Bioactive Saponins from *Astragalus suberi* L. Growing in Yemen

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From the aerial parts of *Astragalus suberi* L., Fabaceae, seven saponins were isolated. Based on spectral data (IR, ^{1}H and ^{13}C NMR and HR-FABMS), the structures were established as 3-*O*-(β-D-glucopyranosyl)-soyasapogenol B (**1**); 3-*O*-(β-D-glucuronopyranosyl)-soyasapogenol B (**2**); 3-*O*-[β-D-galactopyranosyl (1→2)-β-D-glucopyranosyl]-soyasapogenol B (**3**); 3-*O*-[α-L-rhamnopyranosyl (1→2)-β-D-galactopyranosyl (1→2)-β-D-glucopyranosyl]-soyasapogenol B (**4**); 3-*O*-[α-L-rhamnopyranosyl (1→2)-β-D-galactopyranosyl (1→2)-β-D-glucopyranosyl]-11-hydroxy-soyasapogenol B (**5**); 3-*O*-[α-L-rhamnopyranosyl (1→2)-β-D-galactopyranosyl (1→2)-β-D-glucuronopyranosyl]-soyasapogenol B (**6**) and 3-*O*-[α-L-rhamnopyranosyl (1→2)-β-D-galactopyranosyl (1→2)-β-D-glucuronopyranosyl]-complogenin (**7**).

The isolated saponins exhibited antibacterial activities against Gram-positive and Gram-negative bacteria with minimum inhibitory concentration values > 100 μg/ml, antifungal activity against all the strains tested with minimum fungicidal concentration values between 25 and 50 μg/ml and inhibited the growth of Hep-2 (human carcinoma of larynx), with IC_{50} values between 50 μg/ml (compounds **5**–**7**) and 100 μg/ml (compounds **1**–**4**), and Hela (human carcinoma of cervix) cell lines in culture with different IC_{50} values [74 (compound **7**), 98 (compound **5**) and 180 μg/ml (compounds **1**–**4** and **6**)].

Key words: Astragalus suberi L., Triterpenoidal Saponins, Biological Activity

Introduction

The genus *Astragalus* L. belongs to the family Fabaceae of the order Leguminales (Tackholm, 1974; Hutchinson, 1973). Roots of plants from different *Astragalus* species are used as antiperspirant, diuretic, and tonic drug for treatment of diabetes mellitus, leukemia, nephritis, and uterine cancer (Hikino *et al.*, 1976). Triterpene glycosides were isolated from several *Astragalus* species (Calis *et al.*, 1997; Smee *et al.*, 1996; Abdallah *et al.*, 1993; Bedir *et al.*, 1999; Zhiren *et al.*, 1998; Zhang *et al.*, 1992). Some of these glycosides have antitumour activity against some human tumour cell lines and also AIDS antiviral activity (Smee *et al.*, 1996; Abdallah *et al.*, 1993). Although there has been intensive work on triterpene glycosides of *Astragalus* (Smee *et al.*, 1996; Abdallah *et al.*, 1993; Bedir *et al.*, 1998, 1999; Zhiren *et al.*, 1998; Zhang *et al.*, 1992; Calis *et al.*, 1996; Inoue *et al.*, 1992; Hirotani *et al.*, 1994; Tsurumi *et al.*, 1992; Asaad *et al.*, 1995) nothing has been reported about the triterpenoidal saponins from *A. suberi* L. grown in Yemen. In the present study, we describe the isolation and biological study of seven triterpenoidal saponins, which have been identified for the first time from *A. suberi* L. grown in Yemen.

Results and Discussion

The *n*-butanol-soluble fraction of the ethanol extract of the air-dried parts of *Astragalus suberi* L. grown in Yemen received on repeated chromatographic purification on a silica gel column and PTLC yielded seven compounds (**1**–**7**, Table I). These compounds gave a positive Liebermann–Burchard test for triterpenes and Molisch test for sugars and thus appeared to be triterpene glycosides.

Compound **1**, obtained as white powder (from MeOH), m.p. 145–147 °C, $[\alpha]_D^{25}$ + 15° (pyridine c = 1), showed in the HR-FABMS a peak at m/z 643 due to $[M+Na]^+$ calculated for $C_{36}H_{60}O_8$. Acid hydrolysis of compound **1** provided soyasapogenol B (Sun *et al.*, 1992) as sapogenol. The ^{1}H NMR spectrum of compound **1** (500 MHz, pyridine-d_5) displayed the presence of seven tertiary methyl groups at δ 0.7, 0.9, 1.1, 1.3, 1.35 × 2 and 1.47 (all 3H, s), along with an anomeric proton signal at δ 5.2 (1H, d, J = 7.5 Hz) and an olefinic proton signal at δ 5.5

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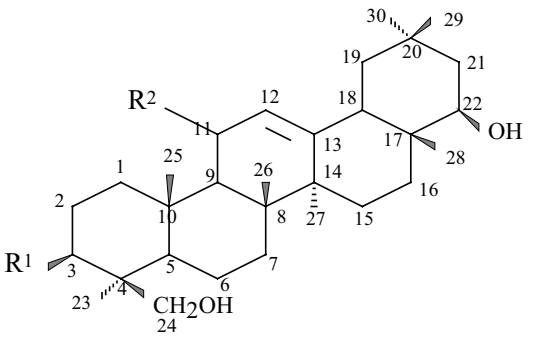
Table I. The structure of the isolated seven compounds.



Compound	R^1	R^2
1	Glu	H
2	Glu A	H
3	Gal (1→2) Glu	H
4	Rha (1→2) Gal (1→2) Glu	H
5	Rha (1→2) Gal (1→2) Glu	OH
6	Rha (1→2) Gal (1→2) Glu A	H
7	Rha (1→2) Gal (1→2) Glu A	= O

Glu = *β*-D-glucopyranosyl;
Glu A = *β*-D-glucuronopyranosyl;
Gal = *β*-D-galactopyranosyl;
Rha = *α*-L-rhamnopyranosyl.

(1H, br s, 12-H). In the ^{13}C NMR spectrum of compound **1** as listed in Table II signals produced from the sugar moiety were assignable to the *O*-*β*-D-glucopyranosyl moiety and the others due to the aglycone part showed a downfield shift at C-3 by 8.2 ppm as compared with that of soyasapogenol B (Sun *et al.*, 1992). Consequently, the structure of compound **1** was identified as 3-*O*-(*β*-D-glucopyranosyl)-soyasapogenol B (Gorbacheva *et al.*, 1996).

Compound **2**, obtained as white powder (from MeOH), $[\alpha]_D^{25}$ + 13° (pyridine *c* = 1), showed a peak at *m/z* 657 due to $[M+Na]^+$ calculated for $C_{36}H_{58}O_9$. Acid hydrolysis of compound **2** provided soyasapogenol B as sapogenol. The ^{1}H NMR spectrum of compound **2** displayed the presence of seven tertiary methyl groups at *δ* 0.84, 1, 1.01, 1.23, 1.3 × 2 and 1.45 (all 3H, s), along with an anomeric proton signal at *δ* 5.17 (1H, d, *J* = 7.7 Hz, Glu A 1-H) and an olefinic proton signal at *δ* 5.31 (1H, br s, 12-H). In the ^{13}C NMR spectrum of **2** as listed in Table II signals caused from the sugar moiety were assignable to the *O*-*β*-D-glucuronopyranosyl moiety and others due to the aglycone part showed a downfield shift at C-3 by 8.2 ppm as compared with that of soyasapogenol B (Sun *et al.*, 1992). Consequently, the structure of compound **2** was characterized as 3-*O*-(*β*-D-glucuronopyranosyl)-soyasapogenol B, whose methyl ester was obtained as a prosapogenol from soybean saponin by mild acid hydrolysis (Berhow *et al.*, 2000).

Compound **3**, obtained as white powder (from MeOH), $[\alpha]_D^{25}$ – 10° (pyridine *c* = 0.5), showed in the HR-FABMS a peak due to $[M+H]^+$ at *m/z* 783, which is higher by 162 mass units than that of compound **1**. Acid hydrolysis of the compound afforded soyasapogenol B (Sun *et al.*, 1992) as sapogenol, glucose and galactose in respect to TLC and the ^{1}H NMR spectrum. Two anomeric proton signals were observed at *δ* 4.98 (1H, d, *J* = 7.5 Hz, Glu 1-H) and 5.82 (1H, d, *J* = 7.5 Hz, Gal 1-H) in the ^{1}H NMR spectrum of compound **3**. A comparative study of the ^{13}C NMR spectrum of **3** with that of compound **1** led to the identification at the sugar moiety, that is, the sugar moiety of **3** possessed a *β*-D-galactopyranosyl (1→2)-*β*-D-glucopyranosyl group. Therefore, the structure of **3** could be represented as 3-*O*-[*β*-D-galactopyranosyl (1→2)-*β*-D-glucopyranosyl]-soyasapogenol B.

Compound **4**, obtained as white powder (from MeOH), $[\alpha]_D^{25}$ – 8° (pyridine *c* = 1), showed in the HR-FABMS a peak due to $[M+H]^+$ at *m/z* 929, which is higher by 308 mass units than that of compound **1**. Acid hydrolysis of compound **4** afforded soyasapogenol B (Sun *et al.*, 1992) as sapogenol, glucose, galactose and rhamnose in respect to TLC and the ^{1}H NMR spectrum. In the ^{1}H NMR spectrum, three anomeric proton signals were observed at *δ* 5.2 (1H, d, *J* = 7.5 Hz, Glu 1-H), 5.81 (1H, d, *J* = 7.4 Hz, Gal 1-H) and 6.3 (1H, s, Rha 1-H). A comparative study of the ^{13}C NMR spectrum of compound **4** as in Table II with that of compound **1** led to the identification at the sugar moiety that is, the sugar moiety of **4** possessed an *α*-L-rhamnopyranosyl (1→2)-*β*-D-galactopyranosyl (1→2)-*β*-D-glucopyranosyl group. Therefore, the structure of **4** could be represented as 3-*O*-[*α*-L-rhamnopyranosyl (1→2)-*β*-D-galactopyranosyl (1→2)-*β*-D-glucopyranosyl]-soyasapogenol B (Ohana *et al.*, 1998).

Compound **5**, obtained as white powder (from MeOH), $[\alpha]_D^{20}$ – 7° (pyridine *c* = 1), showed a peak at *m/z* 945 due to $[M+H]^+$ in the HR-FABMS which is higher by 16 mass units than that of compound **4** for $C_{48}H_{80}O_{18}$. Acid hydrolysis of compound **5** yielded an aglycone with m.p. 240–245 °C, $[\alpha]_D^{20}$ + 80° ($CHCl_3$, *c* = 1), which exhibited infrared (IR) absorption at 3430 cm^{-1} (OH). The HR-FAB-mass spectrum showed a molecular ion peak at *m/z* 475

Table II. ^{13}C NMR chemical shifts of compounds **1**–**7** and aglycone of compound **5** (125 MHz, pyridine-d_5 and TMS as internal standard).

C		**1**	**2**	**3**	**4**	**5**	Aglycone of **5**	**6**	**7**
	1	38	39	39	39	39.5	39.5	38.7	39.3
	2	27	27	28	28	26.7	28	27	27
	3	92	89	91	91	91.1	79.8	91.1	91
	4	44	45	44	44	44.5	43.5	43.8	44.3
	5	56	56	56	56	55.8	55	56	55.8
	6	19	19	18	18	17.7	18	18.5	17.7
	7	33	34	33	33	33.1	33.4	33	33.1
	8	40	40	40	40	44	43.9	40	44
	9	48	48	47	48	61.7	62	48	61.7
	10	36	37	36	37	39	37	36.4	36.8
	11	24	24	24	24	68	68	24.1	198
	12	122	123	122	122	128	128	122.3	127
	13	144	145	144	144	169	169	144.8	168
	14	42	42	42	42	42.4	45.5	42.3	45
	15	26	27	26	27	26.9	26.6	26.4	27
	16	29	29	29	29	28.7	26.6	28.6	28
	17	38	38	38	38	38	37.7	38	38
	18	45	46	46	46	45.8	45.2	45	46
	19	47	47	47	47	46.7	47	46.7	45
	20	31	31	31	31	30.9	31	31	31
	21	42	42	42	42	42.3	42	42.2	42
	22	76	76	76	76	75.6	74.8	75.5	75
	23	23	23	23	23	22.8	23.5	23	23
	24	64	63	64	64	63.4	62.1	63.6	63.4
	25	16	16	16	16	15.8	17	16	17
	26	17	17	17	17	17	18	17	19
	27	26	26	25	26	25.8	23	26	26
	28	21	21	21	21	21.7	21.6	21.7	21
	29	33	33	33	33	33	33	33	33.5
	30	29	29	29	29	28.7	28.4	28.7	29
GluA	1		106					105	105
	2		75					78	78
	3		78					76.6	77
	4		73					73.9	74
	5		78					77.7	78
	6		172					172	172
Glu	1	105		105	105	105			
	2	76		78.5	78.5	78.8			
	3	79		76.5	76.5	76.5			
	4	72		73.4	73.4	73.4			
	5	78		71.7	71.7	71.7			
	6	63		62	62	61.8			
Gal	1			101.7	101.7	102		101.7	101
	2			77	77	77		77.8	76.5
	3			76.5	76.5	76.5		76.6	76.2
	4			71	71	71		71	71
	5			77	77	77		76.4	77
	6			61.8	61.8	61.8		61.5	61.3
Rha	1				102	102		102	102
	2				72	72		72.4	72
	3				73	73		72.4	72.5
	4				74	74		74.3	74.3
	5				69	69		69.4	69
	6				19	19		19	18.7

due to $[M+H]^+$. The ^{13}C NMR spectrum of the aglycone in Table II displayed signals which arise from C-11, 12 and 13 at δ 68, 128 and 169, respectively, and the enolization shifts at C-8, 9, 14, 25, 26 and 27 by 43.9, 62, 45.5, 17, 18, and 23, respectively, in comparison with those of soyasapogenol B (Sun *et al.*, 1992), suggesting the presence of a hydroxyl group at C-11 of the aglycone moiety of soyasapogenol B. The ^1H NMR spectrum of compound **5** exhibited signals due to seven tertiary methyl groups at δ 0.97, 1.09, 1.11, 1.15, 1.24, 1.44, 1.47, (each 3H, s), also showed three anomeric proton signals at 4.98 (1H, d, J = 7.5 Hz, Glu 1-H), 5.82 (1H, d, J = 7.3 Hz, Gal 1-H) and 6.31 (1H, br, s, Rha 1-H). A comparative study of the ^{13}C NMR spectrum of the sugar moiety of compound **5** with that of compound **4** in Table II led to the identification of the sugar moiety of **5** that is, the sugar moiety of **5** possessed an α-L-rhamnopyranosyl (1→2)-β-D-galactopyranosyl (1→2)-β-D-glucopyranosyl group. Therefore, the structure of **5** could be represented as 3-*O*-[α-L-rhamnopyranosyl (1→2)-β-D-galactopyranosyl (1→2)-β-D-glucopyranosyl]-11-hydroxy-soyasapogenol B.

Compound **6** was obtained as a white powder (from MeOH), m.p. 220–222 °C, $[\alpha]_D^{20}$ – 8° (MeOH, c = 1). HR-FABMS gave a molecular ion at *m/z* 965 $[M+Na]^+$ and fragment ions at *m/z* 819 [M+Na-Rha]$^+$, 657 [M+Na-Rha-Gal]$^+$ and 481 [M+Na-Rha-Gal-Glu A]$^+$, indicating the presence of rhamnose-galactose-glucuronic acid-soyasapogenol B. Comparison of the ^{13}C NMR data of **6** with that of sapogenol B (Sun *et al.*, 1992) suggested that the sugar moiety of **6** was linked to C-3 signal (δ 91) of soyasapogenol B was shifted downfield by δ 11 due to glycosidation shift (Brettmier, 1990) as in Table II. The configuration of the anomeric protons of the sugars were assigned on the basis of their $^3J_{1,2}$ values (Rha, 2.2 Hz; Gal, 7.5 Hz; Glu A, 8 Hz) measured by ^1H NMR analysis (Mahato, 1991). Compound **6** was deduced to be 3-*O*-[α-L-rhamnopyranosyl (1→2)-β-D-galactopyranosyl (1→2)-β-D-glucuronopyranosyl]-soyasapogenol B (Sun *et al.*, 1992; Mahato, 1991; Kitagawa *et al.*, 1976).

Compound **7**, obtained as a white powder (from MeOH), $[\alpha]_D^{20}$ – 5° (pyridine, c = 0.5), showed in the HR-FABMS a peak due to $[M+H]^+$ at *m/z* 957 which was higher by 14 mass units than that of **6**. Acid hydrolysis of compound **7** afforded complogenin (Inoue *et al.*, 1992; Kitagawa et *al.*, 1988) as sapogenol, in respect to TLC and the ^1H NMR spectrum. Three anomeric proton signals were observed at δ 4.98 (1H, d, J = 7.5 Hz, Glu A 1-H), 5.82 (1H, d, J = 7.5 Hz, Gal 1-H) and 6.31 (1H, br s, Rha 1-H) in the ^1H NMR spectrum of compound **7**. Comparison of the ^{13}C NMR data of compound **7** in Table II with that of soyasaponin B led to the identification at the sugar moiety, that is, the sugar moiety of **7** possessed an α-L-rhamnopyranosyl (1→2)-β-D-galactopyranosyl (1→2)-β-D-glucuronopyranosyl group. Therefore, the structure of **7** could be represented as 3-*O*-[α-L-rhamnopyranosyl (1→2)-β-D-galactopyranosyl (1→2)-β-D-glucuronopyranosyl]-complogenin (Lee *et al.*, 1999).

The isolated saponins showed activity against Gram-positive and Gram-negative bacteria with MIC (the minimum concentration of the antibiotic that completely inhibited the growth of the microorganism) and MBC values (the first dilution which resulted in a 99.9% decrease from the initial bacterial titer of the starting inoculum) > 100 μg/ml (Reiner, 1974).

With regard to the antifungal activity, the isolated compounds showed activity with MIC and MFC (the first concentration showing no visible growth after incubation time) values of 50 μg/ml against all the strains tested except for *Candida glabrata* with 25 μg/ml comparing with the positive control (amphotericin B), which showed higher activity (0.5–2 μg/ml).

From the study of cytotoxic activity against Hela cells (human carcinoma of cervix) and Hep-2 cells (human carcinoma of larynx), it can be concluded that saponins **1**–**4** have exhibited the same IC_{50} of 100 μg/ml on Hep-2 whereas compounds **5**–**7** showed IC_{50} of 50 μg/ml (Table III). Concerning the activity on Hela cells, the isolated compounds showed different activity in the following order: compound **7** > compound **5** > compounds **1**–**4** and **6** with IC_{50} 74, 98 and 180 μg/ml, respectively (Gonzalez *et al.*, 1999).

Table III. IC_{50} (μg/ml) values of compounds **1**–**7** against Hela and Hep-2 cells (values in parentheses are expressed in μM).

Compound	Hela	Hep-2
1	180 (290)	100 (161)
2	180 (283)	100 (157)
3	180 (230)	100 (127)
4	180 (193)	100 (107)
5	98 (103)	50 (52)
6	180 (191)	50 (53)
7	74 (77)	50 (52)

Experimental

General experimental procedure

Melting points were determined using a Thermosystem FP800 Metler instrument and are uncorrected. Optical rotation was measured on a Zeiss polarimeter Model 53187 equipped with a sodium lamp. Infra-red spectra were generally obtained in KBr discs using a Perkin-Elmer FTIR model 1600 Spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded in pyridine-d_5, on a Bruker AMX NMR spectrometer operating at 500 MHz for ^{1}H, and 125 MHz for ^{13}C NMR. The HR-MS was measured in *meta*-nitrobenzyl alcohol, using a Bioapex FTMS with electrospray ionization. TLC analyses were carried out on precoated silica gel G_{254}, 500 μm with the following developing systems: $CHCl_3$/MeOH (90:10) and $CHCl_3$/MeOH/H_2O (80:20:2). For column chromatography, silica gel 60, 40 μm was used.

Plant material

Air-dried aerial parts (2 kg) of *Astragalus suberi* L. were collected from roads and hill sides in Sana'a region (Wadi-Zahr) in Yemen. The identity of the plant was confirmed by Professor M. El-Monayery, Faculty of Science, El-Azhar University, Egypt. A certified specimen has been deposited at the Pharmacognosy Department, Faculty of Pharmacy, Zagazig University, Egypt.

Extraction and chromatography

The air-dried aerial parts (2 kg) of *Astragalus suberi* L. were exhaustively extracted with 95% EtOH (10 l). The extract was concentrated under reduced pressure. The concentrated extract (70 g) was dissolved in water (1 l) and partitioned into petrol, $CHCl_3$, EtOAc and *n*-BuOH (2 l each), successively. The *n*-BuOH soluble fraction was concentrated under reduced pressure and the residue (10 g) was chromatographed over a silica gel column (300 g, 3 × 150 cm). The elution was carried out with $CHCl_3$, MeOH and H_2O in increasing polarity. Early $CHCl_3$/MeOH (9:1, 500 ml) eluate fractions yielded compound **1** (200 mg); $CHCl_3$/MeOH/H_2O (85:15:1, 500 ml) eluate fractions were subjected to PTLC using the same polarity to yield compound **2** (120 mg); $CHCl_3$/MeOH/H_2O (80:20:1, 800 ml) yielded compound **3** (220 mg), $CHCl_3$/MeOH/H_2O (80:20:2, 800 ml) eluate fractions were re-chromatographed by silica gel CC ($CHCl_3$/MeOH, 70:30) to yield compound **4** (300 mg); $CHCl_3$/MeOH/H_2O (70:30:3, 400 ml) eluate fractions yielded compound **5** (150 mg); $CHCl_3$/MeOH/H_2O (60:40:4, 400 ml) eluate fractions were subjected to PTLC in $CHCl_3$/MeOH/H_2O, (60:40:8) to give compound **6** (130 mg); $CHCl_3$/MeOH/H_2O (50:50:5, 500 ml) eluate fractions yielded compound **7** (250 mg).

3-O-(β-D-Glucopyranosyl)-soyasapogenol B (**1**) (200 mg): Crystallized from MeOH as white powder, m.p. 145–147 °C. – $[\alpha]_D^{25}$ + 15° (pyridine, *c* = 1). – ^{1}H NMR (500 MHz, pyridine-d_5): δ = 0.7, 0.9, 1.1, 1.3, 1.35 × 2, 1.47 (7 × s, 21 H, C-Me), 5.2 (d, *J* = 7.5 Hz, 1H, Glu 1-H), 5.5 (br s, 1H, 12-H). – ^{13}C NMR (125 MHz, pyridine-d_5): see Table II. – HR-FABMS (70 eV): *m/z* (%) = 643 (42) [M$^+$+Na] ,481 (18) [M$^+$+Na–Glu],163 (10), 126 (90), calculated for $C_{36}H_{60}O_8$. – Compound **1** on acid hydrolysis provided a sapogenol identical with soyasapogenol B on TLC and glucose.

3-O-(β-D-Glucuronopyranosyl)-soyasapogenol B (**2**) (120 mg): White powder, m.p. 177–179 °C. – $[\alpha]_D^{20}$ + 13° (pyridine, *c* = 1). – ^{1}H NMR (500 MHz, pyridine-d_5): δ = 0.84, 1, 1.01, 1.23, 1.3 × 2 and 1.45, (7 × s, 21 H, C-Me), 5.17 (d, *J* = 7.7 Hz, 1H, Glu A 1-H), 5.31 (br s, 1H, 12-H). – ^{13}C NMR (125 MHz, pyridine-d_5): see Table II. – HR-FABMS (70 eV): *m/z* (%) = 657 (21) [M$^+$+Na], 481 (34) [M$^+$+Na–Glu A], 176 (10), calculated for $C_{36}H_{58}O_9$. – Compound **2** on acid hydrolysis provided a sapogenol identical with soyasapogenol B on TLC and glucuronic acid.

3-O-[β-D-Galactopyranosyl (1→2)-β-D-glucopyranosyl]-soyasapogenol B (**3**) (220 mg): White powder, m.p. 228–230 °C. – $[\alpha]_D^{25}$ −10° (pyridine, *c* = 0.5). – ^{1}H NMR (500 MHz, pyridine-d_5): δ = 0.97, 1.09, 1.1, 1.15, 1.24, 1.44, 1.47 (7 × s, 21 H, C-Me), 3.4 (dd, *J* = 4.4, 11.7 Hz, 1H, 3-H), 4.98 (d, *J* = 7.5 Hz, 1H, Glu 1-H), 5.8 (br s, 1H, 12-H), 5.82 (d, *J* = 7.5 Hz, 1H, Gal 1-H). – ^{13}C NMR (125 MHz, pyridine-d_5): see Table II. – HR-FABMS (70 eV): *m/z* (%) = 783 (30) [M$^+$+H], 621 (23) [M$^+$+H-Gal], 459 (13) [M$^+$+H-Gal-Glu], 441 (100) [aglycone$^+$+H–H_2O], 181 (100), 163 (10), 126 (90), calculated for $C_{42}H_{70}O_{13}$. – Compound **3** on acid hydrolysis provided a sapogenol identical with soyasapogenol B on TLC, glucose and galactose.

3-O-[α-L-Rhamnopyranosyl (1→2)-β-D-galactopyranosyl (1→2)-β-D-glucopyranosyl]-soyasapogenol B (**4**) (300 mg): White powder, m.p. 218–220 °C (crystallized from MeOH). – $[\alpha]_D^{20}$ – 8° (MeOH, *c* = 1). – ^{1}H NMR (500 MHz, pyridi-

ne-d_5): δ = 0.7 (s, 3H, 25-H), 0.96 (s, 3H, 26-H), 1.0 (s, 3H, 29-H), 1.23 (s, 3H, 28-H), 1.3 (s, 6H, 27-H, 30-H), 1.44 (s, 3H, 23-H), 1.79 (d, J = 6 Hz, 3H, Rha 6-H), 2.19 (d, J = 9 Hz, 1H, 2-H), 2.4 (d, J = 13.5 Hz, 1H, 18-H), 3.25 (d, J = 13 Hz, 1H, 24-H), 3.37 (dd, J = 11 and 3.7 Hz,1H, 3-H), 3.75 (t, 1H, 22-H), 3.95 (t, J = 6 Hz, 1H, Gal 5-H), 4.12 (dd, J = 9.5, 3.1 Hz, 1H, Gal 3-H), 4.83 (d, J = 3.2 Hz, 1H, Rha 2-H), 4.96 (m, 1H, Rha 5-H), 5.2 (d, J = 7.5 Hz, 1H, Glu 1-H), 5.3 (br s, 1H, 12-H), 5.81 (d, J = 7.4 Hz, 1H, Gal 1-H). – ^{13}C NMR (125 MHz, pyridine-d_5): see Table II. – HR-FABMS (70 eV): *m/z* (%) = 929 (29) [M$^+$+H], 783 (29) [M$^+$-Rha+H], 621 (33) [M$^+$-Rha–Gal+H], 459 (12) [M$^+$-Rha–Gal–Glu+H], 249 (70), 215 (43), 182 (13), 163 (19), 146 (15), 143 (100) calculated for $C_{48}H_{80}O_{17}$. – Acid hydrolysis of compound **4** provided sapogenol identical to soyasapogenol B on TLC, glucose, galactose and rhamnose.

3-O-[α-L-Rhamnopyranosyl (1→2)-β-D-galactopyranosyl (1→2)-β-D-glucopyranosyl]-11-hydroxy-soyasapogenol B (**5**) (150 mg): White powder (from MeOH), m.p. 241–243 °C. – $[\alpha]_D^{20}$ – 7° (pyridine, c = 1). – ^{1}H NMR (500 MHz, pyridine-d_5): δ = 0.97, 1.09, 1.11, 1.15, 1.24, 1.44, 1.47, (7 × s, 21H, C-Me), 1.81 (d, J = 6.2 Hz, 3H, Rha 6-Me), 1.6 (d, J = 8.5 Hz, 1H, 9-H), 2.99 (br d, J = 13.6 Hz, 1H, 18-H), 3.4 (dd, J = 4.4, 11.7 Hz, 1H, 3-H), 4.98 (d, J = 7.5 Hz, 1H, Glu 1-H), 5.81 (d, J = 3.5 Hz, 1H, 12-H), 5.82 (d, J = 7.3 Hz, 1H, Gal 1-H), 6.31 (br s, 1H, Rha 1-H). – ^{13}C NMR (125 MHz, pyridine-d_5): see Table II. – HR-FABMS (70 eV): *m/z* (%) = 945 (100) [M$^+$+H], 799 (23) [M$^+$-Rha+H], 653 (26) [M$^+$-Rha-Gal+H], 471 (18) [M$^+$-Rha-Gal-Glu+H] calculated for $C_{48}H_{80}O_{18}$. – Acid hydrolysis of compound **5** afforded 11-hydroxysoysapogenol B as sapogenol in respect to TLC, glucose, galactose and rhamnose.

3-O-[α-L-Rhamnopyranosyl (1→2)-β-D-galactopyranosyl (1→2)-β-D-glucuronopyranosyl]-soyasapogenol B (**6**) (130 mg): White powder (from MeOH), m.p. 220–222 °C. – $[\alpha]_D^{20}$ – 8° (MeOH, c = 1). – IR (film): ν = 3450 (OH), 2940 (C-H), 1730 (C=O), 1640 (C=C), 1460, 1430, 1380, 1280, 1250, 1230 (C-O), 900 cm^{-1}. – ^{1}H NMR (500 MHz, pyridine-d_5): δ = 0.7 (s, 3H, 25-H), 0.96 (s, 3H, 26-H), 1.0 (s, 3H, 28-H), 1.3 (s, 6H, 27-H, 30-H), 1.44 (s, 3H, 23-H), 1.79 (d, J = 6 Hz, 3H, 18-H), 3.25 (d, J = 11.5 Hz, 1H, 24-H), 3.37 (dd, J = 11, 3.7 Hz, 1H, 3-H), 3.75 (t, 1H, 22-H), 3.95 (t, J = 6 Hz, 1H, Gal 5-H), 4.12 (dd, J = 9.5, 3 Hz, 1H, Gal 3-H), 4.83 (d, J = 3 Hz, 1H, Rha 2-H), 4.9 (d, J = 8 Hz, 1H, Glu A 1-H), 4.96 (m, 1H, Rha 5-H), 5.3 (br s, 1H, 12-H), 5.81 (d, J = 7.5 Hz, 1H, Gal 1-H), 6.3 (br s, W ½ = 2.2 Hz, 1H, Rha 1-H). – ^{13}C NMR (125 MHz, pyridine-d_5): see Table II. – HR-FABMS (70 eV): *m/z* (%) = 965 (100) [M$^+$+Na], 943 (20) [M$^+$+H], 819 (20) [M$^+$+Na-Rha], 797 (16) [M$^+$+H-Rha], 657 (27) [M$^+$+Na-Rha-Gal], 481 (16) [M$^+$+Na-Rha-Gal-Glu A], 459 (17) [M$^+$+H-Rha-Gal-Glu A] calculated for $C_{48}H_{78}O_{18}$. – Acid hydrolysis of compound **6** afforded soysapogenol B as sapogenol in respect to TLC, glucuronic acid, galactose and rhamnose.

3-O-[α-L-Rhamnopyranosyl (1→2)-β-D-galactopyranosyl (1→2)-β-D-glucuronopyranosyl]-complogenin (**7**) (250 mg): White powder (from MeOH), m.p. 262–264 °C. – $[\alpha]_D^{20}$ – 5° (pyridine, c = 0.5). – ^{1}H NMR (500 MHz, pyridine-d_5): δ = 0.97, 1.09, 1.11, 1.15, 1.24, 1.44, 1.47, (7 × s, 21H, C- Me), 1.81 (d, J = 6.5 Hz, 3H, Rha 6-Me), 2.5 (s, 1H, 9-H), 2.99 (br d, J = 13.6 Hz, 1H, 18-H), 3.4 (dd, J = 4.5, 11.5 Hz, 1H, 3-H), 4.98 (d, J = 7.5 Hz, 1H, Glu A1-H), 5.81 (s, 1H, 12-H), 5.82 (d, J = 7.5 Hz, 1H, Gal 1-H), 6.31 (br s, 1H, Rha 1-H). – ^{13}C NMR (125 MHz, pyridine-d_5): see Table II. – HR-FABMS (70 eV): *m/z* (%) = 957 (33) [M$^+$+H], 811 (20) [M$^+$-Rha+H], 649 (11) [M$^+$–Rha-Gal+H], 473 (26) [M$^+$-Rha-Gal-Glu A], 456 (18) [aglycone$^+$+H], 438 (76) [aglycone$^+$+H–H_2O], 215 (40), 182 (9), 146 (16), 143 (100) calculated for $C_{48}H_{76}O_{19}$. – Acid hydrolysis of compound **7** afforded complogenin as sapogenol in respect to TLC, glucuronic acid, galactose and rhamnose.

Acid hydrolysis of glycosides

A solution of each saponin (30 mg) in 5 ml of 2 M HCl/MeOH was heated separately at 100 °C for 40 min, and then partitioned with $CHCl_3$/H_2O. The chloroformic layer was checked for the identification of the aglycone and the aqueous layer for the identification of sugar moieties. The aglycones were identified by TLC and comparison with authentic samples using $CHCl_3$/MeOH (8:2) as a developer and 10% H_2SO_4 for detection. Saponins **1**–**4** and **6** gave soyasapogenol B (R_f 0.8), saponin **5** gave 11-hydroxysoyasapogenol B (R_f 0.73) and saponin **7** gave complogenin (R_f 0.66).

The sugars were checked by TLC, which was carried out on silica gel F_{254} plates using *n*-BuOH/PrOH/H_2O (10:5:4) as developer and anisaldehyde/H_2SO_4 for detection. Saponin **1** gave glucose (R_f 0.3), saponin **2** gave glucuronic acid (R_f 0.2), saponin **3** gave galactose (R_f 0.25) and

glucose (R_f 0.3), saponin **4** and **5** gave glucose (R_f 0.3), galactose (R_f 0.25) and rhamnose. (R_f 0.57) while saponins **6** and **7** gave glucuronic acid (R_f 0.2), galactose (R_f 0.25) and rhamnose (R_f 0.57).

Antimicrobial investigation

The antimicrobial investigations were performed against Gram-positive (*Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* CECT 232, *Enterococcus faecalis* CECT 481, *Bacillus subtilis* CECT 39, *Bacillus cereus* CECT 496, *Bacillus pumilus* CECT 29, *Mycobacterium smegmatis* CECT 3030), Gram-negative bacteria (*Escherichia coli* CECT 99, *Proteus mirabilis* CECT 170, *Salmonella veneziana* CECT 450) and yeasts (*Candida albicans* UBC1, *Candida glabrata* ATCC 90030, *Candida kefyr* YO 106) using the agar diffusion technique. The bacteria were maintained in Nutrient Agar (Oxoid) and Brain Heart Infusion Agar for *Mycobacterium* and *Enterococcus* and Sabouraud (Oxoid) medium for yeasts.

MIC values were determined by a two-fold dilution method using nutrient broth or brain heart infusion with Tween 80 (0.06%) for bacteria and Sabouraud liquid medium for yeasts at the final inoculum size of 1.5×10^5 cells/ml. After 24 h, except for *Mycobacterium* (48 h), in agitation at 37 °C the optical density was measured using a micro ELISA reader at 600 nm. The MIC values were recorded as the lowest concentration with no visible growth.

Cytotoxic activity using cell lines

Hela (human carcinoma of cervix) and Hep-2 (human carcinoma of larynx) cell lines were grown as a monolayer in Dulbecco's modified Eagle's medium, DMEM (Gibco), supplemented with 10% fetal calf serum (Gibco) and 1% of penicillin-streptomycin mixture (10,000 IU/ml). The cells were maintained at 37 °C in 5% CO_2 and 90% humidity. The samples dissolved in DMSO were added to the cells at the desired concentration, the same volume of DMSO was used as control. The cytotoxic activity was assayed using the colorimetric MTT [3-(3,5-dimethylthiazol-2-yl)-diphenyl tetrazolium bromide] reduction assay; 2×10^4 cells in 50 μl were added to each well and the optical density was measured using a micro ELISA reader at 550 nm. The percentage viability was plotted against the compound concentrations and the 50% cell viability (IC_{50}) was calculated from the curve (Reiner, 1974). The results are recorded in Table III.

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