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Steroidal saponins from the stem of *Yucca elephantipes*

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Abstract

Ten steroidal saponins with *cis*-fused A/B ring, including a smilagenin glycoside, elephanoside A (**4**), and the five furostanol bisdesmosides, elephanosides B–F (**6**–**10**), were isolated from the stems of *Yucca elephantipes* Regel. (Agavaceae). Their structures were determined by detailed chemical and spectroscopic analysis. All the isolated compounds were tested for their *in vitro* antifungal and antibacterial activities. Only the two known spirostanol glycosides Ys-II (**1**) and Ys-IV (**2**) showed moderate inhibitory activity against the growth of *Candida albicans* and *Cryptococcus neoformans*.

Keywords: *Yucca elephantipes*; Agavaceae; Steroidal saponins; Elephanosides A–F; Antifungal activity

1. Introduction

The genus *Yucca* (Agavaceae) contains about 35–40 species, which are mainly distributed in Central and Northern America. Many of them as ornamental plants are commonly cultivated in the tropical gardens of the World. Yucca extracts are used not only to feed livestock and poultry to improve their growth and productivity (Anthony et al., 1994; Balog et al., 1994), but also to reduce ammonia and odors in poultry excreta on farms (Cheeke, 1999). The steroidal saponins are considered to be closely involved with these actions (Varanda, 1984). Until now, studies on steroidal glycosides from *Yucca* genus have been mainly focused on *Y. gloriosa* (Benidze et al., 1991; Giorgadze and Gurielidze, 1991; Gonzalez et al., 1972; Kemertelidze and Pkheidze, 1972; Kemertelidze and Benidze, 2001; Nakano et al., 1988, 1989, 1991a,b; Pkheidze et al., 1981, 1985, 1991a,b), *Y. schidigera* (Miyakoshi et al., 2000; Oleszek et al., 2001a,b; Piacente et al., 2005), *Y. filamentosa* (Dragalin and Kintia, 1975; Plock et al., 2001), and *Y. aloifolia* (Bahuguna et al., 1991; Benidze et al., 1984; Kishor and Sati, 1990; Kishor et al., 1992).

Yucca elephantipes Regel. is the tallest of the Yuccas, reaching 30 feet in height with glossy bluish green leaves that can grow up to 4 feet long with a narrow width of about 3 inches. Its trunk is somewhat thickened at the base and very rough. It is native to Mexico and introduced to China as an evergreen ornamental plant. The leaf extract of *Y. elephantipes* was reported to have antiviral activity on TMV (Tobacco mosaic virus) (Wang, 2006); however, no chemical study has been reported on this plant. As a continuing work to search for new antifungal natural products from monocotyledons, particularly from the Liliales (Yang et al., 2006; Zhang et al., 2006), a phytochemical investigation on the air-dried stem of *Y. elephantipes* was carried out to afford 10 steroidal glycosides with *cis*-fused A/B ring system. This paper presents the isolation and structure elucidation of these compounds as well as their *in vitro* antifungal and antibacterial activities.

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2. Results and discussion

Repeated column chromatography (including by Diaion HP-20SS, Sephadex LH-20, MCI-gel CHP20P, silica gel and reversed phase silica gel Rp-8) of the *n*-butanol extract of *Y. elephantipes* stem afforded six new steroidal glycosides (**4**, **6**–**10**). In addition, four known compounds were obtained and identified as Ys-II (**1**) (Nakano et al., 1989), Ys-IV (**2**) (Nakano et al., 1989), YS-VIII (**3**) (Nakano et al., 1991b) and (25*R*)-26-*O*-β-D-glucopyranosyl-5β-furostan-3β, 22ξ, 26-triol-3-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-galactopyranoside (**5**) (Skhirtladze et al., 2006), respectively, on the basis of their physical and spectroscopic data and by comparison with reference values (see Chart 1).

Compound **4**, named elephanoside A, showed a positive reaction (green color) to the anisaldehyde reagent, but a negative reaction to Ehrlich reagent, indicating the presence of a spirostanol skeleton (Kiyosawa and Hutoh, 1968). These observations were coincident with the ^{1}H and ^{13}C NMR spectroscopic data, in which four typical methyl proton signals at δ 0.82 (*s*, Me-18), 0.99 (*s*, Me-19), 0.67 (*d*, $J = 4.3$ Hz, Me-27) and 1.14 (*d*, $J = 6.7$ Hz, Me-21), and a characteristic C-22 resonance for a spirostanol skeleton at δ 109.3 were observed (Li et al., 1990, 1992). The negative ion HRESIMS gave a quasi-molecular ion peak at *m/z* 709.4163 $[M-H]^-$, corresponding to a molecular formula $C_{38}H_{62}O_{12}$. The negative ion FABMS also showed a characteristic fragment ion peak at *m/z* 577 $[M-132\ (pentosyl)-H]^-$, indicating the existence of a terminal pentosyl unit in the molecule. The presence of the two anomeric proton signals at δ 4.91 (*d*, $J = 7.6$ Hz), and 5.14 (*d*, $J = 7.0$ Hz) observed in the ^{1}H NMR spectrum suggested that **4** was a spirostanol diglycoside. Upon acid hydrolysis with 2 M HCl, **4** afforded smilagenin [(25*R*)-5β-spirostan-3β-ol] (Agrawal et al., 1985) as aglycone, and D-xylose and D-galactose as sugar residues. The large coupling constants (~7 Hz) of the anomeric protons indicated the β-configuration at the anomeric centers for both D-xylopyranosyl and D-galactopyranosyl units. Connectivities of the sugar moieties were established by the HMBC correlations of the anomeric proton of the xylosyl (δ 5.14) with C-2 of galactosyl (δ 82.4) moieties, and the anomeric proton of the galactosyl (δ 4.91) with that of the C-3 of the aglycone (δ 74.2). Thus, the structure of **4** was elucidated to be (25*R*)-5β-spirostan-3β-ol-3-*O*-β-D- xylopyranosyl-(1 → 2)-β-D-galactopyranoside.

Compounds **6**–**10**, named elephanoside (B–F), were all furostanol glycosides as indicated by the positive reaction (red color) to the Ehrlich reagent (Kiyosawa and Hutoh, 1968) and a characteristic quaternary carbon signal around δ 110 observed in the DEPT spectrum. All of their 25*R* configurations were determined based on their small differences ($\Delta_{ab} = \delta_a - \delta_b$) of the chemical shifts (δ_a, δ_b) of the germinal protons at H_2-26 ($\Delta_{ab} = 0.30$–0.34 ppm). It has been described that Δ_{ab} is usually >0.57 ppm in 25*S* compounds and <0.48 ppm in 25*R* compounds (Agrawal, 2004). The molecular formulas of **6**–**10** were determined to be $C_{51}H_{86}O_{24}$, $C_{51}H_{84}O_{25}$, $C_{45}H_{74}O_{20}$, and $C_{51}H_{86}O_{25}$, respectively, on the basis of analyses of the negative ion HRESIMS and ^{13}C NMR spectra.

The ^{1}H and ^{13}C NMR spectra of **6** showed the presence of 4 characteristic methyls [δ 0.86 (*s*, Me-18), 0.95 (*s*, Me-19), 0.97 (*d*, $J = 6.6$ Hz, Me-27), and 1.33 (*d*, $J = 6.7$ Hz, Me-21)], 11 methylenes [including one oxygen-bearing carbon at δ 75.4 (C-26)], 9 methines [including two oxygen-bearing carbons at δ 75.2 (C-3) and 81.3 (C-16)], and 3 quaternary carbons [including one oxygen-bearing carbon at δ 110.7 (C-22)]. In addition, signals ascribable to four sugar moieties [anomeric protons at δ_H 4.81 (*d*, $J = 7.6$ Hz), 4.84 (*d*, $J = 7.6$ Hz), 5.40 (*d*, $J = 7.6$ Hz) and 5.57 (*d*, $J = 7.7$ Hz)] were also observed. These NMR spectroscopic data were closely related to those of (25*R*)-26-*O*-β-D-glucopyranosyl-5β-furostan-3β,22α,26-triol-3-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-galactopyranoside (**5**) (Skhirtladze et al., 2006), except for the appearance of one more β-glucopyranosyl unit signals. The full assignments of sugar signals were carried out by HSQC and TOCSY experiments. In the HMBC spectrum of **6**, the additional anomeric proton at δ 5.40 (GlcIII H-1) was correlated with δ 83.9 (Gal C-3), indicating its location was on the C-3 position of the inner galactopyranosyl unit. Other HMBC correlations of δ_H 4.81 (GlcII H-1) with δ_C 75.4 (C-26), δ_H 5.57 (GlcI H-1) with δ_C 78.0 (Gal C-2), and δ_H 4.84 (Gal H-1) with δ_C 75.2 (C-3 of aglycone) confirmed the sugar sequence and linkage site to the aglycone. Moreover, enzymatic hydrolysis of **6** with β-glucosidase yielded glucose as the sugar residue and Ys-IV (**2**) (Nakano et al., 1989) as the corresponding spirostanol glycoside, which confirmed that **6** was a furostanol type of compound **2**. Therefore, the

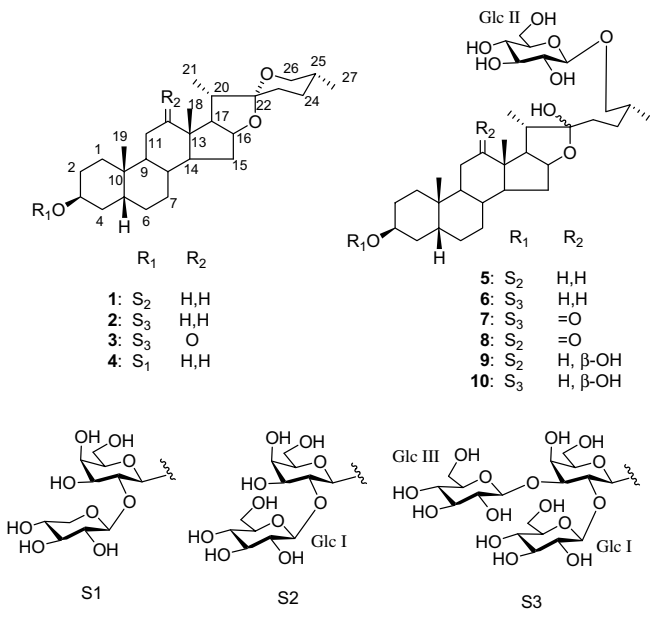

Chart 1. Steroidal saponins **1**–**10** isolated form *Yucca elephantipes*.

structure of **6** was assigned as (25*R*)-26-*O*-β-D-glucopyranosyl-5β-furostan-3β,22ξ,26-triol-3-*O*-β-D-glucopyranosyl-(1 → 2)-*O*-[β-D-glucopyranosyl-(1 → 3)]-β-D-galactopyranoside.

The molecular formula of compound **7** has one more oxygen atom, but two less hydrogen atoms than that of **6**. The ^{13}C NMR spectra of **7** (Tables 1 and 2) were in good agreement with those of **6**, except for obvious differences at C-11 (δ 37.8), C-12 (δ 213.3), C-13 (δ 56.0), and C-17 (δ 55.1), suggesting that one of the methylene at C-12 in **6** was oxygenated to be a carbonyl group in **7**. This was confirmed by the long-range correlations of δ 1.12 (Me-18), 1.42 (H-14), 2.17 (H-11a), 2.35 (H-11b), 2.94 (H-17) with δ 213.3 (C-12) in the HMBC experiment. Enzymatic hydrolysis of **7** with β-glucosidase gave glucose and YS-VIII (**3**) as the corresponding spirostanol glycoside, indicating that **7** was a furostanol type of **3**. Therefore, compound **7** was characterized as (25*R*)-26-*O*-β-D-glucopyranosyl-5β-furostan-3β,22ξ,26-triol-12-one-3-*O*-β-D-glucopyranosyl-(1 → 2)-*O*-[β-D-glucopyranosyl-(1 → 3)]-β-D-galactopyranoside.

Acid hydrolysis of **8** produced D-galactose and D-glucose as sugar residues, and gloriogenin as the sapogenin (Nakano et al., 1991b). Comparison of the NMR spectroscopic data (Tables 1 and 2) with those of **7** and **5** indicated that **8** had a same aglycone as **7** and an identical sugar moiety to **5**. In the HMBC spectrum, three anomeric protons at δ 4.89 (*d*, $J = 7.7$ Hz, Gal H-1), 5.30 (*d*, $J = 7.6$ Hz, GlcI H-1), and 4.83 (*d*, $J = 7.7$ Hz, GlcII H-1) showed obvious correlations with δ 75.3 (C-3), 81.8 (Gal C-2) and 75.3 (C-26), respectively, confirming the sugar sequence and linkages in **8**. Accordingly, the structure of **8** was deduced to be (25*R*)-26-*O*-β-D-glucopyranosyl-5β-furostan-3β,22ξ,26-triol-12-one-3-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-galactopyranoside.

The ^{1}H and ^{13}C NMR spectroscopic data of **9** were very similar to those of **5**, except for some differences in the aglycone part at C-11 (δ 31.5, CH_2), C-12 (δ 79.7, CH), C-13 (δ 47.1, C) and C-18 (δ 11.5, CH_3). The NMR spectroscopic data in the aglycone part of **9** were coincident with those of 12β-hydroxysmilagenin (Nakano et al., 1991a). These observations suggested that **9** had one more hydroxyl attached at the C-12 position, relative to **5**. In the ROESY spectrum of **9**, correlations of δ 3.55 (*t*, $J = 5.6$ Hz, H-12α) with H-14 (δ 1.07, m), H-17 (δ 2.35, *dd*, $J = 6.4$, 8.2 Hz) and CH_3-21 (δ 1.62, *d*, $J = 6.6$ Hz) further confirmed the 12β-hydroxyl group in **9**. Enzymatic hydrolysis of **9** with β-glucosidase afforded glucose and its corresponding spirostanol saponin, (25*R*)-5β-spirostan-3β,12β-diol-3-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-galactopyranoside (YS-XI), which was originally isolated from the caudex of *Yucca gloriosa* (Nakano et al., 1991a). Therefore, compound **9** was assigned as (25*R*)-26-*O*-β-D-glucopyranosyl-5β-furostan-3β,12β,22ξ,26-tetraol-3-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-galactopyranoside.

Table 1
^{13}C NMR spectroscopic data for the aglycone moieties of compounds **4** and **6**–**10** (in pyridine-d_5, δ in ppm)

Position	4	6	7	8	9	10
1	30.2	30.0	30.4	30.7	31.1	30.8
2	27.1	26.9	26.6	26.5	26.8	26.7
3	74.2	75.2	75.2	75.3	75.4	75.4
4	30.7	30.8	30.6	30.8	31.0	30.5
5	36.3	36.6	36.1	36.5	36.8	36.4
6	27.1	26.9	26.8	26.8	26.8	26.8
7	26.8	27.0	26.5	26.4	27.2	27.1
8	35.6	35.3	34.7	34.7	34.7	34.7
9	40.2	40.3	42.1	42.0	39.5	39.5
10	35.3	35.3	35.8	35.7	35.4	35.3
11	21.2	21.2	37.8	37.8	31.5	31.5
12	40.3	40.4	213.3	213.4	79.7	79.7
13	40.9	40.7	56.0	56.0	47.1	47.1
14	56.5	56.4	56.0	56.0	55.3	55.3
15	31.9	32.5	31.8	31.7	32.2	32.2
16	81.3	81.3	79.8	79.8	81.4	81.4
17	63.1	64.1	55.1	55.1	63.8	63.9
18	16.6	16.8	16.2	16.3	11.5	11.4
19	23.9	24.0	23.1	23.2	24.0	23.9
20	42.0	41.3	41.3	41.3	41.7	41.7
21	15.1	16.6	15.3	15.3	15.9	15.7
22	109.3	110.7	110.8	110.8	111.1	111.0
23	32.2	37.3	37.1	37.1	37.4	37.3
24	29.3	28.4	28.4	28.4	28.5	28.5
25	30.6	34.3	34.3	34.3	34.4	34.4
26	66.9	75.4	75.2	75.3	75.3	75.2
27	17.4	17.5	17.5	17.5	17.6	17.6

Table 2
^{13}C NMR spectroscopic data for the sugar moieties of compounds **4** and **6**–**10** (in pyridine-d_5, δ in ppm)

Position	4	6	7	8	9	10
Gal-1	101.4	102.1	101.9	102.5	102.6	102.4
2	82.4	78.0	77.8	81.8	81.7	77.8
3	75.4	83.9	84.1	75.3	75.3	84.2
4	69.9	69.9	69.9	69.9	69.9	69.9
5	76.7	76.5	76.5	77.0	77.0	76.9
6	62.2	63.5	62.9	62.2	62.2	63.6
Xyl-1	107.2					
2	76.6					
3	78.1					
4	71.2					
5	67.6					
GlcI-1		104.5	104.5	106.2	106.1	104.5
2		76.5	76.4	76.7	76.7	76.4
3		78.7	78.4	78.5	78.6	78.6
4		72.6	72.8	71.7	71.7	72.8
5		78.5	77.9	78.1	78.1	78.1
6		62.8	63.5	62.8	62.8	62.9
GlcII-1		105.0	105.0	105.0	105.0	104.9
2		75.2	74.8	75.3	75.6	75.2
3		78.5	78.5	78.7	78.7	78.4
4		71.7	71.8	71.7	71.7	71.8
5		78.0	78.5	78.6	78.6	77.9
6		62.5	62.6	62.8	62.9	62.6
GlcIII-1		105.4	105.3			105.3
2		75.2	75.4			75.2
3		78.6	78.6			78.5
4		71.5	71.6			71.6
5		78.5	78.5			78.4
6		62.4	62.4			62.4

Table 3
Antifungal activity of steroidal saponins **1** and **2** (IC_{50}/MIC/MFC,[a] μg/ml)

	C. albicans ATCC 90028			*C. neoformans* ATCC 90113		
	IC_{50}	MIC	MFC	IC_{50}	MIC	MFC
1	5.0	10.0	20.0	6.0	10.0	20.0
2	15.0	na[b]	na[b]	15.0	20.0	20.0
Amphotericin B	0.15	0.31	1.25	0.55	1.25	1.25

[a] IC_{50}: 50% inhibitory concentration; MIC: minimum inhibitory concentration; MFC: minimum fungicidal concentration.
[b] Not active at the highest test concentration of 20 μg/mL.

The ^1H and ^{13}C NMR spectra of **10** showed the presence of the same aglycone as in **9** and the same sugar moiety as in **6** and **7**. Acid hydrolysis of **10** produced 12β-hydroxysmilagenin (Nakano et al., 1991a). The NMR signal assignments of **10** were facilitated by the HSQC, HMBC, and TOCSY experiments. The 12β-OH configuration in **10** was confirmed by the same ROESY correlations as in **9**. Therefore, the structure of **10** should be (25*R*)-26-*O*-β-D-glucopyranosyl-5β-furostan-3β,12β,22ξ,26-tetraol-3-*O*-β-D-glucopyranosyl-(1 → 2)-*O*-[β-D-glucopyranosyl-(1 → 3)]-β-D-galactopyranoside.

Saponins **1**–**10** were tested for their antifungal activity against *Candida albicans* and *Cryptococcus neoformans* using amphotericin B as a positive control. Only two spirostanol smilagenin glycosides **1** and **2** exhibited moderate antifungal activity (Table 3). The lack of antifungal activity of all furostanol glycosides (**5**–**10**) and two other spirostanol glycosides (**3** and **4**) indicated that both aglycone and sugar moieties are influential factors for the antifungal activity of steroidal saponins. All compounds were also tested for activity against the filamentous fungus *Aspergillus fumigatus*, and the bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, and *Mycobacterium intracellulare* and inactive at the highest test concentration of 20 μg/ml.

3. Concluding remarks

In conclusion, 10 steroidal saponins with *cis*-fused A/B ring were isolated from the stems of *Y. elephantipes*. Of these, six new glycosides (**4**, **6**–**10**) were identified. The *in vitro* antifungal and antibacterial bioassay showed that only two known spirostanol glycosides Ys-II (**1**) and Ys-IV (**2**) had moderate inhibitory activity against the growth of *Candida albicans* and *Cryptococcus neoformans*. Steroidal saponins with *cis*-fused A/B rings are uncommon in nature compared to those with *trans*-fused A/B ring, and have mainly been reported in the genus *Yucca*, especially *Y. gloriosa* and *Y. schidigera*. As a kind of characteristic secondary metabolite, further and systematic chemical work on the glycosides with a *cis*-fused A/B ring from the genus *Yucca* should be carried out in the future to provide more chemotaxonomic information on this genus.

4. Experimental

4.1. General methods

The instruments for the measurement of NMR spectra were Bruker AM-400 (for ^1H NMR and ^{13}C NMR) and DRX-500 (for 2D NMR), with TMS as internal standard and pyridine-d_5 as solvent. Optical rotations were recorded on a SEPA-3000 automatic digital polarimeter. FABMS (negative ion mode) and HRESIMS (negative ion mode) spectra were measured on VG AutoSpe 3000 and API Qstar Pulsar LC/TOF spectrometers, respectively. GC analysis were identical to those described in our previous paper (Zhang et al., 2006). Agilent Technologies HP5890 gas chromatograph was the equipment carrying H_2 flame ionization detector and 30QC2/AC-5 quartz capillary column (30 m × 0.32 mm). The conditions as followings: column temperature: 180 °C/280 °C; programmed increase, 3 °C/min; carrier gas: N_2 (1 ml/min); injection and detector temperature: 250 °C; injection volume: 4 μl, split ratio: 1/50. CC was performed over Diaion HP-20SS (Mitsubishi, Japan), macroporus absorption resin D-101 (Qingdao Makall, China), silica gel (200–300 mesh, Qingdao Makall, China), Sephadex LH-20 (Pharmacia, Sweden), MCI-gel CHP20P (Mitsubishi, Japan) and reversed phase silica gel RP-8 (40–63 μm, Merck, Germany). TLC was carried out on silica gel H (size: 50 × 100 mm, thickness: 0.20–0.25 mm, Qingdao Makall, China), and spots were visualized by spraying with anisaldehyde–H_2SO_4 (for both spirostanol and furostanol saponins) and Ehrlich reagent (for furostanol saponins) followed by heating.

4.2. Plant material

The stems of *Y. elephantipes* were collected from the East Garden of Kunming Institute of Botany, Chinese Academy of Sciences, Yunnan, China. A voucher specimen (KUN No. 0831640) is deposited at the Herbarium of the Institute mentioned above.

4.3. Extraction and isolation

The air-dried stems (4.8 kg) of *Y. elephantipes* were extracted with MeOH (6 L × 3) under reflux for three times. The organic extracts were combined and removed *in vacuo*, and the residue suspended into H_2O was then partitioned with $CHCl_3$ and *n*-BuOH, successively. The *n*-BuOH extract (240 g) was subjected to a silica gel CC eluted with $CHCl_3$–MeOH–H_2O (7.5:2.5:0.4) to afford seven fractions (Frs. 1–7). Fr. 2 (3.5 g) was applied to Sephadex LH-20 and MCI-gel CHP20P columns eluting with MeOH–H_2O (0:1–1:0) to give **4** (14 mg). When Fr. 3 was concentrated *in vacuo*, a precipitate appeared, which was washed with MeOH for three times to afford **1** (6 g). Fr. 4 (11 g) was subjected to MCI-gel CHP20P followed by reversed phase RP-8 CC, eluting with

MeOH–H_2O (40–100%) to give **2** (20 mg) and **3** (40 mg). Fr. 5 (96 g) was applied to a Diaion HP-20SS and reversed phase Rp-8 CC eluting with MeOH–H_2O (4:6–1:0) to afford **5** (228 mg). Repeated CC of Fr. 6 (30 g) over MCI-gel CHP20P (60–100% MeOH in H_2O), silica gel ($CHCl_3$–MeOH–H_2O, 7:3:0.5), Sephadex LH-20 (0–20% MeOH in H_2O) and RP-8 (30–90% aq. MeOH) to furnish **8** (29 mg) and **9** (40 mg). Fr. 7 (64 g) was subjected to a macroporus absorption resin D-101 and MCI-gel CHP20P, eluting with MeOH–H_2O (0:1–1:0), followed by CC on Sephadex LH-20 (10% aq. MeOH) and silica gel ($CHCl_3$–MeOH–H_2O, 7:3:0.5) to afford **6** (20 mg), **7** (56 mg), and **10** (15 mg).

4.3.1. Elephanoside A (*4*)

White amorphous powder, $[\alpha]_D^{23} = -55.8$ (*c* 0.72, pyridine). FABMS (negative ion mode): *m/z* 709 $[M-H]^-$, 577 $[M-132\ (Xyl)-H]^-$. HRESIMS (negative ion mode): *m/z* 709.4155 $[M(C_{38}H_{62}O_{12})-H]^-$(calcd. for 709.4169). ^{1}H NMR (pyridine-d_5, 400 MHz): δ 0.67 (3H, *d*, *J* = 4.3 Hz, Me-27), 0.82 (3H, *s*, Me-18), 0.99 (3H, *s*, Me-19), 1.08 (1H, *m*, H-14), 1.09 (1H, *m*, H-12a), 1.14 (3H, *d*, *J* = 6.7 Hz, Me-21), 1.23 (1H, *m*, H-11a), 1.32 (1H, *m*, H-9), 1.35 (1H, *m*, H-11b), 1.41 (1H, *dd*, *J* = 5.6, 11.2 Hz, H-15a), 1.47 (1H, *m*, H-4a), 1.50 (1H, *m*, H-8), 1.55 (2H, *m*, H-24a,b), 1.68 (2H, *m*, H-23a,b), 1.70 (1H, *m*, H-12b), 1.87 (1H, *m*, H-4b), 1.96 (1H, *m*, H-20), 2.04 (1H, *m*, H-15b), 2.28 (1H, *m*, H-5), 3.51 (1H, *br t*, *J* = 9.6 Hz, H-26a), 3.58 (1H, *br d*, *J* = 9.6 Hz, H-26b), 3.62 (1H, *br t*, *J* = 10.8 Hz, Xyl H-5a), 4.01 (1 H, *m*, Gal H-5), 4.07 (1H, *t*, *J* = 8.4 Hz, Xyl H-2), 4.11 (1H, *t*, *J* = 8.4 Hz, Xyl H-3), 4.24 (1H, *m*, Xyl H-4), 4.38 (1H, *m*, H-3), 4.42 (1H, *m*, Xyl H-5b), 4.56 (1H, *br s*, Gal H-4), 4.57 (1H, *m*, Gal H-2), 4.61 (1H, *m*, H-16), 4.91 (1H, *d*, *J* = 7.6 Hz, Gal H-1), 5.14 (1H, *d*, *J* = 7.0 Hz, Xyl H-1). ^{13}C NMR (pyridine-d_5, 400 MHz): see Tables 1 and 2.

4.3.2. Elephanoside B (*6*)

White amorphous powder, $[\alpha]_D^{23} = -14.5$ (*c* 0.61, MeOH). FABMS (negative ion mode): *m/z* 1081 $[M-H]^-$, 920 $[M-Glc\ (162)]^-$, 757 $[M-Glc\ (162)\times2-H]^-$, 595 $[M-Glc\ (162)\times3-H]^-$. HRESIMS (negative ion mode): *m/z* 1081.5445 $[M(C_{51}H_{86}O_{24})-H]^-$ (calcd. for 1081.5436). ^{1}H NMR (pyridine-d_5, 500 MHz): δ 0.86 (3H, *s*, Me-18), 0.90 (1H, *m*, H-7a), 0.95 (3H, *s*, Me-19), 0.97 (3H, *d*, *J* = 6.6 Hz, Me-27), 1.09 (1H, *m*, H-14), 1.14 (1H, *m*, H-7b), 1.18 (1H, *m*, H-11a), 1.22 (1H, *m*, H-9), 1.26 (1H, *m*, H-12a), 1.27 (1H, *m*, H-11b), 1.33 (3H, *d*, *J* = 6.7 Hz, Me-21), 1.43 (2H, *m*, H-8,2a), 1.62 (1H, *m*, H-24a), 1.69 (1H, *m*, H-12b), 1.87 (2H, *m*, H-2b, 25), 2.03 (3H, *m*, H-23a,b,24b), 2.04 (1H, *m*, H-17), 2.20 (1H, *m*, H-5), 2.21 (1H, *m*, H-20), 3.60 (1H, *dd*, *J* = 4.5, 9.5 Hz, H-26a), 3.81 (1H, *m*, GlcII H-5), 3.92 (1H, *m*, H-26b), 4.15 (1H, *t*, *J* = 10.0 Hz, GlcI H-4), 4.20 (1H, *t*, *J* = 10.0 Hz, GlcIII H-4), 4.24 (1H, *m*, GlcI H-5), 4.29 (1H, *m*, H-3), 4.37 (1H, *m*, Gal H-3), 4.78 (1H, *br s*, Gal H-4), 4.79 (1H, *m*, Gal H-2), 4.81 (1H, *d*, *J* = 7.6 Hz, GlcII H-1), 4.84 (1H, *d*, *J* = 7.6 Hz, Gal H-1), 4.99 (1H, *m*, H-16), 5.40 (1H, *d*, *J* = 7.6 Hz, GlcIII H-1), 5.57 (1H, *d*, *J* = 7.7 Hz, GlcI H-1). ^{13}C NMR (pyridine-d_5, 400 MHz): see Tables 1 and 2.

4.3.3. Elephanoside C (*7*)

White amorphous powder, $[\alpha]_D^{23} = +5.2$ (*c* 0.35, MeOH). FAB-MS (negative ion mode) *m/z*: 1096 $[M]^-$, 933 $[M-Glc\ (162)-H]^-$, 771 $[M-Glc\ (162)\times2-H]^-$, 591 $[M-Glc\ (162)\times3-H_2O-H]^-$. HRESI-MS (negative ion mode) *m/z*: 1095.5198 $[M(C_{51}H_{84}O_{25})-H]^-$ (calcd. for 1095.5229). ^{1}H NMR (pyridine-d_5, 500 MHz): δ 0.89 (1H, *m*, H-7a), 0.95 (3H, *s*, Me-19), 0.97 (3H, *d*, *J* = 6.6 Hz, Me-27), 1.12 (3H, *s*, Me-18), 1.29 (1H, *m*, H-7b), 1.42 (1H, *m*, H-14), 1.55 (3H, *d*, *J* = 6.7 Hz, Me-21), 1.66 (1H, *m*, H-24a), 1.71 (1H, *m*, H-9), 1.76 (1H, *m*, H-8), 1.91 (1H, *m*, H-25), 2.01 (1H, *m*, H-24b), 2.04 (2H, *m*, H-23a,b), 2.17 (1H, *dd*, *J* = 4.1, 13.1 Hz, H-11a), 2.21 (1H, *m*, H-20), 2.24 (1H, *m*, H-5), 2.35 (1H, *m*, H-11b), 2.94 (1H, *t*, *J* = 7.3 Hz, H-17), 3.61 (1H, *dd*, *J* = 6.2, 9.7 Hz, H-26a), 3.94 (1H, *m*, H-26b), 4.02 (1H, *m*, GlcI H-2), 4.17 (1H, *t*, *J* = 9.1 Hz, GlcI H-4), 4.19 (1H, *m*, GlcII H-4), 4.23 (1H, *m*, H-3), 4.37 (1H, *m*, Gal H-3), 4.77 (1H, *d*, *J* = 2.4 Hz, Gal H-4), 4.80 (1H, *d*, *J* = 7.4 Hz, Gal H-1), 4.83 (1H, *d*, *J* = 7.1 Hz, GlcII H-1), 4.91 (1H, *dd*, *J* = 7.3, 14.2 Hz, H-16), 5.40 (1H, *d*, *J* = 7.7 Hz, GlcIII H-1), 5.58 (1H, *d*, *J* = 7.8 Hz, GlcI H-1). ^{13}C NMR (pyridine-d_5, 400 MHz): see Tables 1 and 2.

4.3.4. Elephanoside D (*8*)

White amorphous powder, $[\alpha]_D^{23} = +11.5$ (*c* 0.13, MeOH). FAB-MS (negative ion mode) *m/z*: 933 $[M-H]^-$, 771 $[M-Glc\ (162)-H]^-$, 609 $[M-Glc\ (162)\times2-H]^-$. HRESI-MS (negative ion mode) *m/z*: 933.4691 $[M(C_{45}H_{74}O_{20})-H]^-$ (calcd. for 933.4701). ^{1}H NMR (pyridine-d_5, 400 MHz): δ 0.89 (1H, *m*, H-7a), 0.96 (3H, *s*, Me-19), 0.97 (3H, *d*, *J* = 4.1 Hz, Me-27), 1.12 (3H, *s*, Me-18), 1.28 (1H, *m*, H-7b), 1.36 (1H, *m*, H-2a), 1.42 (1H, *m*, H-14), 1.56 (3H, *d*, *J* = 6.7 Hz, Me-21), 1.66 (1H, *m*, H-24a), 1.69 (1H, *m*, H-9), 1.85 (1H, *m*, H-8), 1.88 (1H, *m*, H-2b), 2.03 (1H, *m*, H-23a), 2.06 (1H, *m*, H-25), 2.19 (1H, *m*, H-24b), 2.20 (2H, *m*, H-5, 23b), 2.21 (2H, *m*, H-11a,20), 2.36 (1H, *m*, H-11b), 2.95 (1H, *t*, *J* = 7.6 Hz, H-17), 3.61 (1H, *m*, H-26a), 3.85 (1H, *m*, GlcII H-5), 3.95 (1H, *m*, H-26b), 4.21 (1H, *t*, *J* = 9.2 Hz, GlcI H-4), 4.22 (1H, *t*, *J* = 9.2 Hz, GlcII H-3), 4.28 (1H, *m*, H-3), 4.33 (1H, *t*, *J* = 9.2 Hz, GlcII H-4), 4.39 (1H, *m*, Gal H-3), 4.57 (1H, *br s*, Gal H-4), 4.68 (1H, *t*, *J* = 8.8 Hz, Gal H-2), 4.83 (1H, *d*, *J* = 7.7 Hz, GlcII H-1), 4.89 (1H, *d*, *J* = 7.7 Hz, Gal H-1), 4.93 (1H, *m*, H-16), 5.30 (1H, *d*, *J* = 7.6 Hz, GlcI H-1). ^{13}C NMR (pyridine-d_5, 400 MHz): see Tables 1 and 2.

4.3.5. Elephanoside E (*9*)

White amorphous powder, $[\alpha]_D^{23} = -14.1$ (*c* 0.32, MeOH). FABMS (negative ion mode): *m/z* 935 $[M-H]^-$, 773 $[M-Glc\ (162)-H]^-$, 609 $[M-Glc\ (162)\times2-3H]^-$. HRESIMS (negative ion mode): *m/z* 935.4848

$[M(C_{45}H_{76}O_{20})-H]^-$ (calcd. for 935.4857). ^{1}H NMR (pyridine-d_5, 400 MHz): δ 0.89 (1H, *m*, H-7a), 0.96 (3H, *d*, $J = 5.8$ Hz, Me-27), 0.97 (3H, *s*, Me-19), 1.07 (1H, *m*, H-14), 1.13 (3H, *s*, Me-18), 1.19 (1H, *m*, H-7b), 1.42 (1H, *m*, H-2a), 1.43 (1H, *m*, H-9), 1.48 (1H, *m*, H-8), 1.49 (1H, *m*, H-11a), 1.56 (1H, *m*, H-15a), 1.62 (3H, *d*, $J = 6.6$ Hz, Me-21), 1.69 (1H, *m*, H-24a), 1.78 (1H, *m*, H-11b), 1.87 (1H, *m*, H-25), 1.88 (1H, *m*, H-2b), 2.03 (1H, *m*, H-24b), 2.06 (1H, *m*, H-15b), 2.07 (2H, *m*, H-23a,b), 2.15 (1H, *m*, H-5), 2.35 (1H, *dd*, $J = 6.4$, 8.2 Hz, H-17), 2.48 (1H, *m*, H-20), 3.55 (1H, *t*, $J = 5.6$ Hz, H-12), 3.60 (1H, *dd*, $J = 6.0$, 9.6 Hz, H-26a), 3.83 (1H, *m*, GlcII H-5), 3.90 (1H, *br t*, $J = 9.6$ Hz, H-26b), 4.19 (1H, *t*, $J = 8.8$ Hz, GlcII H-3), 4.20 (1H, *t*, $J = 8.8$ Hz, GlcII H-4), 4.29 (1H, *m*, H-3), 4.30 (1H, *t*, $J = 8.4$ Hz, GlcI H-4), 4.33 (1H, *m*, Gal H-3), 4.56 (1H, *br s*, Gal H-4), 4.66 (1H, *t*, $J = 8.4$ Hz, Gal H-2), 4.80 (1H, *d*, $J = 7.6$ Hz, GlcII H-1), 4.90 (1H, *d*, $J = 7.6$ Hz, Gal H-1), 5.06 (1H, *m*, H-16), 5.28 (1H, *d*, $J = 7.8$ Hz, GlcI H-1). ^{13}C NMR (pyridine-d_5, 400 MHz): see Tables 1 and 2.

4.3.6. Elephanoside E (*10*)

White amorphous powder, $[\alpha]_D^{23} = -24.8$ (*c* 0.22, MeOH). FABMS (negative ion mode): *m/z* 1097 $[M-H]^-$, 935 $[M-Glc\ (162)-H]^-$, 773 $[M-Glc\ (162)\times 2-H]^-$. HRESIMS (negative ion mode): *m/z* 1097.5355 $[M(C_{51}H_{86}O_{25})-H]^-$ (calcd. for 1097.5385). ^{1}H NMR (pyridine-d_5, 500 MHz): δ 0.89 (1H, *m*, H-7a), 0.96 (3H, *s*, Me-19), 0.97 (3H, *d*, $J = 6.3$ Hz, Me-27), 1.13 (3H, *s*, Me-18), 1.17 (2H, *m*, H-7b, 14), 1.58 (1H, *m*, H-15a), 1.61 (3H, *d*, $J = 6.8$ Hz, Me-21), 1.70 (1H, *m*, H-24a), 1.19 (1H, *m*, H-9), 1.51 (1H, *m*, H-8), 1.90 (1H, *m*, H-25), 2.02 (1H, *m*, H-24b), 2.07 (2H, *m*, H-23a,b), 2.12 (1H, *m*, H-15b), 2.48 (1H, *m*, H-20), 2.21 (1H, *m*, H-5), 2.34 (1H, *t*, $J = 7.5$ Hz, H-17), 3.54 (1H, *dd*, $J = 4.5$, 10.5 Hz, H-12), 3.61 (1H, *dd*, $J = 6.0$, 9.5 Hz, H-26a), 3.91 (1H, *m*, H-26b), 4.15 (1H, *t*, $J = 9.0$ Hz, GlcI H-4), 4.28 (1H, *m*, H-3), 4.79 (1H, *br s*, Gal H-4), 4.84 (1H, *d*, $J = 7.5$ Hz, Gal H-1), 4.80 (1H, *d*, $J = 8.0$ Hz, GlcII H-1), 5.06 (1H, *m*, H-16), 5.37 (1H, *d*, $J = 7.8$ Hz, GlcIII H-1), 5.55 (1H, *d*, $J = 7.7$ Hz, GlcI H-1). ^{13}C NMR (pyridine-d_5, 500 MHz): see Tables 1 and 2.

4.3.7. Acid hydrolysis of compounds *4*, *8*, and *10*

Compounds **4**, **8** or **10** (10 mg each) in 2 M HCl–dioxane (1:1, 2 ml) were individually heated at 95 °C for 6 h. The reaction mixture was diluted with H_2O and extracted with $CHCl_3$. The $CHCl_3$ phases of **4**, **8** and **10** afforded (25*R*)-5β-spirostan-3β-ol (smilagenin) (Agrawal et al., 1985), (25*R*)-5β-spirostan-3β-ol-12-one (gloriogenin) (Nakano et al., 1991b), and 12β-hydroxysmilagenin (Nakano et al., 1991a), respectively, which were identified by direct co-TLC comparison with authentic samples [petroleum ether:-ethyl acetate (7:3), R_f 0.7, 0.4, and 0.2, respectively] and by comparing their NMR data with published values. The aqueous layer was passed through an Amberlite IRA-401 (OH^- form) column, and the eluate was concentrated to dryness to give monosaccharide mixtures for sugar detection by HPTLC [*n*-BuOH–*i*-PrOH–H_2O (10:5:4, homogenous), R_f values of glucose, xylose and galactose were 0.25, 0.40, 0.27, respectively]. The determination of the absolute configuration of the sugars followed the procedure described in our previous paper (Zhang et al., 2006). The retention times of the corresponding trimethylsilylated L-cysteine adducts of D-glucose, D-xylose, D-galactose, L-glucose, L-xylose, and L-galactose were 17.96, 13.06, 18.75, 18.53, 13.72 and 19.31 min, respectively.

4.3.8. Enzymatic hydrolysis of compounds *6*, *7*, and *9*

A solution of **6** (10 mg), **7** (10 mg) or **9** (20 mg) in H_2O (2 ml, 2 ml, 4 ml, respectively) was hydrolyzed with β-glucosidase from almonds (EC No. 2325897) (20 mg, 20 mg, 40 mg, respectively) at 37 °C for 48 h, respectively. The reaction mixtures were partitioned between *n*-BuOH and H_2O. The *n*-BuOH extracts of **6** and **7** were identical with their corresponding spirostanol type compounds **2** and **3**, respectively, which were determined by direct co-TLC detection [$CHCl_3$–MeOH–H_2O (7:3:0.5), R_f 0.35 and 0.32, respectively]. The *n*-BuOH extract of **9** (10 mg) was subjected on a silica gel CC to afford a spirostanol saponin (6 mg), which was identified to be (25*R*)-5β-spirostan-3β,12β-diol-3-*O*-β-D-glucopyranosyl(1 → 2)-β-D-galactopyranoside (YS-XI) by comparison of its NMR spectra with reported data (Nakano et al., 1991a). Co-TLC analysis of the H_2O layer indicated the presence of glucose for all of the compounds **6**, **7**, and **9** (iso-propanol–MeOH–H_2O, 25:1:2, R_f 0.6).

4.3.9. Antifungal and antibacterial bioassays

The testing organisms including *Candida albicans* ATCC 90028, *Cryptococcus neoformans* ATCC 90113, *A. fumigatus* ATCC 90906, *E. coli* ATCC 35218, *P. aeruginosa* ATCC 27853, and *M. intracellulare* ATCC 23068 were obtained from the American Type Culture Collection (Manassas, VA). The former five microbes were performed using a modified version of the NCCLS methods (NCCLS, 2000,2002), while the last one was tested using a modified method of Franzblau et al. (1998). The minimum inhibitory concentration (MIC) is defined as the lowest test concentration that allows no detectable growth, while the minimum fungicidal concentration (MFC) is defined as the lowest test concentration that kills the organism.

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References

Agrawal, P.K., 2004. Dependence of ^{1}H NMR chemical shifts of geminal protons of glycosyloxy methylene (H_2-26) on the orientation of the 27-methyl group of furostane-type steroidal saponins. Magn. Reson. Chem. 42, 990–993.

Agrawal, P.K., Jain, D.C., Gupta, R.K., Thakur, R.S., 1985. Carbon-13 NMR spectroscopy of steroidal sapogenins and steroidal saponins. Phytochemistry 24, 2479–2496.

Anthony, N.B., Balog, J.M., Staudinger, F.B., Walker, R.D., Huff, W.E., 1994. Environmental variability and incidence of ascites. Poult. Sci. 73, 801–809.

Bahuguna, S., Kishor, N., Sati, O.P., Sati, S.P., Sakakibara, J., Kaiya, T., 1991. A new spirostanol glycoside from *Yucca aloifolia*. J. Nat. Prod. 54, 863–865.

Balog, J.M., Anthony, N.B., Wall, C.V., Walker, R.D., Rath, N.C., Huff, W.E., 1994. Blood variables, ascites scores and body and organ weights. Poult. Sci. 73, 810–816.

Benidze, M.M., Dzhikiya, O.D., Vugalter, M.M., Pkheidze, T.A., Kemertelidze, E.P., 1984. New steroidal saponins from *Yucca aloifolia* leaves. Khim. Prir. Soedin., 744–747.

Benidze, M.M., Pkheidze, T.A., Kemertelidze, E.P., 1991. Spirostanol glycosides of *Yucca gloriosas*. Khim. Prir. Soedin., 295–296.

Cheeke, P.R., 1999. Actual and potential applications of *Yucca schidigera* and *Quillaia saponins* in human and animal nutrition. Proc. Am. Soc. Anim. Sci., 1–10.

Dragalin, I.P., Kintia, P.K., 1975. Steroidal saponins of *Yucca filamentosa*. Phytochemistry 14, 1817–1820.

Franzblau, S.G., Witzig, R.S., McLaughlin, J.C., Torres, P., Madico, G., Hernandez, A., Degnan, M.T., Cook, M.B., Quenzer, V.K., Ferguson, R.M., Gilman, R.H., 1998. Rapid, low-technology MIC determination with clinical Mycobacterium tuberculosis isolates by using the microplate Alamar Blue assay. J. Clin. Microbiol. 36, 362–366.

Giorgadze, T., Gurielidze, K., 1991. Isolation of furostanol glycosides from *Yucca gloriosa* L. leaf epidermal layer. Soobshch. Ajad. Nauk Gruz. 144, 81–83.

Gonzalez, A.G., Barriera, R.F., Gonzalez, R.H., Salazar, J.A., Lopez, S.E., 1972. New natural sources of steroidal sapogenins. VII. Gloriogenin and 12β-hydroxysmilagenin, two new steroidal sapogenins from *Yucca gloriosa*. Anal. Quim. 68, 309–317.

Kemertelidze, E., Benidze, M., 2001. Steroidal glycosides of *Yucca gloriosa* L. and their influence on plant growth. Bull. Georgia Acad. Sci. 164, 91–93.

Kemertelidze, E.P., Pkheidze, T.A., 1972. Tigogenin from *Yucca gloriosa* as a possible raw material for the synthesis of steroid hormone preparations. Khim. Farm. Zh. 6, 44–47.

Kishor, N., Sati, O.P., 1990. A new molluschicidal spirostanol glycoside of *Yucca aloifolia*. J. Nat. Prod. 53, 1557–1559.

Kishor, N., Sati, O.P., Sakakibara, J., Kaiya, T., 1992. A spirostanol glycoside from *Yucca aloifolia*. Phytochemistry 31, 706–707.

Kiyosawa, S., Hutoh, M., 1968. Detection of proto-type compounds of diosgenin- and other spirostanol-glycosides. Chem. Pharm. Bull. 16, 1162–1164.

Li, X.C., Wang, D.Z., Yang, C.R., 1990. Steroidal saponins from *Chlorophytum malayense*. Phytochemistry 29, 3893–3898.

Li, X.C., Yang, C.R., Ichikawa, M., Matsuura, H., Kasai, R., Yamasaki, K., 1992. Steroid saponins from *Polygonatum kingianum*. Phytochemistry 31, 3559–3563.

Miyakoshi, M., Tamura, Y., Masuda, H., Mizutani, K., Tanaka, O., Ikeda, T., Ohtani, K., Kasai, R., Yamasaki, K., 2000. Antiyeast steroidal saponins from *Yucca schidigera* (Mohave yucca), a new anti-food-deterioration agent. J. Nat. Prod. 63, 332–338.

Nakano, K., Matsuda, E., Tsurumi, K., Yamasaki, T., Murakami, K., Takaishi, Y., Tomimatsu, T., 1988. The constituents of *Yucca gloriosa*. Phytochemistry 27, 3235–3239.

Nakano, K., Yamasaki, T., Imamura, Y., Murakami, K., Takaishi, Y., Tomimatsu, T., 1989. The steroidal glycosides from the stem of *Yucca gloriosa*. Phytochemistry 28, 1215–1217.

Nakano, K., Hara, K., Murakami, K., Takaishi, Y., Tomimatsu, T., 1991a. 12-Hydroxy steroidal glycosides from the stem of *Yucca gloriosa*. Phytochemistry 30, 1993–1995.

Nakano, K., Midzuta, Y., Hara, Y., Murakami, K., Takaishi, Y., Tomimatsu, T., 1991b. 12-Keto steroidal glycosides from the stem of *Yucca gloriosa*. Phytochemistry 30, 633–636.

NCCLS, 2000a. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically M7-A5. National Committee on Clinical Laboratory Standards 20 (2).

NCCLS, 2000b. Susceptibility testing of mycobacteria, nocardia, and other aerobic actinomycetes; tentative standard – second edition, M24-T2. National Committee on Clinical Laboratory Standards 20 (26).

NCCLS, 2002a. Reference method for broth dilution antifungal susceptibility testing of yeasts; approved standard M27-A2. National Committee on Clinical Laboratory Standards 22, 15.

NCCLS, 2002b. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi; approved standard; M38-A. National Committee on Clinical Laboratory Standards 22, 16.

Oleszek, W., Sitek, M., Stochmal, A., Piacente, S., Pizza, C., Cheeke, P., 2001a. Steroidal saponins of *Yucca schidigera* Roezl. J. Agric. Food Chem. 49, 4392–4396.

Oleszek, W., Sitek, M., Stochmal, A., Piacente, S., Pizza, C., Cheeke, P., 2001b. Resveratrol and other phenolics from the bark of *Yucca schidigera* Roezl. J. Agric. Food Chem. 49, 747–752.

Piacente, S., Pizza, C., Oleszek, W., 2005. Saponins and phenolics of *Yucca schidigera* Roezl: chemistry and bioactivity. Phytochem. Rev. 4, 177–190.

Pkheidze, T.A., Kikoladze, V.S., Kemertelidze, E.P., 1981. Steroidal sapgenin of *Yucca gloriosa* leaves. Khim. Prir. Soedin. 805.

Pkheidze, T.A., Benidze, M.M., Kemertelidze, E.P., 1985. Steroid sapogenins of *Yucca gloriosa* inflorescences. Rast. Resursy 21, 452–455.

Pkheidze, T.A., Gvazava, L.N., Kemertelidze, E.P., 1991a. Luvigenin and hecogenin from leaves of *Yucca gloriosa*. Khim. Prir. Soedin. 432.

Pkheidze, T.A., Gvazava, L.N., Alaniya, M.D., Kemertelidze, E.P., 1991b. Ketosapogenins from leaves of *Yucca gloriosa*. Khim. Prir. Soedin., 244–246.

Plock, A., Beyer, G., Hiller, K., Grundemann, E., Krause, E., Nimtz, M., Wray, V., 2001. Application of MS and NMR to the structure elucidation of complex sugar moieties of natural products: exemplified by the steroidal saponin from *Yucca filamentosa* L.. Phytochemistry 57, 489–496.

Skhirtladze, A., Plaza, A., Montoro, P., Benidze, M., Kemertelidze, E., Pizza, C., Piacente, S., 2006. Furostanol saponins from *Yucca gloriosa* L. Rhizomes. Biochem. Syst. Ecol. 34, 809–814.

Varanda, E.M., 1984. *Yucca* L. species, cultivated in Brazil, as sources of steroidal sapogenins. Rev. Bras. Bot. 7, 37–39.

Wang, Y.H., 2006. Studies on active components against Tobacco Mosaic Virus and chemical constituents of several plants, Ph.D. Dissertation, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, PR China, p. 5.

Yang, C.R., Zhang, Y., Jacob, M.R., Khan, S.I., Zhang, Y.J., Li, X.C., 2006. Antifungal activity of C-27 steroidal saponins. Antimicrob. Agent Chemother. 50, 1710–1714.

Zhang, Y., Li, H.Z., Zhang, Y.J., Jacob, M.R., Khan, S.I., Li, X.C., Yang, C.R., 2006. Atropurosides A–G, new steroidal saponins from *Smilacina atropurpurea*. Steroids 71, 712–719.