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## Secondary metabolites from *Sambucus ebulus*

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**Abstract:** A new nonglycosidic iridoid, sambulin B (**1**), was isolated from the methanol extract of *Sambucus ebulus* L. leaves along with a recently reported new nonglycosidic iridoid, 10-*O*-acetylpatrinoside aglycone (sambulin A) (**2**); 2 flavonoids, isorhamnetin-3-*O*- $\beta$ -D-glucopyranoside (**3**) and isorhamnetin-3-*O*-rutinoside (**4**); and a mixture of 2 flavonoids (**5**), quercetin-3-*O*- $\beta$ -D-glucopyranoside and quercetin-3-*O*- $\beta$ -D-galactopyranoside. Their structures were elucidated by 1-D and 2-D nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry (MS) experiments.

**Key words:** *Sambucus ebulus*, nonglycosidic iridoids, sambulin B, flavonoids

### 1. Introduction

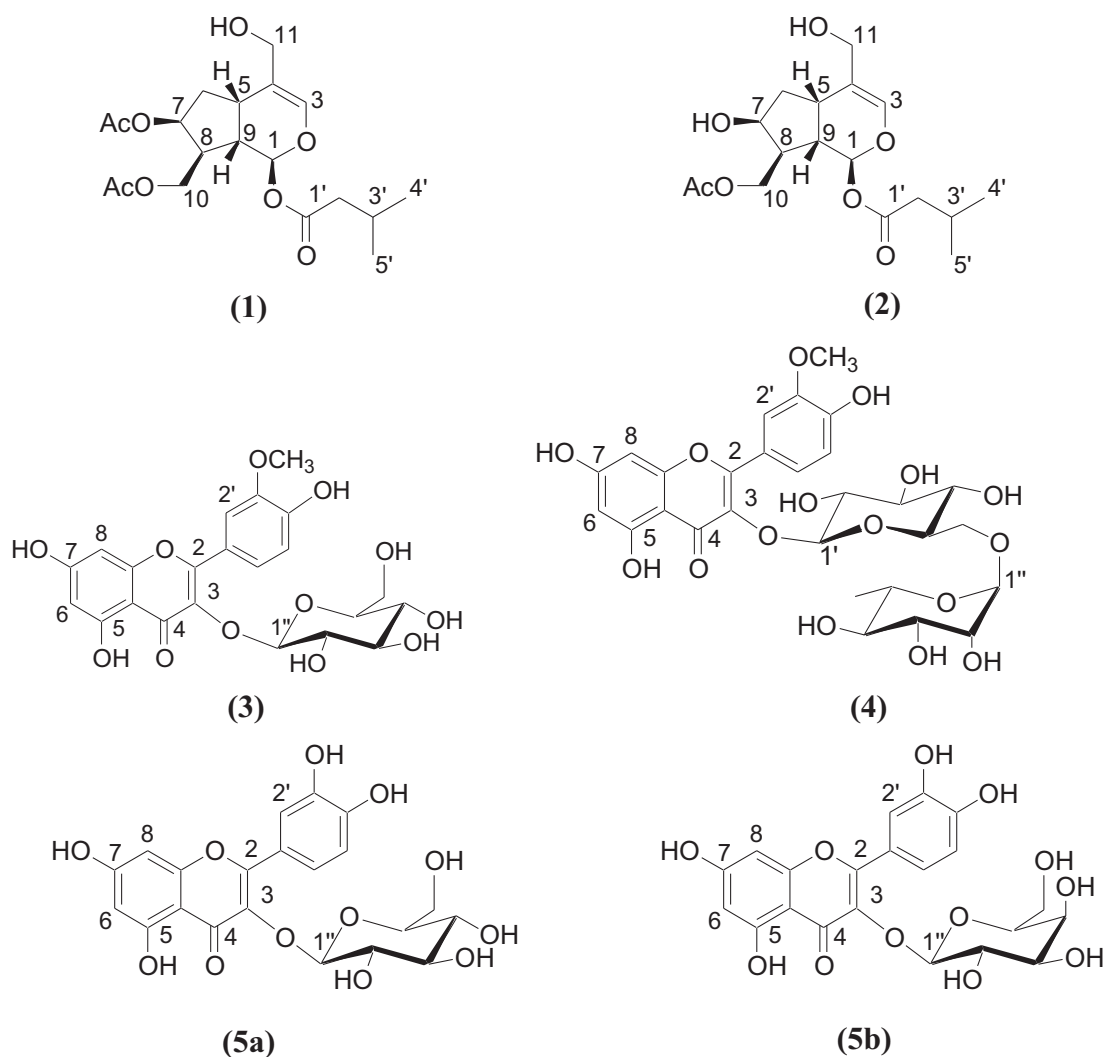
The genus *Sambucus* (Adoxaceae) is represented by 2 species in the flora of Turkey.<sup>1</sup> Among these, *Sambucus ebulus* L. is a perennial herb known locally as mürver, sultanotu, and şahmehlemi.<sup>1,2</sup> In Anatolian folk medicine, *S. ebulus* is particularly used against inflammatory problems, i.e. rheumatic pain, edema, eczema, urticaria, burns, infectious wounds, and hemorrhoids as well as peptic ulcers.<sup>3</sup> Anti-inflammatory, antinociceptive, wound-healing, cytotoxic, antiulcer, and anti-*Helicobacter pylori* effects of *S. ebulus* were reported in several previous studies.<sup>4–8</sup> Simple phenols, flavonoids, anthocyanins, lignans, and iridoids have been shown to be the major secondary metabolites of the genus.<sup>9–13</sup>

We herein present the isolation and structure elucidation of a new nonglycosidic iridoid named sambulin B (**1**), along with an iridoid, 10-*O*-acetylpatrinoside aglycone (sambulin A), and 4 known flavonoids, isorhamnetin-3-*O*- $\beta$ -D-glucopyranoside (**3**), isorhamnetin-3-*O*-rutinoside (**4**), and a mixture of 2 flavonoids, hyperoside (quercetin-3-*O*- $\beta$ -galactoside) and isoquercitrin (quercetin-3-*O*- $\beta$ -glucoside) (**5**). The chemical structures of the compounds are presented in the Figure.

### 2. Results and discussion

The leaves of *S. ebulus* were extracted with MeOH. The crude extract was dispersed in 90% MeOH, and then submitted to partition with organic solvents in increasing polarity. Two nonglycosidic iridoids (**1** and **2**) were isolated from the *n*-hexane and CHCl<sub>3</sub> subextracts, while 2 flavonol glycosides and a mixture of 2 flavonoid glycosides were obtained from the EtOAc subextract.

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**Figure.** Structures of the isolated compounds.

Compound **1** was obtained as a colorless oily substance from the *n*-hexane subextract. The molecular formula,  $C_{19}H_{28}O_8$ , was deduced from the pseudomolecular ion peaks at  $m/z$  407  $[M+Na]^+$  and 791  $[2M+Na]^+$  in the ESI-MS and by inspecting  $^{13}C$  NMR data. It exhibited UV maxima at 241, 274, and 282 nm. The IR spectrum suggested the presence of hydroxyl group ( $3678\text{ cm}^{-1}$ ), olefinic C–H ( $3100\text{ cm}^{-1}$ ), and ester carbonyl ( $1738\text{ cm}^{-1}$ ) functionalities. The  $^1H$  NMR spectrum contained 1 olefinic ( $\delta_H$  6.34), 2 hydroxymethylene ( $\delta_H$  4.20 and 4.16,  $\delta_H$  4.09 and 4.00), 1 methylene ( $\delta_H$  2.15, 1.96), 1 oxymethyne ( $\delta_H$  5.30), 3 methyne ( $\delta_H$  3.01, 2.34, and 2.13), and 1 hemiacetal ( $\delta_H$  5.97) signals (Table 1). Moreover, the  $^1H$  NMR spectrum displayed 2 equivalent secondary methyl resonances at  $\delta$  0.97 (*d*, *J*: 6.4 Hz). In the COSY spectrum, 1 methyne resonance and 1 methylene ( $\delta_H$  2.26) signal conjugated to a carbonyl function were observed in a spin system. These data were indicative of the presence of an isovaleryl moiety. The signals at 22.3 (2C), 25.6, 42.9, and 171.7 in the  $^{13}C$  NMR spectrum supported this assumption.<sup>14</sup> Moreover, the presence of 2 acetoxy groups was evident from the signals at  $\delta_H$  2.05 (3H) and 2.04 (3H) and the corresponding carbon resonances at  $\delta_C$  21.0, 20.8, and 170.2 (2C). The  $^{13}C$  NMR spectrum contained 19 signals; 5 of them were ascribed to an isovaleryl unit,

while 4 of them were arising from 2 acetyl groups. The remaining 10 carbon atoms were in accordance with a C<sub>10</sub>-iridoid core. The esterification sites of the acyl units were established by the long-range correlations in the HMBC. Thus, cross peaks between carbonyl carbon of isovaleryl ( $\delta_C$  171.7) with H-1 ( $\delta_H$  5.97) and carbonyl carbon of one of the acetyl units with H<sub>2</sub>-10 ( $\delta_H$  4.20, 4.16) revealed the locations of acyl units to be at C-1 and C-10, respectively. The NMR data of **1** were very close to those of 10-*O*-acetylpatrinoside, a recently published molecule from the same species by another research group, except for the presence of an additional acetyl unit in compound **1**. When the <sup>1</sup>H NMR spectrum of **1** was carefully inspected, H-7 signal of this appeared to be shifted to downfield about 1 ppm ( $\delta_H$  5.30) compared to 10-*O*-acetylpatrinoside aglycone (**2**).<sup>15</sup> Moreover, the <sup>13</sup>C NMR spectrum of the compound showed that the C-7 signal shifted downfield 2.5 ppm, while the C-6 and C-8 signals shifted upfield around 3 ppm compared to 10-*O*-acetylpatrinoside aglycone (**2**). Therefore, the position of the second acetyl group was determined to be C-7(OH). To determine the relative stereochemistry of the chiral centers in **1**, a ROESY experiment was performed. ROe cross-peaks were observed between H-1 $\alpha$  and H-8 $\alpha$ , and H-7 $\alpha$  and H-8 $\alpha$ , showing that these protons lie on the same side ( $\alpha$ ) of the molecule. In contrast, correlations were observed between H-3 and H-11 $\beta$ , H-5 and H-9, H-5 and H-6 $\beta$ , and H-5 and H-11 $\beta$ , indicating that these protons were on the same side ( $\beta$ ). Based on these findings the structure of compound **1** was elucidated as 7-*O*-acetyl derivative of 10-*O*-acetylpatrinoside aglycone (**2**). A literature survey revealed that compound **1** was a new nonglycosidic iridoid and was named sambulin B. On the other hand, for 10-*O*-acetylpatrinoside aglycone (**2**) we propose the trivial name sambulin A.

**Table 1.** <sup>1</sup>H (600 MHz, CDCl<sub>3</sub>) and <sup>13</sup>C-NMR (150 MHz, CDCl<sub>3</sub>) data and HMBC correlations of sambulin B (1) and 10-*O*-acetylpatrinoside (sambulin A (2)).

	Sambulin B (1)		10- <i>O</i> -acetylpatrinoside aglycon (2)		HMBC (C→H)
Position	$\delta_C$ (ppm)	$\delta_H$ (ppm), <i>J</i> (Hz)	$\delta_C$ (ppm)	$\delta_H$ (ppm) <i>J</i> (Hz)	
1	91.1	5.97 <i>d</i> (4.7)	91.9	5.96 <i>d</i> (4.7)	H-3/H-8
3	138.4	6.34 <i>s</i>	138.0	6.39 <i>s</i>	H-1/H-5/H <sub>2</sub> -11
4	116.9	-	118.0	-	H-3/H-5/H <sub>2</sub> -6/H <sub>2</sub> -11
5	32.0	3.01 <i>m</i>	32.5	3.07 <i>m</i>	H-1/H-3/H-7/H <sub>2</sub> -11
6 $\alpha$	37.2	2.15 <i>m</i>	39.6	2.19 <i>m</i>	H-5
6 $\beta$		1.96 <i>m</i>		1.70 <i>m</i>	
7	74.0	5.30 <i>m</i>	71.5	4.20 <i>m</i>	H-9/H <sub>2</sub> -10
8	43.2	2.34 <i>m</i>	46.6	2.60 <i>m</i>	H <sub>2</sub> -6
9	42.3	2.13 <i>m</i> †	41.1	2.16 <i>m</i> *	H <sub>2</sub> -6/H-8/H <sub>2</sub> -10
10a	62.2	4.20 <i>dd</i> (11.2, 6.9)	62.8	4.49 <i>dd</i> (11.1,6.9)	H-8
10b		4.16 <i>dd</i> (11.2, 6.9)		4.14 <i>dd</i> (11.1, 6.9)	
11a	62.6	4.09 <i>d</i> (12.3)	62.5	4.10 <i>d</i> (12.3)	H-3
11b		4.00 <i>d</i> (12.3)		4.02 <i>d</i> (12.3)	
7-COCH <sub>3</sub>	21.0	2.05 <i>s</i>			
7-COCH <sub>3</sub>	170.2	-			
10-COCH <sub>3</sub>	20.8	2.04 <i>s</i>	21.0	2.09 <i>s</i>	
10-COCH <sub>3</sub>	170.2	-	171.8	-	H <sub>2</sub> -10/COCH <sub>3</sub> (10)
1'	171.7	-	171.7	-	H-1/H <sub>2</sub> -2'
2'	42.9	2.26 <i>m</i>	43.1	2.26 <i>m</i>	H <sub>3</sub> -4'/H <sub>3</sub> -5'
3'	25.6	2.30-2.22 <i>m</i> †	25.6	2.20-2.10 <i>m</i> †	H <sub>2</sub> -2'/ H <sub>3</sub> -4'/H <sub>3</sub> -5'
4'	22.3	0.97 <i>d</i> (6.4)	22.4	0.98 <i>d</i> (6.4)	H <sub>2</sub> -2'/H-3'
5'	22.3	0.97 <i>d</i> (6.4)	22.4	0.98 <i>d</i> (6.4)	H <sub>2</sub> -2'/H-3'

\*Unclear due to overlapping

In addition, 4 known flavonoids were isolated from the ethyl acetate subextract of *S. ebulus* methanol extract. The known compounds were identified by UV, IR, and 1-D and 2-D NMR techniques and by comparison with the previous published data. The structures of **3** and **4** were elucidated as isorhamnetin-3-*O*- $\beta$ -D-glucopyranoside and isorhamnetin-3-*O*-rutinoside (Table 2). The NMR data of these compounds were found to be consistent with the published literature.<sup>16</sup> Inspection of the <sup>1</sup>H NMR spectra of **5** revealed that it was a mixture of 2 flavonoid glycosides. A careful inspection of the spectra led to the identification of quercetin-3-*O*- $\beta$ -D-glucopyranoside and quercetin-3-*O*- $\beta$ -D-galactopyranoside. Thus **5** was identified as an inseparable mixture of hyperoside (quercetin-3-*O*- $\beta$ -galactoside) and isoquercitrin (quercetin-3-*O*- $\beta$ -glucoside). The <sup>13</sup>C and <sup>1</sup>H NMR data of the hyperoside and isoquercitrin are given in Table 3; they were consistent with the previously published data.<sup>17</sup>

**Table 2.** <sup>1</sup>H (600 MHz, CD<sub>3</sub>OD) and <sup>13</sup>C NMR (150 MHz, CD<sub>3</sub>OD) data of isorhamnetin-3-*O*- $\beta$ -D-glucopyranoside (**3**) and isorhamnetin-3-*O*-rutinoside (**4**).

Position	Isorhamnetin-3- <i>O</i> - $\beta$ -D-glucopyranoside (3)		Isorhamnetin-3- <i>O</i> -rutinoside (4)	
	$\delta_H$ , ppm ( <i>J</i> , Hz)	$\delta_C$ , ppm	$\delta_H$ , ppm ( <i>J</i> , Hz)	$\delta_C$ , ppm
2		157.2		159.2
3		130.9		132.9
4		178.0		179.9
5		160.5		164.0
6	6.21 <i>d</i> (2.1)	98.8	6.20 <i>d</i> (1.7)	100.7
7		165.1		163.5
8	6.41 <i>d</i> (2.1)	93.6	6.39 <i>d</i> (1.7)	95.6
9		157.0		159.0
10		104.1		104.9
1'		121.8		123.4
2'	7.91 <i>d</i> (2.1)	112.9	7.94 <i>d</i> (1.5)	116.4
3'		149.5		151.2
4'		147.0		148.9
5'	6.92 <i>d</i> (7.9)	114.7	6.90 <i>d</i> (8.5)	116.5
6'	7.58 <i>dd</i> (7.9, 2.1)	122.4	7.63 <i>dd</i> (8.5, 1.5)	124.5
1''	5.37 <i>d</i> (7.3)	100.2	5.22 <i>d</i> (7.6)	103.0
2''	3.24 <i>dd</i> (7.3, 5.2)	74.4	3.45-3.48 <i>m</i>	76.4
3''	3.46 <i>t</i> (7.3)	76.6	3.35-3.45 <i>m</i>	78.7
4''	3.30 <i>m</i>	69.9	3.35 <i>m</i>	72.1
5''	3.30 <i>m</i>	77.5	3.22 <i>m</i>	77.9
HA-6''	3.56 <i>dd</i> (12.0, 5.6)	61.2	3.55 <i>dd</i> (12.0, 5.1)	68.6
HB-6''	3.72 <i>dd</i> (12.0, 2.3)		3.72 <i>dd</i> (12.0, 2.5)	
OCH <sub>3</sub>	3.94 <i>s</i>	55.4	3.94 <i>s</i>	57.2
1'''			4.53 <i>d</i> (1.7)	102.9
2'''			3.20-3.80	72.4
3'''			3.20-3.80	72.8
4'''			3.20-3.80	74.4
5'''			3.20-3.80	70.3
6'''			1.1 <i>d</i> (6.5)	18.2

Several iridoids have been reported from *Sambucus* species.<sup>12,18,19</sup> Recently 6 new 'Valeriana-type' iridoid glycosides were isolated from *S. ebulus* leaves. However, sambulin B is a new nonglycosidic ester iridoid, isolated from the leaves of *S. ebulus*. Since iridoids are accepted as significant chemotaxonomic markers, the occurrence of

such rare ‘Valeriana-type’ nonglycosidic iridoids might contribute to the chemotaxonomy of the genus *Sambucus* (formerly Caprifoliaceae) within its new family (Adoxaceae).

**Table 3.**  $^1\text{H}$  (600 MHz,  $\text{CD}_3\text{OD}$ ) and  $^{13}\text{C}$  NMR (150 MHz,  $\text{CD}_3\text{OD}$ ) data of quercetin-3-*O*- $\beta$ -D-glucopyranoside (**5a**) and quercetin-3-*O*- $\beta$ -D-galactopyranoside (**5b**).

Position	Quercetin-3- <i>O</i> - $\beta$ -glucopyranoside ( <b>5a</b> )		Quercetin-3- <i>O</i> - $\beta$ -D-galactopyranoside ( <b>5b</b> )	
	$\delta_{\text{H}}$ , ppm ( <i>J</i> , Hz)	$\delta_{\text{C}}$ , ppm	$\delta_{\text{H}}$ , ppm ( <i>J</i> , Hz)	$\delta_{\text{C}}$ , ppm
2		158.8		158.5
3		136.0		136.0
4		179.6		179.5
5		163.1		163.1
6	6.20 <i>d</i> (1.7)	99.9	6.20 <i>d</i> (2.1)	99.9
7		166.7		164.1
8	6.39 <i>d</i> (1.7)	94.8	6.39 <i>d</i> (2.1)	94.8
9		158.6		156.3
10		105.5		103.9
1'		123.1		123.1
2'	7.66 <i>d</i> (2.2)	116.1	7.84 <i>d</i> (2.3)	116.0
3'		145.9		145.9
4'		150.0		149.9
5'	6.84 <i>d</i> (8.5)	117.6	6.86 <i>d</i> (8.8)	117.8
6'	7.63 <i>dd</i> (8.5, 2.2)	123.2	7.59 <i>dd</i> (8.8, 2.3)	121.8
1''	5.25 <i>d</i> (7.6)	104.5	5.15 <i>d</i> (8.2)	102.3
2''	3.42 <i>dd</i> (7.6, 9.0)	75.8	3.53 <i>dd</i> (8.2, 9.3)	73.2
3''	3.48 <i>m</i>	78.4	3.22–3.34 <i>m</i>	75.1
4''	3.35 <i>m</i>	71.3	3.61 <i>d</i> (3.2)	70.1
5''	3.22 <i>m</i>	78.2	3.22–3.34 <i>m</i>	77.2
6''	3.58 <i>dd</i> (12.0, 5.5)	62.6	3.60*	61.9
	3.71 <i>dd</i> (12.0, 2.5)		3.71 <i>dd</i> (12, 2.5)	

\*Unclear due to overlapping

### 3. Experimental

#### 3.1. General

UV spectra were recorded on an HP Agilent 8453 (USA). IR spectra were recorded on a PerkinElmer-2000 FT-IR spectrometer (USA). NMR spectra were recorded on a Varian Mercury-Mx spectrometer (USA) at 600 MHz for  $^1\text{H}$  NMR and at 150 MHz for  $^{13}\text{C}$  NMR, with  $\text{CDCl}_3$  and  $\text{CD}_3\text{OD}$  as solvents. Optical rotations were determined on an Opt. Act. Ltd. AA-5 polarimeter. Kieselgel 60 (0.063–0.200 mm; Merck, Darmstadt, Germany), Sephadex LH-20 (Lipophilic Sephadex, 25–100  $\mu\text{m}$ , Sigma-Aldrich, USA), and Polyamide (Fluka, USA) were used for column chromatography (CC), and precoated Kieselgel 60 F<sub>254</sub> (Merck) plates were used for thin layer chromatography. For medium-pressure liquid chromatography (MPLC) a CombiFlash Companion (Teledyne Isco, USA) apparatus equipped with RediSep columns (C18, 130 and 43 g; Teledyne Isco, USA) was used.

#### 3.2. Plant material

The leaves of *S. ebulus* were collected from Uludağ, Bursa (Turkey) in June 2009. The plants were identified by Prof Dr Erdem Yeşilada (Department of Pharmacognosy, Faculty of Pharmacy, Yeditepe University, İstanbul, Turkey). A voucher specimen (YEF 09017) was deposited at the Herbarium of Yeditepe University.

### 3.3. Extraction and isolation

The air dried and powdered leaves of *S. ebulus* (330 g) were extracted with methanol (MeOH) (2.3 l) over 24 h with intermittent stirring in a water bath (40 °C). The extract was filtered through filter paper and evaporated to dryness under reduced pressure to give crude MeOH extract (60.5 g, yield: 18.3%). The MeOH extract was then redissolved in 200 mL of MeOH in H<sub>2</sub>O (10%) and extracted with *n*-hexane (9 × 200 mL). Combined *n*-hexane extract was evaporated under reduced pressure to yield *n*-hexane subextract (8 g, yield: 13.8%). Then MeOH was removed from the remaining extract and diluted with distilled H<sub>2</sub>O and fractionated by successive solvent extractions with chloroform (CHCl<sub>3</sub>) (4 × 200 mL), ethyl acetate (EtOAc) (4 × 200 mL), and *n*-butanol saturated with H<sub>2</sub>O (*n*-BuOH) (4 × 100 mL). Each extract after solvent extraction was evaporated to dryness under reduced pressure to yield CHCl<sub>3</sub> subextract (22.5 g, yield: 39.2%), EtOAc subextract (2.5 g, yield: 4.3%), and *n*-BuOH subextract (9 g yield: 15.5%), and remaining water (11.5 g, yield: 19.8%).

#### 3.3.1. Isolation of the components from EtOAc subextract

The EtOAc subextract (2 g) was further subjected to column chromatography (CC) over polyamide (25 g) and eluted with H<sub>2</sub>O/MeOH mixtures in different ratios (0%–100%) to yield 4 fractions: SE<sub>EtOAc</sub> I Fr. 1–4 (746 mg), SE<sub>EtOAc</sub> I Fr. 5–10 (400 mg), SE<sub>EtOAc</sub> I Fr. 12–14 (415 mg), and SE<sub>EtOAc</sub> I Fr. 15–20 (227 mg). SE<sub>EtOAc</sub> I Fr. 12–14 was subjected to MPLC over a RediSep Rf Reversed-phase C18 column (130 g) and eluted with mixtures of MeOH/H<sub>2</sub>O (15%–100%) to yield 2 fractions: SE<sub>EtOAc</sub> II Fr. 26–34 and SE<sub>EtOAc</sub> II Fr. 35–49. Further CC of SE<sub>EtOAc</sub> II Fr. 26–34 over SiO<sub>2</sub> (12 g) yielded a mixture (**5**) of hyperoside (quercetin-3-*O*-β-galactoside) and isoquercitrin (quercetin-3-*O*-β-glucoside) by eluting with mobile systems of MeOH/CHCl<sub>3</sub> (from 10% to 15%). The other fraction SE<sub>EtOAc</sub> II Fr. 35–49 yielded isorhamnetin-3-*O*-β-D-glucopyranoside (**3**) by CC over SiO<sub>2</sub> (15 g) and eluting with mobile systems of MeOH/CHCl<sub>3</sub> (from 2% to 20%).

Furthermore, fractions of polyamide CC were further subjected to chromatographic separation by MPLC. SE<sub>EtOAc</sub> I Fr. 15–20 was applied to MPLC over a RediSep flash column (12 g) and eluted with a MeOH/CHCl<sub>3</sub> solvent system (0%–80%). SE<sub>EtOAc</sub> I Fr. 5–10 was applied to a MPLC RediSep 13 g reverse phase C18 column and eluted with MeOH/H<sub>2</sub>O (5%–70%) to yield isorhamnetin-3-*O*-rutinoside (**4**).

#### 3.3.2. Isolation of the components from CHCl<sub>3</sub> subextract

The CHCl<sub>3</sub> (10 g) subextract was applied to CC over SiO<sub>2</sub> (200 g) and eluted with first a solvent system composed of EtOAc/*n*-hexane in different rates (20%–100%) and then with MeOH/CHCl<sub>3</sub> mixtures (10%–50%) to give 4 fractions: SE<sub>CHCl3</sub> I Fr. 2–7 (332 mg), SE<sub>CHCl3</sub> I Fr. 8–13 (794 mg), SE<sub>CHCl3</sub> I Fr. 14–16 (6.4 g), and SE<sub>CHCl3</sub> I Fr. 17–23 (806 mg). SE<sub>CHCl3</sub> I Fr. 14–16 was fractionated by CC over SiO<sub>2</sub> (150 g). Elution was started with CHCl<sub>3</sub> and then mixtures of MeOH/CHCl<sub>3</sub> (2.5%–60%) were used. Four subfractions were obtained: SE<sub>CHCl3</sub> II Fr. 59–63 (1.289 g), SE<sub>CHCl3</sub> II Fr. 71–72 (273 mg), SE<sub>CHCl3</sub> II Fr. 73–74 (180 mg), and SE<sub>CHCl3</sub> I Fr. 78–86 (615 mg). SE<sub>CHCl3</sub> II Fr. 59–63 was applied to MPLC over a RediSep silica flash column (40 g) eluting with acetone/CHCl<sub>3</sub> (15%–90%) and 10-*O*-acetylpatrinoside aglycone (**2**) was obtained.

### 3.3.3. Isolation of the components from hexane subextract

The hexane subextract was applied to CC over Sephadex (16 g) and eluted with  $\text{CHCl}_3/\text{MeOH}$  (50%) to yield 2 fractions:  $\text{SE}_{\text{Hexane}}$  I Fr. 9–12 (38 mg) and  $\text{SE}_{\text{Hexane}}$  I Fr. 13–15 (75 mg). The MPLC (RediSep Silica Flash Column (4 g)) of  $\text{SE}_{\text{Hexane}}$  I Fr. 9–12 with  $\text{EtOAc}/n\text{-hexane}$  (15%–80%) as eluent system yielded sambulin B (1).

### 3.4. Spectroscopic characteristics of the isolated compounds

**Sambulin B (1):** Colorless oily substance;  $\text{C}_{19}\text{H}_{28}\text{O}_8$ ,  $[\alpha]_D^{20} = +39.1$  ( $c = 0.41$ ,  $\text{CHCl}_3$ ); UV ( $\text{CHCl}_3$ ):  $\lambda_{\text{max}}$  241, 274, 282, IR (KBr)  $\nu_{\text{max}}$   $\text{cm}^{-1}$ : 3678, 3100, 1738, 1235, ESI-MS:  $m/z = 407$   $[\text{M}+\text{Na}]^+$ , 791  $[2\text{M}+\text{Na}]^+$ ,  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ ) and  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ ): Table 1.

**10-O-acetylpatrinoside aglycone (sambulin A) (2):** Colorless oily substance;  $\text{C}_{17}\text{H}_{26}\text{O}_7$ ,  $[\alpha]_D^{20} = -4.6$  ( $c = 0.33$ ,  $\text{CHCl}_3$ ); UV ( $\text{CHCl}_3$ ):  $\lambda_{\text{max}}$  241, 274, 282; IR (KBr)  $\nu_{\text{max}}$   $\text{cm}^{-1}$ : 3418, 1736, 1372, 1235; ESI-MS:  $m/z = 343$   $[\text{M}+\text{H}]^+$ ,  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ ) and  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ ): Table 1.

**Isorhamnetin-3-O- $\beta$ -D-glucopyranoside (3):** Yellow amorphous powder;  $\text{C}_{22}\text{H}_{22}\text{O}_{12}$ , UV (MeOH):  $\lambda_{\text{max}}$  264, 354 nm, IR (KBr)  $\nu_{\text{max}}$   $\text{cm}^{-1}$ : 3409, 1653, 1607, HR-ESI-MS:  $m/z = 501.1003$   $[\text{M}+\text{Na}]^+$ ,  $^1\text{H}$  NMR (600 MHz,  $\text{CD}_3\text{OD}$ ) and  $^{13}\text{C}$  NMR (150 MHz,  $\text{CD}_3\text{OD}$ ): Table 2.

**Isorhamnetin-3-O-rutinoside (4):** Yellow amorphous powder;  $\text{C}_{28}\text{H}_{32}\text{O}_{16}$ , UV (MeOH):  $\lambda_{\text{max}}$  266, 354 nm, IR (KBr)  $\nu_{\text{max}}$   $\text{cm}^{-1}$ : 3452, 2934, 1665, 1608, HR-ESI-MS:  $m/z = 647.1583$   $[\text{M}+\text{Na}]^+$ ,  $^1\text{H}$  NMR (600 MHz,  $\text{CD}_3\text{OD}$ ) and  $^{13}\text{C}$  NMR (150 MHz,  $\text{CD}_3\text{OD}$ ): Table 2.

**Mixture of isoquercitrin (5a) and hyperoside (5b) (2/3) (5):** Yellow amorphous powder; UV (MeOH):  $\lambda_{\text{max}}$  266, 352 nm, IR (KBr)  $\nu_{\text{max}}$   $\text{cm}^{-1}$ : 3415, 1655, 1605, HR-ESI-MS:  $m/z = 487.0847$   $[\text{M}+\text{Na}]^+$ ,  $^1\text{H}$  NMR (600 MHz,  $\text{CD}_3\text{OD}$ ) and  $^{13}\text{C}$  NMR (150 MHz,  $\text{CD}_3\text{OD}$ ): Table 3.

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