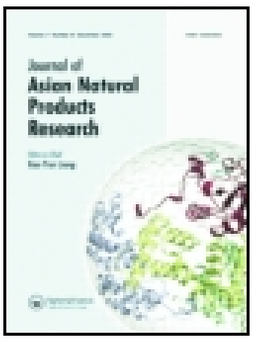

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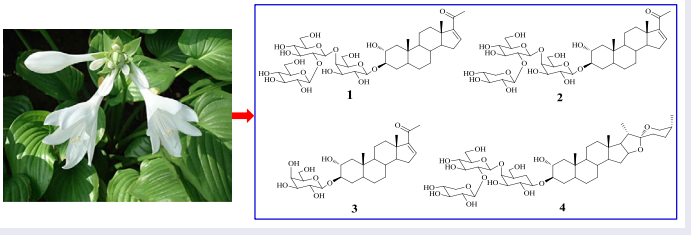
The bioassay-guided isolation of antifungal saponins from *Hosta plantaginea* leaves

Meng-Yue Wang, Ying Peng, Chong-Sheng Peng, Jiang-Yuan Qu and Xiao-Bo Li

School of Pharmacy, Shanghai Jiao Tong University, Shanghai 200240, China

ABSTRACT

Four new steroidal saponins hostaside I (**1**), hostaside II (**2**), hostaside III (**3**), and hostaside IV (**4**), together with five known steroidal saponins (**5–9**), were isolated by the bioassay-guided fractionation from the leaves of *Hosta plantaginea* (Lam.) Aschers, a worldwide well-known ornamental plant. Hostasides I and II showed significant antifungal activities, and they could inhibit the growth of *Candida albicans* and *Fusarium oxysporium* with *MIC* values as low as 4 μg/ml.



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Liliaceae; *Hosta plantaginea*; steroidal saponin; antifungal; *Candida albicans*; *Fusarium oxysporium*

1. Introduction

Hosta plantaginea (Lam.) Aschers, belonging to genus *Hosta* (Liliaceae), is a perennial herb widely cultivated in Europe, Asia, and America as garden plant. Its young leaves and buds are edible, and the leaves, flowers, and rhizomes are commonly used as important folk medicine in China for the treatment of fungal infection, sore throat, mastitis, otitis media, and so on [1,2]. Recently, some steroidal saponins [3–6] and benzylphenethylamine alkaloids [7,8] with the antitumor and antivirus activities were found.

In our continuing efforts to find the bioactive constituents from genus *Hosta* [9–11], some steroidal saponins were isolated from *H. plantaginea* rhizome. In the present study, the antifungal constituents in *H. plantaginea* leaves were investigated for the first time. Using bioassay-guided fractionation, four new saponins hostasides I (**1**), II (**2**), III (**3**), and IV (**4**), together with five known saponins, were isolated, which were (25*R*)-2*α*,3*β*-dihydroxy-5*α*-spirostane 3-*O*-*α*-*L*-rhamnopyranosyl-(1→2)-[*β*-*D*-glucopyranosyl-(1→4)]-*β*-*D*-galactopyranoside (**5**) [12], (25*R*)-2*α*,3*β*,12*β*-trihydroxy-5*α*-spirostane 3-*O*-*α*-*L*-rhamnopyranosyl-(1→2)-*β*-*D*-galactopyranoside

CONTACT Xiao-Bo Li xbli@sjtu.edu.cn

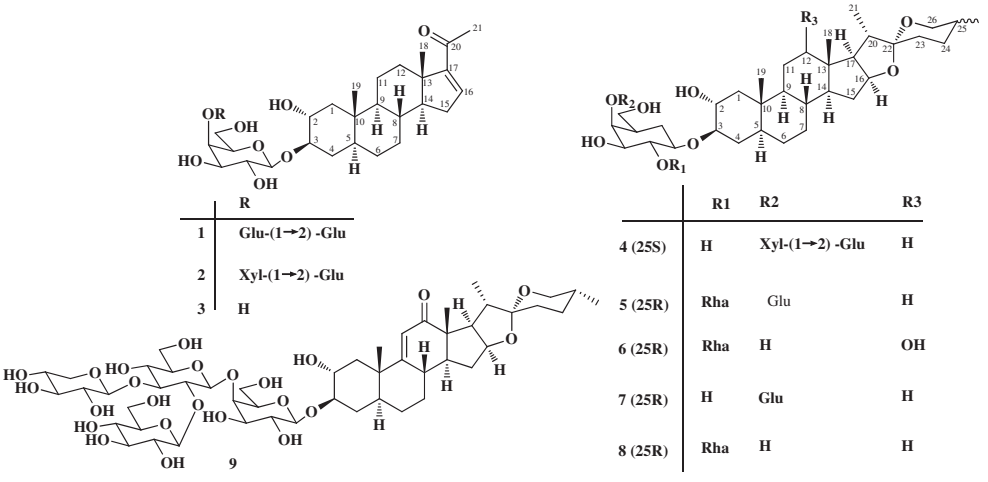

Figure 1. Chemical structures of steroidal saponins **1–9**.

(**6**) [13], (25*R*)-2*α*,3*β*-dihydroxy-5*α*-spirostane 3-O-*β*-*D*-glucopyranosyl-(1→4)-*β*-*D*-galactopyranoside (**7**) [12], (25*R*)-2*α*,3*β*-dihydroxy-5*α*-spirostane 3-*O*-*α*-*L*-rhamnopyranosyl-(1→2)-*β*-*D*-galactopyranoside (**8**) [4], and (25*R*)-2*α*,3*β*-dihydroxy-5*α*-spirost-9-en-12-one 3-*O*-*β*-*D*-glucopyranosyl-(1→2)-[*β*-*D*-xylopyranosyl-(1→3)]-*β*-*D*-glucopyranosyl-(1→4)-*β*-*D*-galactopyranoside (**9**) [14] (Figure 1).

2. Results and discussion

Compound **1** was obtained as white amorphous powder with the molecular formula $C_{39}H_{62}O_{18}$ which was deduced from HR-ESI-MS at *m/z* 841.3832 $[M + Na]^+$. The ^1H NMR spectrum (Tables 1 and 2) indicated the presence of three methyls (singlets at δ_H 0.91, 0.87, and 2.23, each 3H), three anomeric protons [δ_H 4.93 (1H, d, J = 7.7 Hz), 5.15 (1H, d, J = 7.8 Hz), and 5.22 (1H, d, J = 7.5 Hz)], and one vinylic proton (δ_H 6.59 (1H, dd, J = 3.1, 1.7 Hz). The ^{13}C NMR spectrum indicated the presence of one carbonyl carbon (δ_C 196.5) and two vinylic carbon signals (δ_C 144.9 and 155.5, respectively). The above data primarily suggested that **1** was a progesterone triglycoside and its aglycone was further determined to be 2*α*,3*β*-dihydroxy-5*α*-pregna-16-en-20-one. The *α*-orientation of C-5 is deduced by its chemical shift at 44.7. For 5*α* compounds, the chemical shifts of C-5 appear at 43–46; while for 5*β* compounds, the chemical shifts are observed at 35–36.5 [15]. NOESY correlations between H-3 and H-5, H-2 and H-19 indicated the *α*-equatorial configuration of H-3 and *β*-equatorial configuration of H-2. Acid hydrolysis of **1** gave a sapogenin 2*α*,3*β*-dihydroxy-5*α*-pregna-16-en-20-one **(1a)** [16] and a sugar portion. Two monosaccharides identified from the sugar portion by GC analysis were *D*-glucose and *D*-galactose in a ratio of 2:1. The *β*-anomeric configurations for both glucose and galactose were judged from their coupling constants (J > 7.0 Hz). The sugar portion was further confirmed to be *O*-*β*-*D*-glucopyranosyl-(1→2)-*β*-*D*-glucopyranosyl-(1→4)-*β*-*D*-galactopyranosyl unit through HMBC correlations of H-1′/C-3, H- 1″/C-4′, and H-1‴/C-2″. Thus, the structure of **1** was established as 2*α*,3*β*-dihydroxy-5*α*-pregna-16-en-20-one 3-*O*-*β*-*D*-glucopyranosyl-(1→2)-*β*-*D*-glucopyranosyl-(1→4)-*β*-*D*-galactopyranoside, and named hostaside Ⅰ.

Table 1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectral data of the aglycone moieties of compounds **1–4** (*δ* in ppm, in C_5D_5N).

	1		2		3		4	
Position	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
	1.14 dd (12.3, 10.2)	45.5	1.14 dd (12.3, 10.2)	45.5	1.13 dd (12.2, 10.2)	45.6	1.10 dd (12.6, 10.2)	45.6
	2.15 dd (12.3, 4.8)		2.15 dd (12.3, 4.8)		2.14 dd (12.2, 4.6)		2.18 dd (12.6, 4.8)	
2	3.94[a]	70.4	3.95[a]	70.4	3.92–3.93 m	70.6	3.89–3.90 m	70.5
3	3.89–3.90 m	84.4	3.88–3.89 m	84.3	3.88–3.89 m	84.2	3.82–3.83 m	84.6
4	1.45 dd (12.5, 9.0)	34.1	1.45 dd (12.5, 9.0)	34.1	1.44[a]	34.2	1.43 dd (10.8, 5.6)	34.1
	1.83 dd (12.5, 1.6)		1.88 dd (12.5, 1.6)		1.86 dd (12.3, 1.6)		1.83 dd (10.8, 4.2)	
5	0.99[a]	44.7	0.99[a]	44.7	0.98[a]	44.9	1.00[a]	44.7
6	1.00[a]	27.9	1.00[a]	27.9	0.99[a]	28.0	1.01[a]	27.6
	1.11–1.14 m		1.11–1.13 m		1.10–1.12 m		1.08[a]	
7	0.72 dd (15.0, 4.7)	32.0	0.72 dd (15.0, 4.7)	32.0	0.72 dd (15.0, 4.7)	32.1	0.62 dd (12.0, 4.2)	32.1
	1.51–1.53 m		1.51–1.53 m		1.51–1.51 m		1.57–1.59 m	
8	1.61–1.63 m	33.3	1.61–1.63 m	33.3	1.62–1.63 m	33.3	1.63–1.62 m	34.5
9	0.86–0.88 m	55	0.86–0.88 m	55.0	0.85–0.87 m	55.0	0.58 dd (11.2, 4.8)	54.4
10		37.1		37.1		37.1		36.9
11	1.21–1.23 m	21.5	1.21–1.22 m	21.5	1.21–1.22 m	21.5	1.08[a]	21.4
	1.38–1.39 m		1.38–1.39 m		1.38–1.39 m		1.31–1.32 m	
12	1.43 br.d (14.5)	35.3	1.43 br.d (14.5)	35.3	1.44[a]	35.3	0.94–0.95 m	39.9
	2.56 dd (14.5, 3.0)		2.56 dd (14.5, 3.0)		2.56 dd (14.5, 3.0)		1.58 d (11.5)	
13		46.6		46.6		46.6		40.7
14	1.40–1.41 m	56.3	1.40–1.41 m	56.3	1.40–1.42 m	56.3	0.80–0.81 m	56.2
15	1.91 dd (12.0, 1.7)	32.2	1.91 dd (12.0, 1.7)	32.2	1.91 dd (12.0, 1.7)	32.2	0.91–0.92 m	32.1
	2.08–2.11 m		2.08–2.11 m		2.08–2.10 m		1.41–1.43 m	
16	6.59 dd (3.1, 1.7)	144.9	6.58 dd (3.1, 1.7)	144.9	6.59 dd (3.1, 1.7)	144.9	4.48[a]	81.2
17		155.5		155.5		155.5	1.80–1.82 m	62.8
18	0.91 s	16.3	0.92 s	16.3	0.91 s	16.3	0.79 s	16.6
19	0.87 s	13.3	0.87 s	13.3	0.86 s	13.3	0.68 s	13.2
20		196.5		196.5		196.4	1.83[a]	42.2
21	2.23 s	27.1	2.23 s	27.1	2.23 s	27.0	1.85 d (6.6)	14.7
22								109.6
23							1.35–1.36 m	26.2
							1.83[a]	
24							1.29–1.30 m	26.3
							2.05–2.07 m	
25								27.5
26							3.98 dd (10.5, 3.4)	65.0
							3.32 dd (10.5, 10.5)	
27							1.01 d (7.2)	16.2

[a]Overlapped.

Compound **2** was obtained as a white amorphous powder. Its molecular formula was deduced to be $C_{38}H_{60}O_{17}$ by HR-ESI-MS at *m/z* 811.3732 $[M + Na]^+$. The ^{1}H and ^{13}C NMR spectra indicated that the aglycone of **2** was essentially identical to that of **1**. However, the sugar portion was different from **1** in the lack of a glucopyranosyl unit but the presence of an xylopyranosyl unit. Acid hydrolysis of **2** afforded *D*-xylose, *D*-glucose, and *D*-galactose in a ratio of 1:1:1 on the basis of GC analysis. The HMBC correlations of H-1′/C-3,

Table 2. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectral data of the sugar portions of compounds **1–4** (*δ* in ppm, in C_5D_5N).

	1		2		3		4	
Position	δH (*J* in Hz)	δC	δH (*J* in Hz)	δC	δH (*J* in Hz)	δC	δH (*J* in Hz)	δC
	β-D-Gal		*β-D*-Gal		*β-D*-Gal		*β-D*-Gal	
1′	4.93 d (7.7)	103.8	4.93 d (7.7)	103.8	4.96 d (7.8)	103.9	4.94 d (7.7)	103.9
2′	4.47 dd (9.0, 7.7)	72.8	4.47[a]	72.8	4.52 dd (9.3, 7.8)	72.2	4.49[a]	72.7
3′	4.18 dd (9.0, 2.7)	75.5	4.18 dd (9.0, 2.7)	75.5	4.36 dd (9.3, 3.0)	75.1	4.17 dd (9.6, 2.4)	75.6
4′	4.57 d (2.7)	80.8	4.57 (*d,* 2.7)	80.4	4.58 d (3.0)	70.0	4.58 d (2.4)	80.4
5′	4.07–4.09 m	75.7	4.07–4.08 m	75.8	4.12–4.13 m	77.2	4.07–4.08 m	75.8
6′	4.74 dd (9.6, 9.6)	60.4	4.74 dd (9.6, 9.6)	60.4	4.59 *dd* (9.3, 9.3)	61.1	4.75 dd (9.6, 9.6)	60.4
	4.22[a]		4.22 dd (9.6, 4.8)		4.32 dd (9.3, 4.2)		4.24 dd (9.6, 4.8)	
	β-D-Glu		*β-D*-Glu				*β-D*-Glu	
1″	5. 5.15 d (7.8)	105.2	5.18 d (7.8)	105.2			5.19 d (7.8)	105.2
2″	4.15 dd (8.6, 7.8)	86.2	4.13 dd (8.4, 7.8)	86.5			4.14 dd (8.4, 7.8)	86.5
3″	4.27 dd (8.6, 8.6)	78.3	4.27 dd (8.4, 8.4)	78			4.27 dd (8.4, 8.4)	78.1
4″	3.96[a]	71.9	3.96[a]	72.3			3.96[a]	72.3
5″	3.95[a]	78.1	4.01[a]	78.1			4.01[a]	78.1
6″	4.63 d (10.2)	63.1	4.63 d (10.2)	63.3			4.64 d (10.0)	63.4
	4.12–4.13 m		4.11–4.12 m				4.11–4.12 m	
	β-D-Glu		*β-D*-xyl				*β-D*-xyl	
1‴	5.22 d (7.5)	107.0	5.08 d (6.0)	104.8			5.09 d (5.8)	104.7
2‴	4.06 d (9.0, 7.2)	76.6	4.03–4.05 m	76.5			4.03–4.04 m	76.5
3‴	4.12 dd (9.0, 9.0)	77.5	4.02[a]	77.9			4.02[a]	77.9
4‴	4.22[a]	70.3	3.95[a]	70.4			3.96[a]	70.4
5‴	3.80–3.81 m	78.8	4.48[a]	67.4			4.49[a]	67.4
			3.67 d (10.6)				3.68 d (11.0)	
6‴	4.60 dd (12.4, 3.2)	61.5						
	4.37 dd (12.4, 3.6)							

[a]Overlapped.

Table 3. The MIC values (μg/ml) of steroidal saponins against *Candida albicans* and *Fusarium oxysporium*.

Sample	*Candida albicans*	*Fusarium oxysporium*
1	4	8
2	4	8
3	32	64
9	16	>64
nystatin	4	16

H-1″/C-4′, and H-1‴/C-2″ indicated the sugar portion to be *O-β-D*-xylopyranosyl-(1→2)-*β-D*-glucopyranosyl-(1→4)-*β-D*-galactopyranosyl unit. Thus, the structure of **2** was established as 2*α*,3*β*-dihydroxy-5*α*-pregna-16-en-20-one 3-*O-β-D*-xylopyranosyl-(1→2)-*β-D*-glucopyranosyl-(1→4)-*β-D*-galactopyranoside, and named hostaside II.

Compound **3** was obtained as a white needle solid. Its HR-ESI-MS gave a quasi-molecular ion at *m/z* 517.2774 $[M + Na]^+$, corresponding to the molecular formula $C_{27}H_{42}O_8$. The ^{1}H and ^{13}C NMR spectra revealed that the aglycone of **3** was essentially identical to that of **1**, and **3** only possessed a galactopyranosyl (Tables 1 and 2). Acid hydrolysis of **3** afforded **1a**

and *D*-galactose. The HMBC experiment showed correlation between the proton signal at δ_H 4.96 (H-1′, galactosyl group) and the carbon signal at δ_C 84.2 (C-3, aglycone). Thus, the structure of **3** was elucidated as 2*α*,3*β*-dihydroxy-5*α*-pregna-16-en-20-one 3-O-*β*-*D*-galactopyranoside, and named hostaside III.

Compound **4** was obtained as white amorphous powder with the molecular formula $C_{44}H_{70}O_{18}$, deduced from the HR-ESI-MS at *m/z* 909.4458 $[M + Na]^+$. The ^1H NMR spectrum indicated the presence of two tertiary methyl groups [δ_H 0.79 and 0.68 (each 3H, s)], two secondary methyl groups [δ_H 1.85 (d) and 1.01 (d)], and three anomeric proton [δ_H 4.94, 5.19, and 5.09 (each 1H, *d*)] signals. So, **4** was determined to be a steroidal triglycoside with (25*S*)-2*α*,3*β*-dihydroxy-5*α*-spirostane (neogitogenin) as aglycone. The 25*S* configuration was deduced through the comparison of characteristic chemical shifts for C-23, C-24, C-25, C-26, and C-27 with those data reported [17]. However, acid hydrolysis of **4** gave both neogitogenin with 25 *S*-configuration and gitogenin with 25 *R*-configuration in a ratio of 1:1. Since 25 *S* steroidal aglycones were known to easily change to 25 *R* aglycones with acid treatment [18], the configuration at the 25-position of **4** was primarily deduced to be *S*. The further enzymatic hydrolysis of **4** only gave neogitogenin (**4a**) [19], confirming that the aglycone of **4** was 25 *S* neogitogenin. Acid hydrolysis of **4** also gave *D*-xylose, *D*-glucose, and *D*-galactose in a ratio of 1:1:1 on the basis of GC analysis. The *β*-anomeric configurations for xylose, glucose, and galactose were judged from their coupling constants. The sugar portion was further confirmed to be *O*-*β*-*D*-xylopyranosyl-(1→2)-*β*-*D*-glucopyranosyl-(1→4)-*β*-*D*-galactopyranosyl unit by comparing the ^1H and ^{13}C NMR spectra with those of **2** and confirmed through HMBC correlations of H- 1′/C-3, H-1″/C-4′, and H-1‴/C-2″. Thus, the structure of **4** was established as (25*S*)-2*α*,3*β*-dihydroxy-5*α*-spirostane 3-*O*-*β*-*D*-xylopyranosyl-(1→2)-*β*-*D*-glucopyranosyl-(1→4)-*β*-*D*-galactopyranoside, and named hostaside IV.

Saponins **1–3** with progesterone as aglycone and **9** with manogenin as aglycone were all observed to significantly inhibit the growth of fungi *Candida albicans* and *Fusarium oxysporium* (Table 3). However, their aglycones (**1a** and **4a**) and the saponins with spirostanol and isospirostanol as aglycones (**4–8**) didn't show the obvious activity. The results revealed that the antifungal activity of saponins was related to both the structures of aglycones and the sugar chains.

3. Experimental

3.1. General experimental procedures

Melting point was determined on an XT4A micro melting point apparatus (unchecked, Shanghai Iwise Scientific Instrument Co., Ltd., Shanghai, China). Optical rotations were measured on a JASCO P-2000 polarimeter (JASCO, Tokyo, Japan). UV spectra were measured on a UV1102 spectrophotometer (Shanghai Tianmei Scientific Instrument Co., Ltd., Shanghai, China). IR spectra were recorded on an Equinox 55 FTIR spectrophotometer (Bruker, Karlsruhe, Germany). ESI-MS analyses were achieved on an Agilent 6410 triple-quad mass spectrometer (Agilent, Santa Clara, USA). GC analysis was performed on a Shimadzu GC-2010 gas chromatograph (Shimadzu, Tokyo, Japan) equipped with an H_2 flame ionization detector and a DB-5 quartz capillary column (30 m × 0.25 mm × 0.25 μm). ^1H and ^{13}C NMR spectra were recorded on Bruker Avance DRX-600 spectrometers with a

5 mm $^{13}C/^{1}H/^{15}N$ TCI CryoProbe (Bruker, Karlsruhe, Germany). HPD 100 macroporous resin (16–60 mesh, Tianjin Haiguang Chemical Group Co., Ltd., Tianjin, China), silica gel (200–300 mesh, Qingdao Haiyang Chemical Group Co., Ltd., Qingdao, China), and ODS (40–63 μm, Merck, Darmstadt, Germany) were used for column chromatography (CC). Nystatin and naringinase were purchased from China National Medicines CO. Ltd (Shanghai, China). L-cysteine methyl ester and hydrochloride and 1-trimethylsilylimidazole were purchased from Sigma-Aldrich (St. Louis, USA). Potato dextrose agar media *C. albicans* and *F. oxysporium* were provided by Shanghai Institute of Pharmaceutical Industry. Preparative HPLC chromatography was performed on a Water 2535 preparative liquid chromatography system (Waters, Milford, USA), equipped with an auto-sampler, an Xbridge Prep C_{18} OBD column (19.0 × 150 mm, 5 μm), and a WFC III collector. Semi-preparative HPLC chromatography was performed on a Shimadzu LC 2010 AHT liquid chromatography system (Shimadzu, Tokyo, Japan), equipped with a Nova-Pak HR C_{18} column (7.9 × 300 mm, 6 μm).

3.2. Plant material

The leaves of *Hosta plantaginea* were collected in July 2013 from Chongqing, China, and authenticated by one of the authors (Mengyue Wang). A voucher specimen (CQ20130722) has been deposited at the herbarium at the School of Pharmacy, Shanghai Jiao Tong University, Shanghai, China.

3.3. Bioassay-guided isolation

The air-dried leaves of *H. plantaginea* (1.2 kg) were powdered and extracted by refluxing with 10 L 95% alcohol for three times (2 h each). The alcohol extract was completely evaporated under reduced pressure to afford a residue (A, 260 g). Part of A (255 g) was suspended in water (750 ml), and then successively partitioned with CH_2Cl_2, EtOAc, and *n*-BuOH (each 500 ml × 3). The CH_2Cl_2, EtOAc , and *n*-BuOH layers were dried under vacuum to afford CH_2Cl_2 fraction (CA, 54 g), EtOAc fraction (EA, 31 g), and *n*-BuOH fraction (BA, 63 g), respectively. The water layer remained was dried under vacuum to afford water layer fraction (WA, 104 g). Fractions A, CA, EA, BA, and WA were subjected to the antifungal (*C. albicans* and *F. oxysporium*) evaluation by agar diffusion method at the same concentration of 100 μg/ml in methanol [20]. The most active fraction BA was subjected to the further fractionation.

Part of BA (60 g) was fractionated by macroporous resin CC (8.0 × 90 cm) successively eluted with water, 30, 60, and 90% ethanol (2000 ml each). The eluents were completely evaporated under vacuum to afford water portion (BA0, 6.9 g), 30% ethanol portion (BA30, 27.4 g), 60% ethanol portion (BA60, 17.3 g), and 90% ethanol portion (BA90, 7.4 g), respectively. Portions BA0, BA30, BA60, and BA90 were subjected to the antifungal evaluation at the same concentrations of 100 μg/ml again. The most active portion BA60 was subjected to the further chemical investigation.

Part of BA60 (15.4 g) was fractionated by ODS CC (5.5 × 60 cm; MeOH/H_2O; 20:80 to 75:25) to give *Frs.1–21* (200 ml each). *Fr.3* was further purified by silica gel CC (CH_2Cl_2/MeOH/H_2O; 60:35:2) to obtain **9** (146 mg). *Fr.9* and *Fr.10* were combined and further purified by prep. HPLC (MeCN/H_2O 20:80; 8.0 ml/min; 210 nm) to obtain fraction *Frs.9.1–9.4* (collected by peaks). *Fr.9.3* was further purified by semi-prep. HPLC (MeCN/H_2O 20:80;

3.0 ml/min; 210 nm) to afford **1** (t_R 10.3 min, 31 mg) and **2** (t_R 11.1 min, 40 mg). *Fr.14* was further purified by ODS CC (3.5 × 60 cm; MeCN/H_2O 35:65 to 55:45) to afford **4** (23 mg) and **5** (78 mg). *Fr.17* and *Fr.*18 were combined and further purified on silica gel CC (CH_2Cl_2/MeOH 9:1 to 8:2) to obtain **6** (31 mg), **7** (20 mg), and **8** (19 mg). *Fr.20* was further purified by semi-preparative HPLC (MeCN/H_2O 30:70; 2.0 ml/min; 210 nm) to afford **3** (t_R 18.7 min, 32 mg).

3.3.1. Hostaside I (1)

White amorphous powder (MeOH); mp 271–273 °C; $[\alpha]_D^{20}$ −18.8 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε): 240 (3.74) nm; IR (KBr) ν_{max}: 3407 (OH), 2932 (CH), 1660 (C = O), 1585 (C = C), 1380, 1232, 1164, 1074, 924, 892 cm^{-1}; ^1H and ^{13}C NMR spectral data see Tables 1 and 2. HR-ESI-MS: *m/z* 841.3832 $[M + Na]^+$ (calcd for $C_{39}H_{62}O_{18}Na$, 841.3834).

3.3.2. Hostaside II (2)

White power (MeOH); mp 266–267 °C; $[\alpha]_D^{20}$ −15.6 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε): 239 (3.77) nm; IR (KBr) ν_{max}: 3405 (OH), 2931 (CH), 1660 (C=O), 1585 (C=C), 1379, 1231, 1162, 1073, 924, 892 cm^{-1}; ^1H and ^{13}C NMR spectral data see Tables 1 and 2. HR-ESI-MS: *m/z* 811.3732 $[M + Na]^+$ (calcd for $C_{38}H_{60}O_{17}Na$, 811.3728).

3.3.3. Hostaside III (3)

White amorphous powder; mp 245–247 °C; $[\alpha]_D^{20}$ −7.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε): 239 (3.98) nm; IR (KBr) ν_{max}: 3402 (OH), 2931(CH), 1659 (C=O), 1584 (C=C), 1375, 1230, 1163, 1074, 924, 892 cm^{-1}; ^1H and ^{13}C NMR spectral data see Tables 1 and 2. HR-ESI-MS: *m/z* 517.2774 $[M + Na]^+$ (calcd for $C_{27}H_{42}O_8Na$, 517.2769).

3.3.4. Hostaside IV (4)

White powder; mp 275–277 °C; $[\alpha]_D^{20}$ −11.3 (*c* 0.10, MeOH); IR (KBr) ν_{max}: 3410 (OH), 2935 (CH), 1452, 1378, 1240, 1057, 980, 921, 898, 865 cm^{-1} (intensity: 898 > 921); ^1H and ^{13}C NMR spectral data see Tables 1 and 2. HR-ESI-MS: *m/z* 909.4458 $[M + Na]^+$ (calcd for $C_{44}H_{70}O_{18}Na$, 909.4462).

3.4. Acid hydrolysis of 1, 2, 3, and 4

A solution of sample (9 mg) in HCl/MeOH (4:1, 10 ml) was refluxed at 90 °C for 2 h. Then, the reaction mixture was diluted with H_2O and extracted with CH_2Cl_2 (15 ml × 2). The CH_2Cl_2 layer was washed with water to neutrality, concentrated under vacuum and then subjected to silica gel CC (CH_2Cl_2/MeOH 95:5) to give aglycone. The water layer was neutralized with 10% NaOH solution and concentrated *in vacuo* to dryness to give sugar fraction. The sugar fraction was dissolved in anhydrous pyridine (2.0 ml) followed by adding of L-cysteine methyl ester hydrochloride (5 mg). The mixture was stirred at 60 °C for 1.5 h and trimethylsilylated with 1-trimethylsilylimidazole (0.3 ml) for 2 h. The mixture was partitioned between *n*-hexane and H_2O (2 ml each), and the organic layer was analyzed by GC (column temperature, 100/280 °C, programmed increase, 10 °C/min; carrier gas, N_2, 1.5 ml/min; injector and detector temperature, 280 °C; injection volume, 1 μl; split ratio, 10:1) [3]. Identification of *D*-glucose, *D*-galactose, and *D*-xylose was detected by co-injection of the hydrolysate with standard silylated samples.

3.5. Enzymatic hydrolysis of 4

Compound **4** (7.5 mg) was treated with naringinase (90.0 mg) in HCOOH/KOAc buffer (pH 4.5, 3.0 ml) at room temperature for 5 days. The reaction mixture was purified by CC on silica gel (2 × 15 cm) eluted with CH_2Cl_2/MeOH (95:5) to yield aglycone (1.4 mg).

3.6. Antifungal activity assay of saponins

The primary antifungal screening was carried out using agar disk-diffusion, at an initial concentration of 64 μg/ml in methanol [20]. The active compounds with inhibition zones more than 10 mm were subjected to the further *MIC* investigation by the microdilution method [21].

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Disclosure statement

No potential conflict of interest was reported by the authors.

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