s 26.7, CH ₃ 0.91, s 27.6, CH ₃ 0.93, s 27.4, CH ₃ 29 0.70, s 15.5, CH ₃ 0.70, s 160, CH ₃ 0.74, s 15.8, CH ₃ 30a 3.88 64.2, CH ₂ 3.88 d (7.2) 64.2, CH ₂ 0.83, s 9.5, CH ₃ 30b 378, d (72)				1.24		1.24				
br d (8.0) 24 4.99, d (8.0) 121.0,CH 5.08, br d (8.0) 125.9,CH 5.08, br d (8.0) 5.15, br t (7.0) 122.5,CH 25 - 135.2, C - 133.5, C - 133.5, C - 131.1, C 26 1.61, s 17.9, CH ₃ 1.61, s 17.9, CH ₃ 1.61, s 17.9, CH ₃ 1.61, s 17.8, br s 17.8, CH ₃ 27 1.65, s 25.0, CH ₃ 1.65, s 25.0, CH ₃ 1.65, s 25.0, CH ₃ 1.65, br s 25.7, CH ₃ 28 0.91, s 26.7, CH ₃ 0.91, s 27.6, CH ₃ 0.91, s 27.4, CH ₃ 29 0.70, s 15.5, CH ₃ 0.70, s 160, CH ₃ 0.70, s 160, CH ₃ 0.74, s 15.8, CH ₃ 30a 3.88 64.2, CH ₂ 3.88 d (7.2) 64.2, CH ₂ 0.83, s 9.5, CH ₃	23	4.73,	67.8,CH	4.59	67.2,CH	4.59	67.2,CH	2.13, m	27.2, CH ₂	
24 4.99, d (8.0) 121.0,CH 5.08, br d (8.0) 125.9,CH 5.08, br d (8.0) 125.9,CH 5.15, br t (7.0) 122.5,CH 25 - 135.2, C - 133.5, C - 133.5, C - 131.1, C 26 1.61, s 17.9, CH ₃ 1.61, s 17.9, CH ₃ 1.61, s 17.9, CH ₃ 1.58, br s 17.8, CH ₃ 27 1.65, s 25.0, CH ₃ 1.65, s 25.0, CH ₃ 1.65, s 25.0, CH ₃ 1.65, br s 25.7, CH ₃ 28 0.91, s 26.7, CH ₃ 0.91, s 27.6, CH ₃ 0.91, s 27.6, CH ₃ 0.93, s 27.4, CH ₃ 29 0.70, s 15.5, CH ₃ 0.70, s 160, CH ₃ 0.74, s 15.8, CH ₃ 30a 3.88 64.2, CH ₂ 3.88 d (7.2) 64.2, CH ₂ 0.83, s 9.5, CH ₃ 30b 378, d (72) 378, d (72) 378, d (72) - - - -		br d (8.0)								
d (8.0) br d (8.0) 25 - 135.2, C - 133.5, C - 133.5, C - 131.1, C 26 1.61, s 17.9, CH ₃ 1.61, s 17.9, CH ₃ 1.61, s 17.9, CH ₃ 1.58, br s 17.8, CH ₃ 27 1.65, s 25.0, CH ₃ 1.65, s 25.0, CH ₃ 1.65, s 25.0, CH ₃ 1.65, br s 25.7, CH ₃ 28 0.91, s 26.7, CH ₃ 0.91, s 27.6, CH ₃ 0.91, s 27.4, CH ₃ 29 0.70, s 15.5, CH ₃ 0.70, s 160, CH ₃ 0.70, s 160, CH ₃ 0.74, s 15.8, CH ₃ 30a 3.88 64.2, CH ₂ 3.88 d (7.2) 64.2, CH ₂ 3.88 d (7.2) 64.2, CH ₂ 0.83, s 9.5, CH ₃	24	4.99,	121.0,CH	5.08, br d (8.0)	125.9,CH	5.08,	125.9,CH	5.15, br t (7.0)	122.5,CH	
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$		d (8.0)				br d (8.0)				
26 1.61, s 17.9, CH ₃ 1.61, s 17.9, CH ₃ 1.79, CH ₃ 1.58, br s 17.8, CH ₃ 27 1.65, s 25.0, CH ₃ 1.65, s 25.0, CH ₃ 1.65, s 25.0, CH ₃ 1.65, br s 25.7, CH ₃ 28 0.91, s 26.7, CH ₃ 0.91, s 27.6, CH ₃ 0.91, s 27.6, CH ₃ 0.91, s 27.4, CH ₃ 29 0.70, s 15.5, CH ₃ 0.70, s 160, CH ₃ 0.70, s 160, CH ₃ 0.74, s 15.8, CH ₃ 30a 3.88 64.2, CH ₂ 3.88 d (7.2) 64.2, CH ₂ 3.88 d (7.2) 64.2, CH ₂ 3.88 d (7.2) 0.83, s 9.5, CH ₃	25	—	135.2, C	—	133.5, C	-	133.5, C	-	131.1, C	
27 1.65, s 25.0, CH ₃ 1.65, s 25.0, CH ₃ 1.65, s 25.0, CH ₃ 1.65, br s 25.7, CH ₃ 28 0.91, s 26.7, CH ₃ 0.91, s 27.6, CH ₃ 0.91, s 27.6, CH ₃ 0.91, s 27.4, CH ₃ 29 0.70, s 15.5, CH ₃ 0.70, s 160, CH ₃ 0.70, s 160, CH ₃ 0.74, s 15.8, CH ₃ 30a 3.88 64.2, CH ₂ 3.88 d (7.2) 64.2, CH ₂ 3.88 d (7.2) 64.2, CH ₂ 0.83, s 9.5, CH ₃	26	1.61, s	17.9, CH ₃	1.61, s	17.9, CH ₃	1.61, s	17.9, CH ₃	1.58, br s	17.8, CH ₃	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	27	1.65, s	25.0, CH ₃	1.65, s	25.0, CH ₃	1.65, s	25.0, CH ₃	1.65, br s	25.7, CH ₃	
29 0.70, s 15.5, CH ₃ 0.70, s 16.0, CH ₃ 0.70, s 16.0, CH ₃ 0.74, s 15.8, CH ₃ 30a 3.88 64.2, CH ₂ 3.88 d (7.2) 64.2, CH ₂ 3.88 d (7.2) 64.2, CH ₂ 0.88, s 9.5, CH ₃ 30b 3.78 d (7.2) 3.78 d (7.2) 3.78 d (7.2) 3.78 d (7.2) - -	28	0.91, s	26.7, CH ₃	0.91, s	27.6, CH ₃	0.91, s	27.6, CH ₃	0.93, s	27.4, CH ₃	
30a 3.88 64.2, CH ₂ 3.88 d (7.2) 64.2, CH ₂ 3.88 d (7.2) 64.2, CH ₂ 0.83, s 9.5, CH ₃ 30b 3.78 d (7.2) 3.78 d (7.2) 3.78 d (7.2) 3.78 d (7.2) -	29	0.70, s	15.5, CH ₃	0.70, s	16.0, CH ₃	0.70, s	16.0, CH ₃	0.74, s	15.8, CH ₃	
30b 378 d(72) = 378 d(72) = 378 d(72) = -	30a	3.88	64.2, CH ₂	3.88 d (7.2)	64.2, CH ₂	3.88 d (7.2)	64.2, CH ₂	0.83, s	9.5, CH ₃	
5.70, u (1.2) 5.70, u (1.2)	30b	3.78, d (7.2)		3.78, d (7.2)		3.78, d (7.2)		_		
31 – 169.5, C – – – – – – – –	31	-	169.5, C	-	-	-	-	-	-	
32 2.05, s 20.3, CH ₃	32	2.05, s	20.3, CH ₃	-	-	-	-	-	-	

Overlapped ¹H-NMR signals are reported without multiplicities.

^b ¹³C shifts were extracted from HSQC and HMBC data.

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Table 2 ¹H and ¹³C spectroscopic data for the aglycon moieties of **7–10** (DMSO- d_6) (δ in ppm, *J* in Hz).

No.	lo. 7		8		9		10	
	$\delta_H{}^a$	δ _C , type	δ_H^a	δ _C ^b , type	$\delta_{H}{}^{a}$	δ _c ^b , type	$\delta_{H}{}^{a}$	δ _C ^b , type
1	1.57	38.8. CH ₂	1.58	38.5. CH ₂	1.57	38.9.CH2	1.58	39.0. CH ₂
	0.89	, . 2	0.94	, . 2	0.92	, 2	0.94	, 2
2	1.89	26.0. CH ₂	1.93. m	25.5. CH ₂	1.91	25.6. CH ₂	1.91	25.6. CH ₂
	1.50	, . 2	1.53	, . 2	1.52	, . 2	1.52	, 2
3	3.01, dd (11.4, 4.0)	87.8, CH	3.03. dd (11.5, 4.0)	87.8, CH	3.02, dd (11.5, 4.0)	87.9, CH	3.05, dd (11.4, 4.0)	87.8, CH
4	-	40.6, C	_	38.5, C	_	38.0, C	-	39.3, C
5	0.68, br d (12.0)	55.6, CH	0.73	55.7, CH	0.70, m	55.5, CH	0.71, br d (11.5)	56.1, CH
6	1.37	17.6, CH ₂	1.44	17.0, CH ₂	1.42	17.2, CH ₂	1.45	17.1, CH ₂
	1.32		1.39		1.33		1.38	
7	1.42	34.6, CH ₂	1.55	35.0, CH ₂	1.59	35.0, CH ₂	1.44	35.4, CH ₂
	0.98		1.35		1.40		1.25	
8	-	40.6, C	_	39.6, C	_	39.3, C	-	40.2, C
9	1.22, m	50.3, CH	1.37, m	50.6, CH	1.25, m	50.6, CH	1.28, m	50.2, CH
10	-	36.5, C	_	36.7, C	_	36.4, C	-	37.5, C
11	1.40	20.7, CH ₂	1.51	20.6, CH ₂	1.41	21.0, CH ₂	1.44	20.5, CH ₂
	1.13		1.28		1.10		1.16	
12	1.67	25.2, CH ₂	1.81, m	26.3, CH ₂	1.74	27.0, CH ₂	1.75	24.8, CH ₂
	1.18		1.30		1.21		1.26	
13	2.02, m	34.9, CH	2.24, ddd (11.0, 10.3, 3.5)	34.1, CH	1.69, m	39.2, CH	2.07, m	37.5, CH
14	-	52.4, C	_	46.6, C	_	49.4, C	-	53.1, C
15	5.06, br s	76.7, CH	3.95	80.0, CH	3.86, dd (8.5, 8.5)	71.2, CH	4.16, br s	82.0, CH
16	-	109.9, C	_	216.1, C	1.82	32.9, CH ₂	-	118.7, C
					1.24			
17	1.75, d (11.4)	62.5, CH	1.70, d (10.3)	53.5, CH	1.67	42.9, CH	1.94, d (12.0)	61.0, CH
18	1.02, s	15.9, CH ₃	1.11, s	15.2, CH ₃	0.96, s	14.9, CH ₃	1.00, s	15.9, CH ₃
19	0.82, s	16.4, CH ₃	0.85, s	15.9, CH ₃	0.80, s	16.0, CH ₃	0.81, s	16.3, CH ₃
20	-	76.3, C	_	72.3, C	_	75.3, C	-	76.1, C
21	1.15, s	27.2, CH ₃	1.06, s	15.8, CH ₃	0.94, s	20.1, CH ₃	1.18, s	26.9, CH ₃
22a	3.70	87.6, CH	2.32, dd (13.3, 8.0)	42.3, CH ₂	3.20	75.3, CH	3.99	86.1, CH
22b	-		2.15, dd (13.3, 6.0)		-		-	
23a	2.16, m	27.1, CH ₂	5.49, ddd (15.7, 8.0, 6.0)	121.1,CH	2.08, ddd (13.0, 7.0, 3.0)	30.0, CH ₂	1.60, m	34.6, CH ₂
23b	-		_		1.80, m		1.50	
24	5.15, br t (7.0)	122.3,CH	5.56, d (15.7)	142.0,CH	5.21, br t (7.0)	122.6,CH	3.95, br s	70.6,CH
25	-	131.4, C	_	68.2, C	-	129.8, C	-	148.9, C
26	1.58, br s	17.8, CH ₃	1.14, s	29.8, CH ₃	1.54, s	17.4, CH ₃	1.66, s	17.7, CH ₃
27a	1.65, br s	25.6, CH ₃	1.14, s	29.8, CH ₃	1.65, s	25.1, CH ₃	4.89, br s	109.0, CH ₂
27b	-		_		-		4.71, br s	
28	0.93, s	27.2, CH ₃	0.94, s	26.8, CH ₃	0.93, s	27.0, CH ₃	0.94, s	27.1, CH ₃
29	0.75, s	16.0, CH ₃	0.75, s	15.1, CH ₃	0.73, s	15.3, CH ₃	0.74, s	15.4, CH ₃
30	1.00, s	10.1, CH ₃	0.69, s	9.2, CH ₃	0.82, s	9.4, CH ₃	0.81, s	8.7, CH ₃
31	-	169.9, C	_	-	_	-	-	-
32	1.98, s	21.6, CH ₃	-	-	_	-	-	_

^a Overlapped ¹H-NMR signals are reported without multiplicities.

^b ¹³C shifts were extracted from HSQC and HMBC data.

resonances, the relative configuration at C-24 could not be determined by a ROESY spectrum. Spatial proximity of H₃-21 and H₃-26 was inferred from a selective ROESY experiment (Fig. S40) that indicated an α -orientation of the isopropylene group. The new aglycon of **10** was thus established as (3 β ,15 α ,16 α ,22R,24S)-3,15,16-triol-16,22:20,24-diepoxy-dammar-25(27)-ene, and named siconigenin. Hence, saponin **10** was identified as siconigenin 3-O- α -L-rhamnopyranosyl-(1 \rightarrow 4)- α -L-rhamnopyranosyl-(1 \rightarrow 2)- β -D-glucopyranoside (Fig. 2).

From a biogenetic perspective, the aglycon of **10** can be rationalized as being formed from aglycons of **8** and **9** via 20,22dihydroxy,16-keto-dammar-24,25-ene (**a**) as a putative intermediate (Fig. 4). This intermediate would then undergo cyclization to a hemiketal, and subsequent nucleophilic attack of the C-22 hydroxy function onto the 24,25 olefin, followed by oxidation/reduction steps would lead to the aglycon of **10**.

Saponin profiles of *Z. spina-christi* leaves of different geographical origin were compared in order to evaluate possible qualitative and semi-quantitative differences that might occur. HPLC-ESIMS chromatograms of the *n*-BuOH portion of hydroalcoholic extracts of four samples are shown in Fig. S67. Besides the sample from Dezful (Iran) that was used in the phytochemical characterization, samples from Caesarea (Israel), Nurabad (Iran), and a commercial sample purchased in a bazar in Tehran (Iran) were analyzed (Fig. 5). Jujubogenin glycosides **1–4** were the major saponins in samples from Caesarea and Nurabad, as compared to glycosides of lotogenin, sidragenin, konarigenin, and siconigenin (compounds **6–10**). The sample purchased at the bazar contained the lowest concentration as well as diversity of triterpene glycosides. Jujubogenin glycoside **5** was a major saponin in the sample from Dezful, but was not found in the other three samples. Lotogenin glycosides **6** and **7** were absent in the sample from the bazar. Sidrigenin and siconigenin glycosides **8** and **10** were present in all samples in comparable concentrations, whereas konarigenin glycoside **9** was detected only in the samples from Dezful and Nurabad (Fig. 5).

The structure of the previously undescribed flavonol glycoside **11** (Fig. 2) was established as follows. A molecular ion was observed at m/z 901.2469 [M-H]⁻ in the HRESIMS spectrum corresponding to a molecular formula of C₄₂H₄₆O₂₂ (calcd for C₄₂H₄₅O₂₂: 901.2481). The UV spectrum was typical of a flavonol, with UV absorption maxima at 273 and 312 nm. The ¹H-NMR and ¹³C-NMR data (Table 5) showed characteristic signals of quercetin, with three proton resonances at δ 7.62 (dd, J = 8.5 and 2.0 Hz), 7.50 (d, J = 2.0 Hz), and 6.80 (d, J = 8.5 Hz), and two *meta*-coupled

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Table 3	
¹ H and ¹³ C spectroscopic data for the sugar moieties of 3 -	-6 (DMSO- d_6) (δ in ppm, J in Hz).

Sugar	No	3		4		5		6	
		δ_{H}^{e}	δ_{C}^{f} , type	δ_{H}^{e}	δ_{C}^{f} , type	δ_{H}^{e}	δ_{C}^{f} , type	δ_{H}^{e}	δ _C , type
Glc ^a	1′	4.33, d (7.7)	102.8, CH	4.33, d (7.7)	102.8,CH	4.33, d (7.7)	102.8,CH	4.24, d (7.4)	104.0,CH
	2′	3.06, dd (8.5, 7.7)	73.2, CH	3.06, dd (8.5, 7.7)	73.2, CH	3.06, dd (8.5, 7.7)	73.2, CH	3.28, dd (9.0, 7.4)	75.0, CH
	3′	3.17, dd (9.0, 8.5)	76.6, CH	3.17, dd (9.0, 8.5)	76.6, CH	3.17, dd (9.0, 8.5)	76.6, CH	3.34, dd (9.0, 9.0)	78.3, CH
	4′	3.11, dd (9.0, 9.0)	69.3, CH	3.11, dd (9.0, 9.0)	69.3, CH	3.11, dd (9.0, 9.0)	69.3, CH	3.09	70.4, CH
	5′	3.14,	76.6, CH	3.14,	76.6, CH	3.14,	76.6, CH	3.09	76.6, CH
		ddd (9.0, 5.0, 2.0)		ddd (9.0, 5.0, 2.0)		ddd (9.0, 5.0, 2.0)			
	6a′	3.65, br d (11.2, 2.0)	60.8, CH ₂	4.31 (11.2, 2.0)	64.7, CH ₂	4.31 (11.2, 2.0)	64.7, CH ₂	3.65, br d (11.0, 2.0)	61.1, CH ₂
	6b′	3.44, dd (11.2, 5.0)		4.09 (11.2, 5.0)		4.09 (11.2, 5.0)		3.45, dd (11.0, 5.0)	
	31	-	_	-	167.8, ^g C	-	168.7, ^g C	_	_
	32	-	_	1.99, s	21.6, CH ₃	3.50	70.7, CH ₂	-	_
	33	-	-	-	-	-	168.7, ^g C	-	_
Rha I ^b	1″	-		-		-		5.34, br s	98.9, CH
	2″	-		-		-		3.63, br d (3.6)	70.7, CH
	3″	-		-		-		3.68, dd (9.0, 3.6)	71.2, CH
	4″	-		-		-		3.33, dd (9.0, 9.0)	78.4, CH
	5″	-		-		-		3.97, dq (9.0, 6.0)	65.9, CH
	6″	-		-		-		1.11, d (6.0)	18.3, CH ₃
Rha II ^b	1‴	-		-		-		5.03, br s	101.0,CH
	2‴	-		-		-		3.71, br d (3.6)	70.7, CH
	3‴	-		-		-		3.37	70.9, CH
	4‴	-		-		-		3.19, dd (9.0, 9.0)	72.0, CH
	5‴	-		-		-		3.49, dd (9.0, 6.0)	68.8, CH
	6‴	-		-		-		1.10, d (6.0)	17.8, CH ₃
Ara ^c	1″	4.29, d (6.2)	103.6,CH	4.29, d (6.2)	103.6,CH	4.29, d (6.2)	103.6,CH	-	
	2″	3.73, dd (8.5, 6.2)	71.9, CH	3.73, dd (8.5, 6.2)	71.9, CH	3.73, dd (8.5, 6.2)	71.9, CH	-	
	3″	3.70, dd (8.5, 3.0)	81.6, CH	3.70, dd (8.5, 3.0)	81.6, CH	3.70, dd (8.5, 3.0)	81.6, CH	-	
	4″	3.87, m	66.6, CH	3.87, m	66.4, CH	3.87, m	66.4, CH	-	
	5a''	3.68	64.3, CH ₂	3.68	64.3, CH ₂	3.68	64.3, CH ₂	-	
d	5b''	3.39		3.39		3.39			
Fuc	1‴	5.31, br s	100.0,CH	5.31, br s	100.0,CH	5.31, br s	100.0,CH	-	
	2‴	3.63, br d (6.0)	70.1, CH	3.63, br d (6.0)	70.1, CH	3.63, br d (6.0)	70.1, CH	-	
	3‴	3.60, m	65.0, CH	3.60, m	65.0, CH	3.60, m	65.0, CH	-	
	4‴	3.56, m	72.1, CH	3.56, m	72.1, CH	3.56, m	72.1, CH	-	
	5‴	4.11, br q (6.2)	66.0, CH	4.11, br q (6.2)	66.0, CH	4.11, br q (6.2)	66.0, CH	-	
	6‴	1.07, d (6.2)	16.5, CH ₃	1.07, d (6.2)	16.0, CH ₃	1.07, d (6.2)	16.0, CH_3	-	

^a Glc = β -D-glucopyranosyl.

^b Rha = α -L-rhamnopyranosyl.

^c Ara = α -L-arabinopyranosyl.

^d Fuc = β -D-fucopyranosyl.

^e Overlapped ¹H-NMR signals are reported without multiplicities.

^f ¹³C shifts were extracted from HSQC and HMBC data.

^g Interchangeable spin systems of sugars were resolved by 1D selective TOCSY and 2D HSQC-TOCSY.

resonances at δ 6.37 (br s) and 6.19 (br s) attributable to rings B and A, respectively. Three anomeric protons at δ 5.53 (d, J = 7.8 Hz), 5.12 (br s), and 4.40 (br s) indicated the presence of 3 sugars. By $^{1}H^{-1}H^{-1}$ COSY, 1D selective TOCSY, and 2D HSQC-TOCSY experiments the three sugars spin systems were assigned to one β -galactose and two α -rhamnose units (Table 5). The β -galactosyl residue was characterized by J values of H-1" (δ 5.53, d, J_{H-1/H-2} = 7.8 Hz) and H-3" (δ 3.62, dd, $J_{\text{H-3/H-2}} = 9.0$ Hz, $J_{\text{H-3/H-4}} = 3.5$ Hz). The two rhamnosyl residues were identified from the vicinal coupling constants of H-2^{'''}/H-2^{''''} (dd, ${}^{3}J_{H-2/H-3} = 3.2$ Hz, ${}^{3}J_{H-2/H-1} = br$ s), and the α configuration was established on the basis of the ¹³C-NMR shifts of C-3^{'''}/C-3^{''''} (δ 68.0 and 70.5) and C-5^{'''}/C-5^{''''} (δ 65.5 and 67.8) (Agrawal et al., 1985). The linkage position of sugar residues was established by HMBC and 2D ROESY data (Figs. S41 and S45). HMBC correlations between H-1" (δ 5.53) and C-3 (δ 135.0) indicated that the galactose unit was attached to the hydroxyl group at C-3 of the flavonoid. Likewise, the HMBC correlation between H-1^{$\prime\prime\prime$} (δ 5.12) and C-2" (δ 75.1) established the fragment of α -rhamnopyranosyl- $(1 \rightarrow 2)$ - β -galactopyranoside. The downfield shift of H-4^{'''} (δ 4.83) and its HMBC correlation with a carboxyl carbon (δ 165.7) revealed that the Rha I unit was esterified at C-4^{'''} by a trans-p-coumaroyl moiety. The attachment of the second α-rhamnose was established by a NOESY contact between the methylenic proton of CH₂-6b^{''} of Gal (δ 3.25, m) and H-1^{'''} (δ 4.40, br s) of Rha II. Hence, compound

11 was identified as quercetin 3-*O*-(4-*O*-*trans*-*p*-coumaroyl)- α -L-rhamnopyranosyl-(1 \rightarrow 2)-[α -L-rhamnopyranosyl-(1 \rightarrow 6)]- β -D-galactopyranoside.

Phenolic compounds **12–21** were identified on the basis of UV, ESIMS, and NMR data comparison as: $3',5'-\text{di-}C-\beta$ -glucosylphloretine (**12**) (Ogawa et al., 2001), quercetin 3-O- β -xylopyranosyl-(1 \rightarrow 2)- α -rhamnopyranoside 4'-O- α -rhamnopyranoside (**13**) (Nawwar et al., 1984), epigallocatechin (**14**) (Liao et al., 2014), gallocatechin (**15**) (Nomizu et al., 2008), quercetin 3-O- α -rhamnopyranosyl-(1 \rightarrow 2)- β -galactopyranosyl-(1 \rightarrow 6)- α -rhamnopyranosyl-(1 \rightarrow 2)- β -galactopyranoside (**16**) (Yasukawa et al., 1989), prodelphinidin (**17**) (Fujii et al., 2013), quercetin (**18**) (Shul'ts et al., 2012), kaempferol 3-O-robinobioside (**19**) (Pawlowska et al., 2009), kaempferol 3-O-rutinoside (**20**) (Kazuma et al., 2003), quercetin 3-O- α -arabinosyl-(1 \rightarrow 2)- α -rhamnoside (**21**) (Nielsen et al., 2005).

3. Conclusions

A phytochemical profiling of *Z. spina-christi* leaves led to the characterization of 10 dammarane-type saponins and 12 known polyphenols. Eight saponins (**3–10**) are reported here for the first time. For the three previously undescribed aglycons in saponins **8–10**, a possible biosynthetic pathway leading from **8** and **9** to the unprecedented *bis*-tetrahydrofurane moiety in **10** is proposed.

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Table 4

Indie 1		
¹ H and ¹³ C spectroscopic data	for the sugar moieties of 7-10	(DMSO- d_6) (δ in ppm, J in Hz).

Sugar	No	7		8		9		10	
		δ_{H}^{c}	δ _C , type	δ_{H}^{c}	δ_{C}^{d} , type	δ_{H}^{c}	δ _c ^d , type	δ_{H}^{c}	δ_{C}^{d} , type
Glc ^a	1′	4.23, d (7.4)	103.9,CH	4.25, d (7.4)	103.5,CH	4.25, d (7.7)	103.3,CH	4.25, d (7.4)	103.5,CH
	2′	3.24, dd (9.0, 7.4)	76.6, CH	3.28, dd (9.0, 7.4)	75.0, CH	3.27, dd (9.0, 7.7)	74.9, CH	3.28, dd (9.0, 7.4)	75.0, CH
	3′	3.31, dd (9.0, 9.0)	78.2, CH	3.34, dd (9.5, 9.0)	77.9, CH	3.33, dd (9.5, 9.0)	78.0, CH	3.34, dd (9.0, 9.0)	78.3, CH
	4′	3.09	70.5, CH	3.09	70.2, CH	3.09	70.1, CH	3.07	70.2, CH
	5′	3.09	76.1, CH	3.09	76.2, CH	3.09	76.0, CH	3.09	76.2, CH
	6a′	3.64, br d (11.0)	61.1, CH ₂	3.65, br d (11.0)	60.8, CH ₂	3.65, br d (11.0)	60.5, CH ₂	3.66, br d (11.0)	61.1, CH ₂
	6b′	3.47, dd (11.0, 5.0)		3.44, dd (11.0, 5.0)		3.45, dd (11.0, 5.0)		3.45, dd (11.0, 5.0)	
Rhal ^b	1″	5.24, br s	99.8, CH	5.34, br s	98.7, CH	5.33, brs	98.4, CH	5.33, br s	98.7, CH
	2″	3.73, br d (3.6)	70.7, CH	3.62, br d (3.6)	70.5, CH	3.64, dd (3.6)	70.3, CH	3.63, br d (3.6)	70.7, CH
	3″	3.49, dd (9.0, 3.6)	71.2, CH	3.68, dd (9.0, 3.6)	70.7, CH	3.68, dd (9.0, 3.6)	70.6, CH	3.68, dd (9.0, 3.6)	71.1, CH
	4″	3.19, dd (9.0, 9.0)	78.4, CH	3.34, dd (9.0, 9.0)	78.0, CH	3.33, dd (9.0, 9.0)	78.0, CH	3.34, dd (9.0, 9.0)	78.2, CH
	5″	3.81, dq (9.0, 6.0)	65.9, CH	3.97, dq (9.0, 6.0)	65.6, CH	3.96, dq (9.0, 6.0)	65.5, CH	3.98, dq (9.0, 6.0)	65.9, CH
	6″	1.07, d (6.0)	17.9, CH ₃	1.11, d (6.0)	17.7, CH ₃	1.10, d (6.0)	17.8, CH ₃	1.10, d (6.0)	17.6, CH ₃
Rhall ^b	1‴	-		5.06, br s	100.5,CH	5.06, br s	100.4,CH	5.06, br s	100.8,CH
	2‴	-		3.69, br d (3.5)	70.4, CH	3.71, br d (3.5)	70.3, CH	3.71, br d (3.5)	70.6, CH
	3‴	-		3.39, dd (9.0, 3.5)	70.4, CH	3.38, dd (9.0, 3.5)	70.4, CH	3.38, dd (9.0, 3.5)	70.5, CH
	4‴	-		3.19, dd (9.0, 9.0)	71.9, CH	3.19, dd (9.0, 9.0)	71.6, CH	3.19, dd (9.0, 9.0)	72.0, CH
	5‴	-		3.49, dq (9.0, 6.0)	68.3, CH	3.48, dq (9.0, 6.0)	68.2, CH	3.49, dq (9.0, 6.0)	68.8, CH
	6‴	_		1.11, d (6.0)	17.3, CH ₃	1.10, d (6.0)	17.4, CH ₃	1.10, d (6.0)	17.6, CH ₃

Spin systems of sugars were resolved by 1D selective TOCSY and 2D HSQC-TOCSY.

^a Glc = β -D-glucopyranosyl.

^b Rha = α -L-rhamnopyranosyl.

^c Overlapped ¹H-NMR signals are reported without multiplicities.

^d ¹³C shifts were extracted from HSQC and HMBC data.

Semiquantitative analysis of saponin profiles in four geographically distinct leaf samples showed that lotogenin glycosides were the major saponins and occurred in all samples analyzed, while glycosides of the other aglycons were more restricted in occurrence.

4. Experimental section

4.1. Solvents and chemicals

For extraction and column chromatography technical grade solvents (Scharlau, Spain) were used after distillation, and HPLC grade solvents (Macron, Norway) were used for HPLC. HPLC grade water was obtained from an EASY-pure II water purification system. DMSO was from Scharlau (Spain). Deuterated solvents were purchased from Armar Chemicals (Switzerland). Sephadex LH-20 was purchased from GE Healthcare. Silica gel 60 (40–63 μ m, 70–230 mesh ASTM) was from Merck (Germany).

4.2. Plant material

Z. spina-christi leaves were originating from the province of Khuzestan, Dezful County, Iran. Leaves were collected in February 2014 by Mr. Hamid Ranjbar, and identified by Dr. Ali Sonboli, Department of Biology, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Tehran, Iran. A voucher specimen (MPH-2409) has been deposited at the Herbarium of the Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Tehran, Iran. A voucher specimen (MPH-2409) has been deposited at the Herbarium of the Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Tehran. The samples originating from Caesarea (Israel), Nurabad (Iran), and a sample purchased from a bazar in Tehran (Iran) were provided by Mibelle AG, Buchs, Switzerland.

4.3. NMR spectroscopy

NMR spectra were recorded on a 500 MHz Avance IIITM spectrometer (Bruker BioSpin) equipped with a 1 mm TXI microprobe (¹H-NMR) or a 5 mm BBO probe (¹³C-NMR) at 18.0 and 23.0 °C, respectively. Standard pulse sequences of the software package Topspin 3.0 were used.

4.4. HPLC analysis

HPLC profiling of extracts was carried out on a HPLC system (Shimadzu, USA) (system 1) consisting of a degasser, binary high pressure mixing pump, column thermostat, PDA detector (SDP-M20A) with thermostatted UV cell, and a triple quadrupole MS with ESI interface (ESIMS) (LCMS-8030) coupled via T split. Both positive and negative ion HPLC-ESIMS spectra were simultaneously recorded in the range of m/z 160 to 1500; capillary voltage: 4.5 V; scan speed: 6000 u/sec; event time: 0.150 s. The second line of the T-splitter was connected to an Alltech 3300 evaporative light scattering detector. ELSD conditions were as follows: nitrogen flow: 2.5 l/min, temperature: 55 °C, detector gain: 8. Data acquisition and analysis were performed with LabSolutions software (Shimadzu, USA).

High-resolution spectra were recorded with a micrOTOF (Bruker Daltonic, Germany) mass spectrometer with ESI interface (Bruker) connected to an 1100 series HPLC (Agilent, USA) (system 2). Spectra were recorded in both positive and negative mode in the range of m/z 160 to 1500; capillary voltage: 4.0 V in positive and 3.0 V in negative mode. For calibration, a reference solution of sodium formate 0.1% (m/v) in *iso*-PrOH/H₂O (1:1) containing 5 mM NaOH was used. Data acquisition and processing were performed using HyStar 3.0 software (Bruker).

The *n*-BuOH fraction was dissolved in MeOH at a concentration of 10 mg/ml and filtered. Separation conditions for the *n*-BuOH fraction were: SunFire C₁₈ column (3.5 μ m, 150 \times 3.0 mm I.D.) (Waters, Ireland) equipped with a guard column (20.0 \times 3.0 mm I.D.); mobile phase A: H₂O with 0.1% (v/v) formic acid, mobile phase B: MeCN with 0.1% (v/v) formic acid; gradient profile: 15% B isocratic for 2 min, 15–70% B in 18 min, 100% B in 5 min; flow rate: 0.4 ml/min; column temperature: 25.0 °C; sample injection volume: 6 μ l.

4.5. Extraction and isolation

Dried leaves of *Z. spina-christi* (900 g) were ground with a RLB mill (Rotor AG, Switzerland) and extracted by maceration with

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Fig. 4. Proposed biosynthesis of aglycon in saponin 10.



Fig. 5. 2D heat map representing semi-quantitative distribution of saponins $1\!-\!10$ in leaf samples of four different origins.

EtOAc (2×2 l), followed by 35% EtOH v/v (3×4 l). After evaporation 30 g of EtOAc and 119 g of 35% ethanolic extracts were obtained. For the ethanolic extract, fifteen aliquots of 7 g each were

suspended in distilled water (600 ml), ultrasonicated for 15 min, and partitioned with *n*-BuOH (3×200 ml). After evaporation a total of 64 g of aqueous and 24 g of *n*-BuOH fraction were obtained (Fig. S68).

A portion of the *n*-BuOH fraction (12 g) was separated on a Sephadex LH-20 column (100 \times 7 cm, I.D.) eluted with MeOH at a flow rate of 1.5 ml/min using the B-688 pump (Büchi, Switzerland). Fractions were collected with a SuperFrac fraction collector (Pharmacia Biotech, Sweden). A total of 11 fractions (A-K) were combined on the basis of TLC patterns. The saponin containing fraction A (2.9 g) was further separated on an open column (75×4 cm, I.D.) packed with silica gel 60 (40–63 µm, 300 g). Separation was performed with a step gradient of CHCl₃-MeOH-H₂O 80:20:2 (1 l), 75:30:3 (1 l), and 65:35:3.5 (1 l) at a flow rate of 5 ml/min. The eluate was combined in fractions A1-A13 based on TLC pattern. Fractions A2 (15.7 mg), A3 (44.1 mg), A5 (38.4 mg), A6 (23.3 mg), A7 (98.2 mg), A9 (72.8 mg) A11 (59.7 mg), A12 (45.5 mg) were selected for final purification of compounds. This step was achieved by HPLC utilizing an Alliance 2690 instrument (Waters, USA) coupled via a T-splitter to an Alltech 2000ES (USA) evaporative light scattering detector (system 3). Separations were carried out at 25 °C on a SunFire C₁₈ column (5 μ m, 150 \times 10 mm, I.D.) (Waters, Ireland) equipped with a pre-column (10 \times 10 mm, I.D.). Gradients of H₂O/ MeCN were used. The flow-rate was 4 ml/min. Finally, we obtained compound 1 (3.2 mg) and compound 2 (5.1 mg) from A3; compound 3 (2.8 mg) from A2; compounds 4-5 (2.1 mg) from A6; compound 6 (2.2 mg) from A7; compound 7 (2.8 mg) from A5; compound **8** (1.7 mg) from A9: compound **9** (1.7 mg) from A11-A12: compound **10** (1.3 mg) from A11. Sephadex fractions B-K contained polyphenolic compounds. Compound 11 (4.7 mg) from fraction D (300.1 mg), **12** (4.3 mg) and **13** (6.2 mg) from fraction E (273.4 mg), and 14 (14.8 mg) and 15 (12.7 mg) from fraction H (283.6 mg) were isolated by means of semi-preparative HPLC. Compounds 16-22 were identified in fractions B-C, F-G, I-K comparing their UV spectra and MS spectroscopic data with those available in literature.

4.5.1. 22α -Acetoxy-christinin A (**3**)

Amorphous white solid; $[\alpha]^{25}_{Na}$ -27.9 (c 0.05, MeOH); for ¹³C NMR and ¹H NMR spectroscopic data, see Table 1 and Table 3; (-)-ESIMS *m*/*z* 969; (-)-HRESIMS *m*/*z* 969.5150 [M-H]⁻ (calcd for C₄₉H₇₇O₁₉: 969.5137).

4.5.2. Christinin A1 (4); christinin A2 (5)

Amorphous white solid; for ¹³C NMR and ¹H NMR spectroscopic data, see Tables 1 and 3; (-)-ESIMS m/z 953; (-)-HRESIMS m/z 953.5161 [M-H]⁻ (calcd for C₄₉H₇₇O₁₈: 953.5188) (**4**). ESIMS m/z 997; (-)-HRESIMS m/z 997.5072 [M-H]⁻ (calcd for C₅₀H₇₇O₂₀: 997.5086) (**5**).

4.5.3. Lotoside III (6)

Amorphous white solid; $[\alpha]^{25}_{Na}$ -50.4 (c 0.12, MeOH); for ¹³C NMR and ¹H NMR spectroscopic data, see Tables 1 and 3; (-)-ESIMS *m/z* 943; (-)-HRESIMS *m/z* 943.5363 [M-H]⁻ (calcd for C₄₈H₇₉O₁₈: 943.5345).

4.5.4. 15-Acetoxy-lotoside IV (7)

Amorphous white solid; $[\alpha]^{25}_{Na}$ -36.2 (c 0.31, MeOH); for ¹³C NMR and ¹H NMR spectroscopic data, see Tables 2 and 4; (-)-ESIMS *m*/*z* 839; (-)-HRESIMS *m*/*z* 839.4849 [M-H]⁻ (calcd for C₄₄H₇₁O₁₅: 839.4871).

4.5.5. Sidrigenin 3-O- α -L-rhamnopyranosyl- $(1 \rightarrow 4)$ - α -L-

rhamnopyranosyl- $(1 \rightarrow 2)$ *-* β *-D-glucopyranoside* (**8**)

Amorphous white solid; $[\alpha]^{25}_{Na}$ -76.8 (c 0.14, MeOH); for ¹³C NMR and ¹H NMR spectroscopic data, see Tables 2 and 4; (-)-ESIMS

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¹H and ¹³C spectroscopic data of **11** (DMSO- d_6) (δ in ppm, *J* in Hz).

No.	aglycon				sugars	
	δ_{H}	δ _C ^c , type			δ_{H}^{e}	δ_{C}^{c} , type
2	_	156.1, C	Gal ^a	1″	5.53, d (7.8)	99.1, CH
3	_	135.0, C		2″	3.86, dd (9.0, 7.5)	75.1, CH
4	_	d		3″	3.62, dd (9.0, 3.5)	73.5, CH
5	_	d		4″	3.62, d (3.7)	68.3, CH
6	_	d		5″	3.57	73.1, CH
7	6.19, br s	98.7, CH		6a''	3.59	65.0, CH ₂
8	_	165.0, C		6b''	3.25	
9	6.37, br s	93.4, CH				
10	_	100.5, C	Rha I ^b	1‴	5.12, br s	100.0,CH
1′	_	d		2‴	3.85, dd (3.2, br s)	70.7, CH
2′	7.50, d (2.0)	115.5, CH		3‴	3.87, dd (9.0, 3.2)	68.0, CH
3′	_	145.4, C		4‴	4.83, dd (9.0, 9.0)	73.6, CH
4′	_	147.8, C		5‴	4.14, dq (9.0, 6.0)	65.5, CH
5′	6.80, d (8.5)	115.0, CH		6‴	0.73, d (6.0)	16.5, CH ₃
6′	7.62, dd (8.5, 2.0)	121.2, CH				
1'''''	_	125.5, C	Rha II ^b	1''''	4.40, br s	99.8, CH
2'''''	7.36, d (8.0)	130.0, CH		2''''	3.42, dd (3.2, br s)	70.1, CH
3'''''	6.79, d (8.0)	115.3, CH		3''''	3.32, dd (9.0, 3.2)	70.5, CH
4'''''	_	159.5, C		4''''	3.10, dd (9.0, 9.0)	71.5, CH
5'''''	6.79, d (8.0)	115.3, CH		5''''	3.37, dq (9.0, 6.0)	67.8, CH
6'''''	7.36, d (8.0)	130.0, CH		6''''	1.06, d (6.0)	17.5, CH ₃
7'''''	7.35, d (16.0)	143.8, CH				
8'''''	6.23, d (16.0)	114.0, CH				
9'''''	-	165.7, C				

Spin systems of sugars were resolved by 1D selective TOCSY and 2D HSQC-TOCSY.

^a Gal = β -D-galactopyranosyl.

^b Rha = α -L-rhamnopyranosyl.

^c ¹³C shifts were extracted from HSQC and HMBC data.

^d Not detected.

^e Overlapped ¹H-NMR signals are reported without multiplicities.

m/z 943; (-)-HRESIMS m/z 943.5339 [M-H]⁻ (calcd for C₄₈H₇₉O₁₈: 943.5345).

4.5.6. Konarigenin 3-O- α -L-rhamnopyranosyl- $(1 \rightarrow 4)$ - α -L-rhamnopyranosyl- $(1 \rightarrow 2)$ - β -D-glucopyranoside (**9**)

Amorphous white solid; $[\alpha]^{25}_{Na}$ -30.4 (c 0.16, MeOH); for ¹³C NMR and ¹H NMR spectroscopic data, see Tables 2 and 4; (-)-ESIMS *m/z* 929; (-)-HRESIMS *m/z* 929.5563 [M-H]⁻ (calcd for C₄₈H₈₁O₁₇: 929.5552).

4.5.7. Siconigenin 3-O- α -L-rhamnopyranosyl- $(1 \rightarrow 4)$ - α -L-rhamnopyranosyl- $(1 \rightarrow 2)$ - β -D-glucopyranoside (**10**)

Amorphous white solid; $[\alpha]^{25}_{Na}$ -30.7 (c 0.26, MeOH); for ¹³C NMR and ¹H NMR spectroscopic data, see Tables 2 and 4; (-)-ESIMS *m*/*z* 941; (-)-HRESIMS *m*/*z* 941.5167 [M-H]⁻ (calcd for C₄₈H₇₇O₁₈: 941.5188).

4.5.8. Quercetin 3-O-(4-O-trans-p-coumaroyl)- α -L-

rhamnopyranosyl- $(1 \rightarrow 2)$ - $[\alpha$ -L-rhamnopyranosyl- $(1 \rightarrow 6)$]- β -D-galactopyranoside (**11**)

Amorphous yellow solid; $[\alpha]^{25}_{Na}$ -57.2 (c 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 273 (2.95), 312 (4.01) nm; ¹H NMR and ¹³C NMR data, see Table 5; (-)-ESIMS *m/z* 901; (-)-HRESIMS *m/z* 901.2469 [M-H]⁻ (calcd for C₄₂H₄₅O₂₂: 901.2481).

4.6. Acid hydrolysis and sugar analysis

Hydrolysis of sugars and GC-MS analysis of derivatives was performed according to Abbet et al. (2011). Compounds **1** (0.8 mg), **6** (1.5 mg), **9** (1.7 mg), and **11** (1.2 mg) were hydrolyzed separately. After heating at 100 °C for 1 h in 2 M TFA (1 ml), the mixture was extracted with CH_2Cl_2 (3 × 1.0 ml). The aq. phase was freeze-dried, and re-dissolved in dry pyridine (200 µl) containing 5 mg/ml L-

cysteine methyl ester hydrochloride. The reaction mixture was heated at 60 °C for 1 h, followed by silylation with hexamethyldisilazane and chlorotrimethylsilane (Fluka) in pyridine (3:1:10, 300 µl) at 60 °C for 30 min (Chai et al., 2007). After silvlation, pyridine was evaporated, and the solid residue extracted with *n*hexane. GC-MS analysis was performed on a 5890 Series II gas chromatograph coupled to a HP 5971A mass detector (Hewlett Packard, USA). The separation was carried out on a DB-225 MS column (30 m \times 0.25 mm, I.D., Waters, USA); column temp. 150 °C for 2 min, then gradient of 58 °C/min to 210 °C, then 10 °C/min to 240 °C. Comparison of the retention times of derivatized reference sugars with those obtained from samples resulted in L-arabinose (Rt 24.48 min), D-glucose (Rt 28.64 min). D-xylose (Rt 24.47 min), and D-fucose (Rt 25.96 min) in compound 1, and D-glucose (Rt 28.64 min) and L-rhamnose (Rt 25.66 min) in compound 6. Sugars in 9 and 11 were analyzed at a different time and were identified as D-glucose and L-rhamnose (9), and L-rhamnose (24.49 min) and D-galactose (26.04 min) (11). Derivatives of L-arabinose (Rt 24.48 min) and D-xylose (Rt 24.47 min) had very similar retention times resulting in peak overlapping. Confirmation of L-arabinose and D-xylose in 1 was obtained in a subsequent spiking experiment with derivatized reference sugars leading to unambiguous separation (D-arabinose, Rt 24.92 min); L-xylose, Rt 24.84 min) (Figs. S47–S66).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http:// dx.doi.org/10.1016/j.phytochem.2017.02.028.

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