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CHEMICAL CONSTITUENTS AND CYTOTOXICITY OF *ASPIDISTRA LETREAE*

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Abstract. A phytochemical investigation of whole *Aspidistra letreae* plants led to the isolation of 2*H*-chromen-2-one (**1**), α-tocopherol (**2**), (*E*)-phytol (**3**), asparenydiol (**4**) and (25*S*)-spirost-1β,3α,5β-triol (**5**). Their structures were determined on the basis of NMR spectral evidences and in comparison with the reported data. Of these, asparenydiol (**4**) was isolated from the genus *Aspidistra* for the first time. This is also the first report on the separation and structural determination of (25*S*)-spirost-1β,3α,5β-triol (**5**) as a pure compound. The methanol extract from the whole plants of *Aspidistra letreae* exhibits moderate cytotoxicity against the LU-1, HeLa, MDA-MB-231, Hep-G2, and MKN-7 human cancer cell lines with IC_{50} values ranging from 52.58 ± 3.65 to 64.78 ± 4.89 μg/mL.

Keywords: Asparagaceae, *Aspidistra letreae*, asparenydiol, (25*S*)-spirost-1β,3α,5β-triol, cytotoxicity

1 Introduction

Since *Aspidistra* was discovered in 1822 as a genus that belongs to the Asparagaceae family, more than 150 species have been identified in this family worldwide. They are mainly distributed in the subtropical regions of Asia, from Assam (India) to the southern part of Japan in the east and the Malaysia Peninsula in the south, and play a central role in plant species diversity in Southern China and Northern Vietnam [1]. *Aspidistra* species have been used as folk medicine in many countries to make tonics, expectorants, and diuretics [2], as well as to treat fractures, congestion, snakebites [3], and abscesses, traumatic injuries, pain, and coughs [4]. Previous phytochemical studies of the genus have mainly focused on three species: *A. elatior*, *A. typica*, and *A. sichuanensis*. The compounds isolated from this genus (lectins, homoisoflavones, steroidal saponins) show broad pharmacological activities, including antifungal [5], antiviral, antitumor [6], antibacterial [7], inhibition of HIV viral replication [3], and cytotoxicity [8].

Aspidistra letreae Aver. is a new species in the genus *Aspidistra* that was recently discovered by Averyanov et al. [9]. This plant is a terrestrial perennial herb distributed in Central Vietnam. Herein, the isolation and structural determination of compounds **1**–**5**, as well as the cytotoxicity of isolates and methanol extract from the whole plants of *A. letreae*, are described.

2 Material and methods

2.1 Plant material

Whole *A. letreae* plants were collected from Quang Tri province, Vietnam (N16°57'53.0" E106°52'18.5")

in January 2019, and were identified by one of the authors (Mr. Anh Tuan Le). A voucher specimen (VL-TD-01) was deposited at the Faculty of Pharmacy, University of Medicine and Pharmacy, Hue University, Vietnam.

2.2 General experiment procedures

NMR spectra were recorded on a Bruker Avance 500 spectrometer (Bruker, MA, USA), with TMS as an internal reference. Column chromatography was performed by using silica gel (60 N, spherical, neutral, 40–50 μm, Kanto Chemical Co., Inc., Tokyo, Japan), YMC RP-18 (Fuji Silysia Chemical Ltd., Kasugai, Aichi, Japan). Analytical TLC was performed on pre-coated silica gel 60F_{254} and RP-18 F_{254} plates (0.25 or 0.50 mm thickness, Merck KGaA, Darmstadt, Germany).

Cell culture flasks and 96-well plates were from Corning Inc. (Corning, NY, USA). An ELISA Plate Reader (Bio-Rad, California, USA) was used to measure the absorbance of the cells in the *in vitro* cytotoxicity assay.

2.3 Extraction and isolation

The dried *A. letreae* powder (2.015 kg) was extracted three times with 8 L of MeOH at room temperature, to yield 370 g of a dark solid extract. This extract was suspended in water and successively partitioned with *n*-hexane and ethyl acetate (EtOAc) (three times each, 2.5 L) to obtain the *n*-hexane (AH, 224.3 g), EtOAc (AE, 43.0 g) and water (AW, 91.2 g) portions, after removal of the solvents *in vacuo*.

The AH extract (224.3 g) was chromatographed on a silica gel column, eluted with an *n*-hexane/cetone gradient system (100:0, 40:1, 20:1, 10:1, 5:1, 1:1, 0:100 v/v, each 1 L) to obtain 7 sub-fractions (AH1–AH7). Fraction AH1 (3.4 g) produced a precipitate of crude crystals, which was recrystallized in *n*-hexane to yield **1** (1.5 g) as a major component in this species. Fraction AH2 (5.7 g) was chromatographed on a silica gel column, eluted with an *n*-hexane/EtOAc (10:1, v/v) mixture to give 13 sub-fractions (AH2.1–AH2.13). Fraction AH2.2 (120 mg) was chromatographed on a YMC RP-18 column, eluted with an acetone/water (20:1, v/v) mixture to yield **2** (10.4 mg). Fraction AH2.5 (440 mg) was loaded onto a YMC RP-18 column, eluted with an acetone/MeOH/water (5:5:1, v/v) mixture to yield **3** (7.2 mg). Fraction AH2.10 (190 mg) was subjected to a YMC RP-18 column, eluted with a MeOH/water (2:1, v/v) mixture to yield **4** (1.3 mg).

The AE extract was separated on a silica gel column, eluted with an CH_2Cl_2/MeOH gradient system (100:0, 40:1, 20:1, 10:1, 5:1, 2:1, 1:1, 0:100 v/v, each 1.0 L) to give 8 sub-fractions (AE1–AE8). Fraction AE6 (5.7 g) was chromatographed on a silica gel column, eluted with an *n*-hexane/acetone/MeOH (4:1:0.2, v/v) mixture to obtain 8 smaller fractions (AE6.1–AE6.8). The crude precipitate from fraction AE6.2 was washed with MeOH to afford **5** (1.8 mg).

2.4 Sulforhodamine B assay for evaluating cytotoxic activity

Stock cultures were grown in T-75 flasks, containing 10 mL of Dulbecco's modified eagle medium (DMEM) with 2 mM L-glutamine, 1.5 g/L sodium bicarbonate, and 10% fetal bovine serum (FBS). The media were changed at 48-hour intervals. The cells were dissociated with 0.05% trypsin-EDTA, sub-cultured every 3–5 days at a ratio of 1:3, and incubated at 37 °C under a humidified 5% carbon dioxide atmosphere. The human cancer cell lines, LU-1 (lung adenocarcinoma), HeLa (cervical carcinoma), MDA-MB-231 (breast adenocarcinoma), Hep-G2 (liver hepatocellular carcinoma), and MKN-7 (gastric adenocarcinoma) were cultivated in a humidified atmosphere of 5% CO_2 at 37 °C for 48

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h. Cell viability was examined by using the sulforhodamine B (SRB) method for cell density determination, which is based on the measurement of cellular protein content [10].

The viable cells were seeded in the growth medium (180 μL) in 96-well microplates (4×10^4 cells per well) and allowed to attach overnight. Test samples were carefully added into each well of the 96-well plates, and the cultivation continued under the same conditions for another 72 h. Thereafter, the medium was removed, and the remaining cell monolayers were fixed with cold 20% (w/v) trichloroacetic acid for 1 h at 4 °C. The fixed cells were stained with a 1X SRB staining solution at room temperature for 30 min, and then the unbound dye was removed by washing repeatedly with 1% (v/v) acetic acid. The protein-bound dye was dissolved in a 10 mM Tris base solution for the optical density determination at 515 nm on an ELISA Plate Reader (Bio-Rad). As a blank sample, DMSO 10% was used, while ellipticine was used as a positive control. The cytotoxicity was measured at doses of 100, 20, 4, and 0.8 μg/mL, and the half-maximal inhibitory concentration (IC_{50}) was calculated by using the program TableCurve, Version 4.0. All experiments were prepared in triplicate. The inhibition rate (IR) of cells was calculated according to the following formula

$$IR\ \% = \{100\ \% - [(A_t - A_0)/(A_c - A_0)] \times 100\}$$

where A_t is the average optical density value at day 3; A_0 is the average optical density value at time-zero; A_c is the average optical density value of the blank DMSO control sample.

3 Results and discussion

Compound **1** is obtained as a white powder. The ^{1}H NMR spectrum of **1** exhibits characteristic signals of four aromatic protons belonging to a 1,2-disubstituted benzene ring at δ_H 7.54 (*m*, H-7), 7.51 (*dd*, *J* = 1.6, 8.8 Hz, H-5), 7.33 (*d*, *J* = 8.4 Hz, H-8) and 7.29 (*m*, H-6). In addition, other aromatic protons are observed at δ_H 7.73 (*d*, *J* = 9.6 Hz, H-4) and 6.42 (*d*, *J* = 9.6 Hz, H-3). The ^{13}C NMR and HSQC spectra of **1** show nine signals, including six methine (δ_C 116.7, 116.9, 124.5, 127.9, 131.9, 143.5) and three quaternary carbons (δ_C 118.9, 154.0, 160.8) (Table 1). The HMBC correlations of H-3/H-4 to C-2 (δ_C 160.8)/C-10 (δ_C 118.9) and H-4 to C-9 (δ_C 154.0) lead us to construct the δ-lactone ring in **1**. This residue is linked to a benzene ring at C-9/C-10 via HMBC correlations between H-5/H-6/H-8 and C-10 and between H-5/H-7/H-8 and C-9. According to the aforementioned observations, compound **1** is determined to be 2*H*-chromen-2-one (known as coumarin) (Fig. 1) [11].

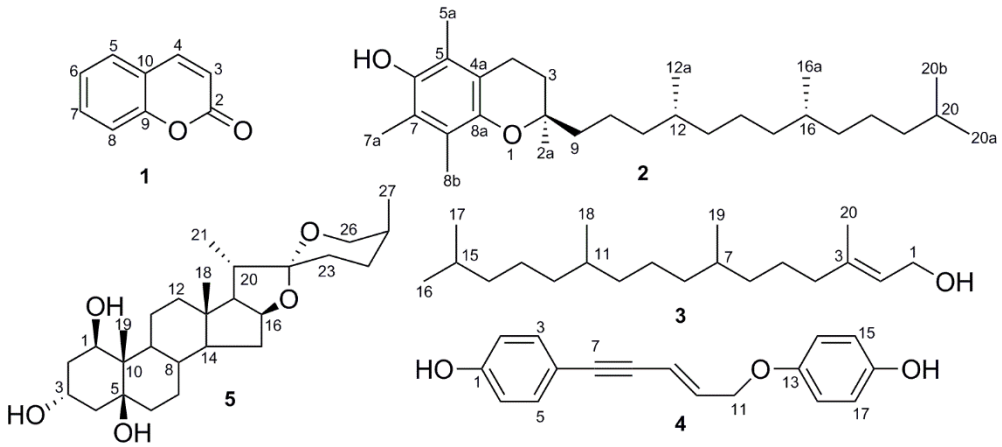

Fig. 1. Chemical structures of isolated compounds **1–5**

Compound **2** is obtained as a pale yellow oil. The ^{1}H NMR spectrum shows the presence of four angular methyl groups [δ_H 2.16 (3H), 2.11 (6H), 1.23 (3H)], four secondary methyl groups [δ_H 0.85 (6H, *d*, *J* = 6.5 Hz), 0.87 (6H, *d*, *J* = 6.5 Hz)], and one hydroxy group [δ_H 4.18 (*br. s*)]. The analysis of the ^{13}C NMR spectrum of **2** demonstrates six sp^2 carbons (δ_C 117.4, 118.5, 121.1, 122.6, 144.6, 145.6) and one oxygenated sp^3 carbon (δ_C 74.6). The appearance of six sp^2 carbons and the lack of olefinic and/or aromatic protons imply that compound **2** has a hexa-substituted benzene ring in the molecule. Meanwhile, the carbon signals (δ_C 19.7–39.9) correspond to a saturated hydrocarbon chain. According to the above evidence, compound **2** is verified to be α-tocopherol [12].

Compound **3** is isolated as a colorless oil. The ^{1}H NMR of **3** shows typical signals of one olefinic proton [δ_H 5.41 (*qt*, *J* = 7.0, 1.5 Hz)], one oxymethylene group [δ_H 4.15 (*d*, *J* = 7.0 Hz)], and five methyl groups [δ_H 0.86–0.88 (H_3-16–H_3-19) and 1.67 (*s*, H_3-20)]. The ^{13}C NMR contains twenty carbon resonances. Among those, the signals at δ_C 123.1 (C-2), 140.3 (C-3) are assigned to a tri-substituted double bond, whereas the presence of an oxygenated methylene group is deduced by a carbon signal at δ_C 59.5 (C-1). Therefore, compound **3** is identified to be (*E*)-phytol [13].

Compound **4** is obtained as a pale yellow powder. The appearance of two *para*-disubstituted benzene rings is derived from signals at δ_H 7.26 (2H, *d*, *J* = 8.5 Hz), 6.82 (2H, *d*, *J* = 8.5 Hz), 6.75 (2H, *d*, *J* = 8.5 Hz) and 6.73 (2H, *d*, *J* = 8.5 Hz). Moreover, the signals of two *trans* olefinic protons [δ_H 6.28 (*td*, *J* = 16.0, 5.5 Hz), 6.04 (*d*, *J* = 16.0 Hz)] and two oxymethylene protons [δ_H 4.56 (2H, *d*, *J* = 5.5 Hz)] are found in the ^{1}H NMR spectrum. The analysis of the ^{13}C NMR and HSQC spectra of **4** reveals one oxymethylene (δ_C 69.7), ten sp^2 methine [δ_C 116.5 (2C), 116.9 (2C), 117.1 (2C), 134.0 (2C), 113.3, 138.4], and six quaternary carbons (δ_C 86.2, 91.6, 115.3, 152.7, 153.3, 159.1). Notably, three down-field signals [δ_C 152.7, 153.3, 159.1] are assigned to oxygenated sp^2 carbons. The HMBC correlations are seen between H-11 (δ_H 4.56) and C-9 (δ_C 113.3)/C-10 (δ_C 138.4)/C-13 (δ_C 153.3), leading to the assignment of a double bond at Δ^9 as well as *para*-quinol moiety at C-11. Similarly, the triple bond at Δ^7 is confirmed by the HMBC correlations from H-3/H-5 (δ_H 7.26) to C-7 (δ_C 91.6). Consequently, compound **4** is determined as 4-[(3*E*)-5-(4-hydroxyphenoxy)-pent-3-en-1-ynyl]phenol and is given a trivial name as asparenydiol [14]. This compound was previously isolated from several *Asparagus* species, such as *A. officinalis* [14] and *A. cochinchinensis* [15]. However, this is the first report on the isolation of asparenydiol from the *Aspidistra* genus.

Table 1. 1H and ^{13}C NMR data of compounds **1–4** [δ (ppm), *J* (Hz)]

Position	**1** (in $CDCl_3$)		**2** (in $CDCl_3$)		**3** (in $CDCl_3$)		**4** (in CD_3OD)	
	δ_C[a]	δ_H[b]	δ_C[c]	δ_H[d]	δ_C[c]	δ_H[d]	δ_C[c]	δ_H[d]
1	–	–	–	–	59.5	4.15 *d* (7.0)	159.1	–
2	160.8	–	74.6	–	123.1	5.41 *qt* (7.0, 1.5)	116.5	6.75 *d* (8.5)
2a			23.8	1.23 *s*				
3	116.7	6.42 *d* (9.6)	31.6	1.78 *m*	140.3	–	134.0	7.26 *d* (8.5)
4	143.5	7.73 *d* (9.6)	20.8	2.60 *t* (7.0)	39.9	1.99 *m*	115.3	–
4a			117.4	–				
5	127.9	7.51 *dd* (8.8, 1.6)	118.5	–	25.2	1.39*	134.0	7.26 *d* (8.5)

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Position	**1** (in $CDCl_3$)		**2** (in $CDCl_3$)		**3** (in $CDCl_3$)		**4** (in CD_3OD)	
	δ_C[a]	δ_H[b]	δ_C[c]	δ_H[d]	δ_C[c]	δ_H[d]	δ_C[c]	δ_H[d]
5a			11.3	2.11 *s*				
6	124.5	7.29 *m*	144.6	–	36.7	1.32*	116.5	6.75 *d* (8.5)
6–OH			–	4.18 *br. s*				
7	131.9	7.54 *m*	121.1	–	32.7	1.39*	91.6	–
7a			12.2	2.16 *s*				
8	116.9	7.33 *d* (8.4)	122.6	–	37.5	1.07*	86.2	–
8a			145.6	–				
8b			11.8	2.11 *s*				
9	154.0	–	39.9	1.54 *m*	24.5	1.23*	113.3	6.04 *d* (16.0)
10	118.9	–	21.1	1.43 *m*, 1.38 *m*	37.4	1.07*	138.4	6.28 *td* (16.0, 5.5)
11			37.3	1.08 *m*, 1.25 *m*	32.9	1.39*	69.7	4.56 *d* (5.5)
12			32.8	1.39 *m*	37.3	1.07*	–	–
12a			19.7	0.85 *d* (6.5)				
13			37.4	1.08 *m*, 1.25 *m*	24.8	1.28*	153.3	–
14			24.5	1.18 *m*	39.4	1.16*	117.1	6.82 *d* (8.5)
15			37.5	1.08 *m*, 1.25 *m*	28.0	1.52 *m*	116.9	6.73 *d* (8.5)
16			32.7	1.39 *m*	22.7	0.88*	152.7	–
16a			19.8	0.85 *d* (6.5)				
17			37.5	1.08 *m*, 1.25 *m*	22.6	0.88*	116.9	6.73 *d* (8.5)
18			24.8	1.28 *m*	19.8	0.86*	117.1	6.82 *d* (8.5)
19			39.4	1.13 *m*	19.7	0.86*		
20			28.0	1.53 *m*	16.2	1.67 *s*		
20a			22.7	0.87 *d* (6.5)				
20b			22.6	0.87 *d* (6.5)				

[a]100 MHz, [b]400 MHz, [c]125 MHz, [d]500 MHz, *overlapped signals.

Compound **5** is afforded as a white amorphous powder. The ^{1}H NMR spectrum of **5** (Table 2) shows typical signals corresponding to two angular methyl groups [δ_H 0.71 (*s*, H_3-18), 1.03 (*s*, H_3-19)], two secondary methyl groups [δ_H 0.93 (*d*, *J* = 7.0 Hz, H_3-21), 1.01 (*d*, *J* = 7.0 Hz, H_3-27)], three oxymethine groups [δ_H 3.74 (*br. s*, H-1), 4.04 (*m*, H-3), 4.28 (*m*, H-16)], and one oxymethylene group [δ_H 3.21 (*br. d*, *J* = 11.0 Hz, H-26a), 3.77 (*dd*, *J* = 11.0, 2.5 Hz, H-26b)]. The ^{13}C NMR shows 27 signals. In combination with an HSQC experiment, these signals are classified into four methyl, ten methylene, nine methine, and four quaternary carbons. Moreover, the ^{13}C NMR spectrum contains the signals corresponding to one dioxygenated carbon (δ_C 108.8), five oxygenated sp^3 carbons (δ_C 61.7, 64.2, 74.1, 75.9, 80.2). The above data of **5** are indicative of a spirostanol. All proton and carbon signals of **5** are evidenced by the analysis of 2D-NMR, including HSQC, HMBC, and COSY (Fig. 2). The HMBC correlations of H_3-19 (δ_H 1.03) to C-1 (δ_C 74.1)/C-5 (δ_C 75.9)/C-10 (δ_C 41.7), 1-OH (δ_H 5.11) to C-1/C-10, and 5-OH (δ_H 4.92) to C-4 (δ_C 42.1)/C-5/C-6 (δ_C 35.2)/C-10 confirm the attachment of two hydroxyl groups at C-1 and C-5, respectively. The third hydroxyl group is located at

C-3 (δ_C 61.7) via the correlations from 3-OH (δ_H 4.35) to C-2 (δ_C 37.0)/C-3/C-4. These findings are further supported by the COSY cross-peaks of H-1 (δ_H 3.74) to 1-OH (δ_H 5.11)/H-2 (δ_H 1.81, 1.43), H-3 (δ_H 4.04) to H-2/H-4 (δ_H 1.92, 1.47), and 3-OH to H-3. The planar structure of **5** is thus determined as spirost-1,3,5-triol.

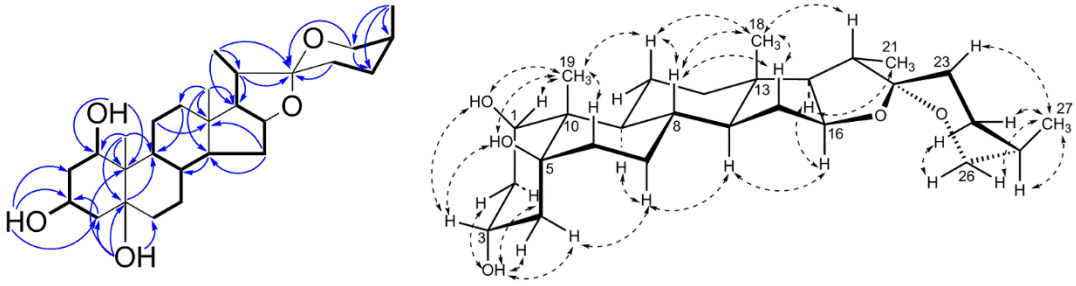

Fig. 2. Key HMBC ($^1H \rightarrow ^{13}C$, arrows), COSY (bold lines) and NOESY (dashed arrows) correlations of **5**

Table 2. NMR data of compound **5** and reference compounds [δ (ppm), *J* (Hz)]

Position	(25*R*,*S*)–Spirost–1β,3α,5β–triol[#]		**5** (in DMSO–d_6)	
	(25*R*) isomer	(25*S*) isomer		
	δ_C[a]	δ_C[a]	δ_C[a]	δ_H[b]
1	75.9	75.9	74.1	3.74 *br. s*
1–OH				5.11 *d* (6.0)
2	38.9	38.9	37.0	1.81*, 1.43 *m*
3	63.7	63.7	61.7	4.04 *m*
3–OH				4.35 *d* (5.5)
4	44.0	44.0	42.1	1.92 *br d* (12.5), 1.47*
5	77.4	77.4	75.9	–
5–OH				4.92 *s*
6	36.7	36.7	35.2	1.59 *m*, 1.17 *br d* (13.0)
7	29.2	29.2	28.0	1.47*, 0.98 *m*
8	35.4	35.4	34.3	1.55 *m*
9	45.8	45.8	44.3	1.25*
10	43.3	43.3	41.7	–
11	21.7	21.7	20.4	1.25*
12	40.3	40.3	39.6	1.65*, 1.08 *m*
13	41.1	41.1	40.0	–
14	56.6	56.6	55.4	1.12*
15	32.5	32.5	31.4	1.90*, 1.12*
16	81.5	81.6	81.2	4.28 *m*
17	63.4	63.2	61.7	1.66*

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Position	(25*R*,*S*)–Spirost–1β,3α,5β–triol[#]		**5** (in DMSO–d_6)	
	(25*R*) isomer	**(25*S*) isomer**		
	δ_C[a]	δ_C[a]	δ_C[a]	δ_H[b]
18	17.0	17.0	16.1	0.71 *s*
19	13.7	13.7	12.5	1.03 *s*
20	42.4	42.9	41.5	1.76 *m*
21	15.4	15.2	14.4	0.93 *d* (7.0)
22	109.6	110.1	108.8	–
23	**32.2**	**26.8**	**25.3**	1.90*, 1.35 *m*
24	**29.6**	**26.5**	**25.5**	1.81*, 1.25*
25	**31.0**	**27.9**	**26.4**	1.65*
26	**67.3**	**65.5**	**64.2**	3.77 *dd* (2.5, 11.0); 3.21 *br. d* (11.0)
27	**17.7**	**16.6**	**15.9**	1.01 *d* (7.0)

[#]Data (in C_5D_5N) taken from ref. [16], [a]125 MHz, [b]500 MHz, *overlapped signals.

The stereochemistry of **5** was determined on the basis of the NOESY experiment (Fig. 2). The NOESY correlations of H-3/H_3-19 to 1-OH/5-OH and 3-OH to H-2/H-4 establish a *cis*-fused juncture of the A/B ring in **5** as well as the β-orientation of H-3, 1-OH and 5-OH [16, 17]. Whilst, the NOESY correlations between H_3-27 (δ_H 1.01) and H-23β (δ_H 1.35)/H-24β (δ_H 1.25)/H-26β (δ_H 3.77) confirm the β-axial orientation of Me-27 in the chair conformation of the F ring. The *S* configuration is thus suggested for chiral center C-25. This is strengthened by comparing the difference in the δ_H values of two protons at C-26 ($\Delta_{ea} = \delta_{H\text{-}26e} - \delta_{H\text{-}26a}$ = 0.56) of **5** with those of two isomers (25*S*: $\Delta_{ea} > 0.35$, 25*R*: $\Delta_{ea} < 0.20$) [18]. As a consequence, compound **5** is determined to be (25*S*)-spirost-1β,3α,5β-triol. It is worth noting that (25*S*)-spirost-1β,3α,5β-triol (**5**) is isolated and determined as the pure form for the first time. In a previous study, this compound was described as a mixture of (25*R*,*S*)-spirost-1β,3α,5β-triol from the rhizome of *Tupistra chinensis* [16].

Table 3. Cytotoxicity of methanol extract and isolated compounds against cancer cell lines

Cell lines	IC_{50}[a] (μg/mL)			
	Methanol extract	**1**	**2**	Ellipticine[b]
LU-1	56.23 ± 3.67	>100	>100	0.31 ± 0.07
HeLa	52.58 ± 3.65	>100	>100	0.43 ± 0.05
MDA-MB-231	62.96 ± 2.64	>100	>100	0.48 ± 0.08
Hep-G2	54.73 ± 2.09	>100	>100	0.34 ± 0.07
MKN-7	64.78 ± 4.89	>100	>100	0.39 ± 0.02

[a] IC_{50} (concentration that inhibits 50% of cell growth), [b] Positive control.

The cytotoxicity of the methanol extract and isolated compounds against the growth of five human cancer cell lines was tested by using the SRB assay, and the results are shown in Table 3. As seen in the table, the methanol extract exhibits moderate cytotoxicity against all tested cell lines with the IC_{50} values ranging from 52.58 ± 3.65 to 64.78 ± 4.89 μg/mL, whereas pure compounds **1**, **2** are inactive (IC_{50} values > 100 μg/mL). Compounds **3**–**5** were not tested on the cytotoxicity because the amount (less than 2 mg) of compounds **4**, **5** is no longer sufficient for testing activity, while the other compound is rather well known [19].

4 Conclusion

The chemical constituents and cytotoxicity of *Aspidistra letreae* are reported for the first time in the present study. Five compounds, including 2*H*-chromen-2-one, α-tocopherol, (*E*)-phytol, asparenydiol, and (25*S*)-spirost-1β,3α,5β-triol were isolated from whole *Aspidistra letreae* plants. Importantly, asparenydiol was isolated from the genus *Aspidistra* for the first time. This is also the first report on the isolation and structural determination of (25*S*)-spirost-1β,3α,5β-triol as a pure compound from nature. Besides, the methanol extract exhibits a moderate cytotoxic effect against various human cancer cell lines.

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