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RESEARCH ARTICLE

A new triterpene glycoside from the stems of *Lagerstroemia indica*

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Abstract A bioassay-guided fractionation and chemical investigation of the stems of *Lagerstroemia indica* resulted in the isolation and identification of a new triterpene glycoside, lagerindiside (**1**), along with nine known triterpenes (**2**–**10**). The structure of this new compound was elucidated on the basis of 1D and 2D nuclear magnetic resonance spectroscopic data analysis as well as chemical method. The cytotoxic activities of the isolates (**1**–**10**) were evaluated by determining their inhibitory effects on four human tumor cell lines (A549, SK-OV-3, SK-MEL-2, and HCT15) using a sulforhodamine B bioassay. Compounds **3** and **4** showed potent cytotoxicity on the tumor cell lines with IC_{50} values ranging from 3.38 to 6.29 μM.

Keywords *Lagerstroemia indica* · Lythraceae · Triterpene glycoside · Cytotoxicity

Introduction

Lagerstroemia indica (Lythraceae) is a decorative shrub, widely distributed in Korea, Japan, and China (Lee 1996). The root of this plant has been used as an astringent, detoxicant and diuretic (Lee et al. 2011). Previous phytochemical investigations on *L. indica* have reported the isolation of alkaloids (Kim et al. 2009b), triterpenes (Lee et al. 2011), and flavonoids (Vinod et al. 2010). In our continuing search for bioactive constituents from Korean medicinal plants, we recently reported the isolation of phenolic derivatives and their anti-inflammatory and cytotoxic activities (Woo et al. 2015). Our interest in the research on new bioactive constituents from the stems of *L. indica* further led us to investigate this plant since the our screening test confirmed that $CHCl_3$ and EtOAc fraction of the source showed significant cytotoxicity against A549, SK-OV-3, SK-MEL-2, and HCT-15 using a sulforhodamine B (SRB) bioassay. Using the bioactivity-guided isolation techniques, a new triterpene glycosides (**1**), along with nine known triterpenes (**2**–**10**) were isolated from the active fractions (Fig. 1) and evaluated the cytotoxicities of all isolates (**1**–**10**). In this paper, we report the isolation, structural elucidation (**1**–**10**), and cytotoxicity of all the isolated compounds.

Materials and methods

General experimental procedure

Thin-layer chromatography (TLC) was performed using Merck precoated Silica gel F_{254} plates and reversed-phase (RP)-18 F_{254}s plates. Spots were detected on TLC under ultraviolet (UV) light or by heating after spraying with 10 % H_2SO_4 in C_2H_5OH (v/v). Packing material of molecular sieve column chromatography was Sephadex LH-20 (Pharmacia Co. Ltd). Low-pressure LC was performed over a LiChroprep Lobar-A RP-C_{18} (240 × 10 mm i.d.) column with a FMI QSY-O pump (ISCO). Semi-

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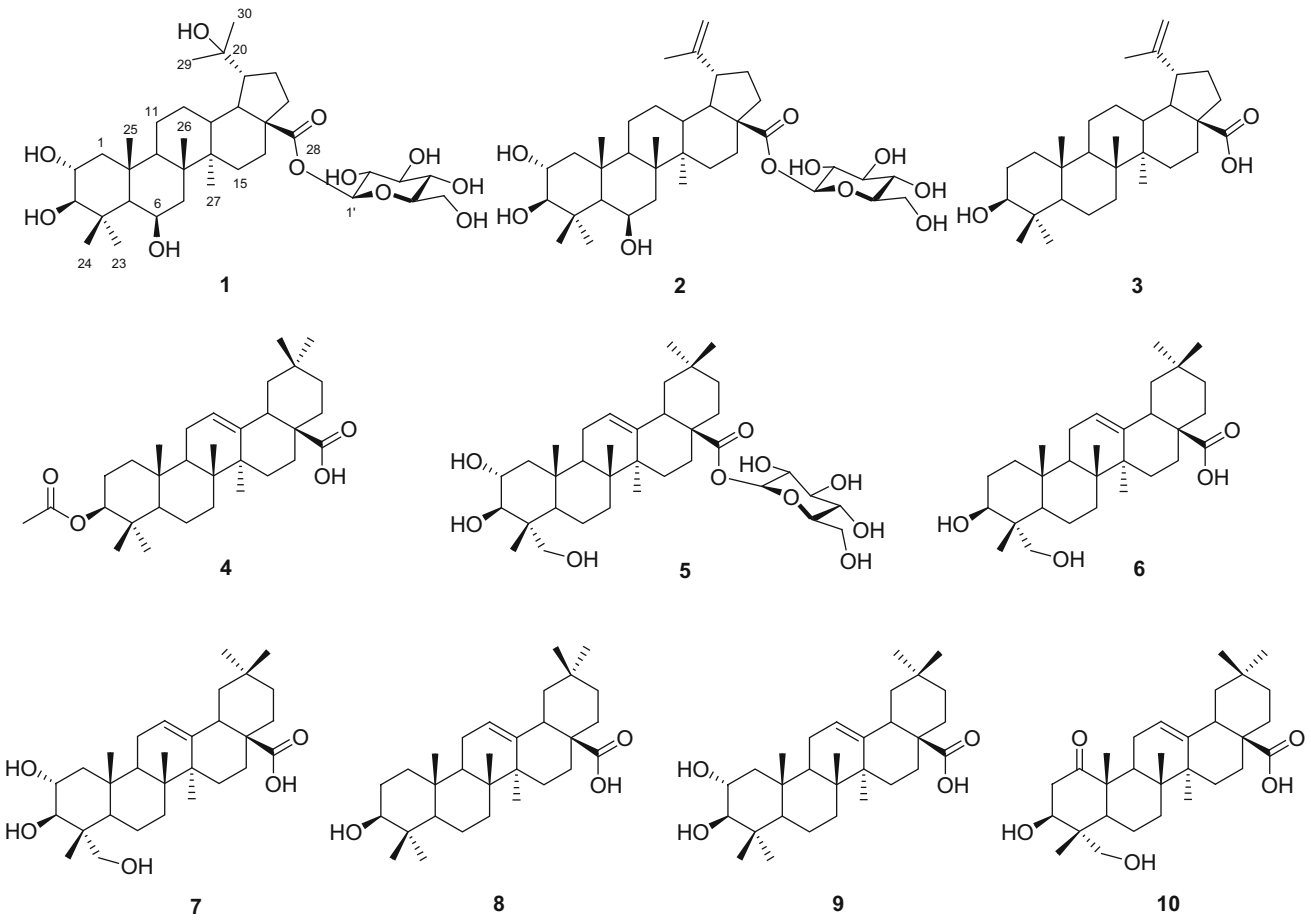

Fig. 1 Structures of compounds **1–10**

preparative high performance liquid chromatography (HPLC) was performed using a Gilson 306 pump (Gilson, Middleton, WI) with a Shodex refractive index detector (Shodex, New York, NY) and Econosil® RP- C_{18} 10 μ column (250 × 10 mm). Optical rotations were obtained on a JASCO P-1020 Polarimeter (Jasco, Easton, MD). Infrared (IR) spectra were recorded on a Bruker IFS-66/S FT-IR spectrometer. Nuclear magnetic resonance (NMR) spectra, including ^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC), hetero nuclear multiple bond correlation (HMBC), were recorded on a Varian UNITY INOVA NMR spectrometer operating at 700 MHz (^{1}H) and 175 MHz (^{13}C), with chemical shifts given in ppm (δ). High resolution (HR)-fast atom bombardment (FAB) mass spectrometry (MS) and FABMS spectra were obtained on a JEOL JMS 700 mass spectrometer.

Plant materials

The stems of *L. indica* (5 kg) were collected from Goesan in Chungcheongbuk-do, Korea, in May 2012. The plants were authenticated by one of the authors (K. R. Lee). A voucher specimen (SKKU-NPL-1203) of the plant was deposited in the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Korea.

Extraction and isolation

The stems of *L. indica* (5 kg) were extracted with 80 % MeOH (60 L) three times under reflux. The filtrate was evaporated under reduced pressure to yield a MeOH extract (300 g), which was suspended in water (800 mL, 5 times) and solvent-partitioned to afforded *n*-hexane (17.3 g, yield 5.73 %), $CHCl_3$ (9.1 g, yield 3.03 %), EtOAc (8.9 g, yield 2.96 %), and BuOH (55.0 g, yield 18.33 %) fractions. Each fraction was evaluated for cytotoxicity against human tumor cell lines A549, SK-OV-3, SK-MEL-2, and HCT-15, using the SRB bioassay. It was found that the $CHCl_3$ and ethyl acetate-soluble fractions showed cytotoxic activity against all the cell lines.

The $CHCl_3$ fraction (9.1 g) was separated over a silica gel column (230–400 mesh, 300 g) with a solvent system of $CHCl_3$/MeOH (30:1 to 1:1) to obtain four fractions (A–

D). Fraction A (2.2 g) was chromatographed on a silica gel column eluted with $CHCl_3$/MeOH (60:1 to 1:1) to yield four fractions (A1–A4). Fraction A2 (1.0 g) was separated over a RP-C_{18} open column (230–400 mesh, 150 g) with a solvent system of 100 % MeOH to obtain seven subfractions (A21–A27). Subfraction A25 (35 mg) was purified by semi-preparative reversed-phase HPLC using a 250 × 10 mm^2 i.d., 10 μm, Econosil RP-C_{18} column (Alltech) with a solvent system of 97 % MeOH (1 L, flow rate; 2 mL/min) to isolate compounds **3** (5 mg, yield 0.00167 %) and **8** (4 mg, yield 0.00134 %). Subfraction A26 (15 mg) was purified by semi-preparative reversed-phase HPLC with a solvent system of 97 % MeOH to afford compound **4** (3 mg, yield 0.001 %). Fraction A4 (900 mg) was separated over a RP-C_{18} open column (230–400 mesh, 150 g) with a solvent system of 90 % MeOH to obtain five subfractions (A41–A45). Subfraction A44 (10 mg) and A45 (17 mg) were purified by semi-preparative reversed-phase HPLC with a solvent system of 70 % MeOH to afford compounds **6** (4 mg, yield 0.00134 %) and **9** (3 mg, yield 0.001 %), respectively.

The EtOAc fraction (8.9 g) was separated over a RP-C_{18} open column (230–400 mesh, 300 g) with a solvent system of 30–100 % MeOH to obtain ten fractions (A–J). Fraction E (1.0 g) was chromatographed on a Sephadex LH-20 column (450 g, 3 × 90 cm) and eluted with 80 % MeOH to yield five subfractions (E1–E5). Subfraction E2 (220 mg) was chromatographed on a Sephadex LH-20 column (80 % MeOH) and purified by semi-preparative reversed-phase HPLC with a solvent system of 40 % MeCN to afford compound **1** (9 mg, yield 0.003 %). Subfraction E3 (22 mg) was applied to LPLC on a LiChroprep Lobar-A RP-C_{18} column eluted with 80 % MeOH and purified by semi-preparative reversed-phase HPLC with a solvent system of 70 % MeOH to afford compound **5** (7 mg, yield 0.00234 %). Fraction F (470 mg) was separated over a RP-C_{18} open column (230–400 mesh, 100 g) with a solvent system of 70–100 % MeOH and purified by semi-preparative reversed-phase HPLC (55 % MeCN) to afford compound **2** (25 mg, yield 0.00834 %). Fraction G (40 mg) was purified by semi-preparative reversed-phase HPLC with a solvent system of 60 % MeCN to afford compounds **7** (2 mg, yield 0.00067 %) and **10** (2 mg, yield 0.00067 %).

Compound (**1**): colorless gum, $[\alpha]_D^{25}$—8.5 (*c* 0.09, MeOH); IR (KBr) ν_{max} 2973, 2944, 2870, 2830, 1707, 1052, 1032, 1022 cm^{-1}; ^{1}H NMR (700 MHz, C_5D_5N) and ^{13}C NMR (175 MHz, C_5D_5N) data, see Table 1; HR-FAB-MS 691.4033 *m/z* $[M + Na]^+$ (calcd for $C_{36}H_{60}O_{11}Na$, 691.4033).

Maslinic acid (**9**): colorless gum, ^{1}H-NMR (500 MHz, CD_3OD) δ 5.27 (1H, t, J = 3.5 Hz, H-12), 3.62 (1H, m, H-2), 2.91 (1H, d, J = 10.0 Hz, H-3), 1.17 (3H, s, Me-23), 1.02 (3H, s, Me-27), 1.01 (3H, s, Me-24), 0.95 (3H, s, Me-30), 0.91 (3H, s, Me-25), 0.82 (3H, s, Me-26), 0.81 (3H, s, Me-29); ^{13}C-NMR (500 MHz, CD_3OD) δ 179.0 (C-28), 144.4 (C-13), 121.7 (C-12), 83.2 (C-3), 68.0 (C-2), 55.4 (C-5), 47.7 (C-9), 47.3 (C-1), 46.2 (C-17), 46.0 (C-19), 41.7 (C-14), 41.5 (C-18), 39.4 (C-4), 39.3 (C-8), 38.0 (C-10), 33.8 (C-21), 32.8 (C-7, 29), 32.7 (C-22), 30.5 (C-20), 28.9 (C-23), 27.8 (C-15), 25.7 (C-27), 23.5 (C-11), 23.3 (C-16, 30), 18.4 (C-6), 17.2 (C-26), 17.1 (C-24), 16.4 (C-25); FAB-MS *m/z* 473 $[M+H]^+$.

Table 1 ^{1}H- and ^{13}C-NMR data of compound **1** in C_5D_5N

Position	δ_H	δ_C	Position	δ_H	δ_C
1	2.41 m, 1.30 m	50.5	19	2.69 m	50.0
2	4.27 m	69.1	20	–	72.3
3	3.42 d (9.0)	84.3	21	2.21 m	30.8
4	–	40.8	22	2.13 m, 1.65 m	36.9
5	1.09 m	56.6	23	1.45 s	28.9
6	4.81 brs	68.0	24	1.76 s	19.2
7	1.98 m, 1.77 m	42.9	25	1.62 s	19.5
8	–	44.0	26	1.86 s	17.7
9	1.65 m	52.1	27	1.13 s	15.7
10	–	38.7	28	–	175.6
11	1.78 m	22.4	29	1.43 s	31.6
12	1.75 m	29.2	30	1.36 s	27.3
13	3.01 m	37.9	1′	6.44 d (8.5)	95.5
14	–	41.1	2′	4.21 m	74.2
15	2.38 m	30.8	3′	4.05 m	79.4
16	2.73 m, 1.60 m	32.6	4′	4.40 m	71.2
17	–	57.1	5′	4.32 m	78.9
18	2.02 m	49.1	6′	4.44 m	62.2

NMR data were obtained at 700 MHz for ^{1}H and 175 MHz for ^{13}C

3*β*,23-Dihydroxy-1-oxo-olean-12-en-28-oic acid (**10**): colorless gum, ^{1}H-NMR (500 MHz, C_5D_5N) δ 5.50 (1H, t, J = 3.5 Hz, H-12), 4.55 (1H, dd, J = 12.0, 5.0 Hz, H-3), 4.12 (1H, d, J = 11.0 Hz, H-23*α*), 3.71 (1H, d, J = 11.0 Hz, H-23*β*), 3.44 (1H, t, J = 12.0 Hz, H-2*β*), 3.26 (1H, dd, J = 14.0, 4.0 Hz, H-18*β*), 2.79 (1H, dd, J = 11.5, 5.0 Hz, H-2*α*), 2.72 (1H, dt, J = 18.0, 5.5, 4.5 Hz, H-11*α*), 2.65 (1H, dd, J = 11.0, 6.0 Hz, H-9), 1.36 (3H, s, Me-25), 1.23 (3H, s, Me-27), 1.17 (3H, s, Me-24), 1.08 (3H, s, Me-26), 0.99 (3H, s, Me-30), 0.93 (3H, s, Me-29);^{13}C-NMR (500 MHz, C_5D_5N) δ 212.9 (C-1), 179.8 (C-28), 143.9 (C-13), 123.1 (C-12), 72.6 (C-3), 65.8 (C-23), 52.2 (C-10), 47.2 (C-5), 46.6 (C-17), 45.9 (C-19), 44.9 (C-2), 43.6 (C-4), 42.2 (C-18), 42.0 (C-14), 39.5 (C-8), 39.4 (C-9), 34.1 (C-21), 33.1 (C-29), 32.9 (C-22), 32.7 (C-7), 30.7 (C-20), 28.1 (C-15), 25.8 (C-27), 25.5 (C-11), 23.6 (C-

30), 23.5 (C-16), 17.9 (C-26), 17.7 (C-6), 15.4 (C-25), 13.5 (C-24); FAB-MS *m/z* 487 $[M+H]^+$.

Acid hydrolysis of 1 and sugar determination

Compound **1** (1.0 mg) was refluxed with 1 mL of 1 N HCl for 2 h at 100 °C. The hydrolysate was extracted with EtOAc and the extract was evaporated *in vacuo*. The H_2O layer was neutralized by passage through an Amberlite IRA-67 column (Sigma, St. Louis, MO) and evaporated to give D-glucose identified by co-TLC ($CHCl_3$:MeOH:H_2O = 2:1:0.2, R_f value 0.2) with an authentic sample and measurement of optical rotation {$[\alpha]_D^{25}$ +54.0 (c = 0.05, H_2O)}.

Cytotoxicity assay

A sulforhodamine B bioassay (SRB) was used to determine the cytotoxicity of each compound on four cultured human cancer cell lines (Skehan et al. 1990). The assays were performed at the Korea Research Institute of Chemical Technology. The cell lines used were A549 (non small cell lung adenocarcinoma), SK-OV-3 (ovarian cancer cells), SK-MEL-2 (skin melanoma), and HCT15 (colon cancer cells). Doxorubicin (Sigma Chemical Co., ≥98 %) was used as a positive control.

Results

Structure elucidation

Purification of the $CHCl_3$ and EtOAc-soluble fractions by multiple chromatographic steps isolated a new triterpene glycoside (**1**), together with nine known triterpenes (**2**–**10**). The known compounds were identified as quadranoside I (**2**) (Adnyana et al. 2000), betulinic acid (**3**) (Choi et al. 2006), 3*β*-acetoxyolean-12-en-28-acid (**4**) (Kwon et al. 2008), arjunolic acid 28-*O*-glucopyranoside (**5**) (Jayasinghe et al. 1993), hederagenin (**6**) (He et al. 2003), arjunolic acid (**7**) (Kim et al. 2008), oleanolic acid (**8**) (Seebacher et al. 2003), maslinic acid (**9**) (Taniguchi et al. 2002), and 3*β*,23-dihydroxy-1-oxo-olean-12-en-28-oic acid (**10**) (Okada et al. 2003) by comparing the ^{1}H-, ^{13}C-NMR, and MS spectral data with the literature values.

Compound **1** was obtained as a colorless gum. The molecular formula was determined to be $C_{36}H_{60}O_{11}Na$ from the molecular ion peak $[M+Na]^+$ at *m/z* 691.4033 (calcd for $C_{36}H_{60}O_{11}Na$, 691.4033) in the positive-ion HR-FAB-MS. The ^{1}H-NMR spectrum showed signals for seven tertiary methyl groups at δ_H 1.86 (s, Me-26), 1.76 (s, Me-24), 1.62 (s, Me-25), 1.45 (s, Me-23), 1.43 (s, Me-29), 1.36 (s, Me-30) and 1.13 (s, Me-27) and three oxygenated methine protons at δ_H 4.81 (brs, H-6), 4.27 (m, H-2), and 3.42 (d, *J* = 9.0 Hz, H-3). In the ^{13}C NMR (including DEPT) spectrum, a total of 30 carbon signals, composed of 7 methyls (δ_C 31.6 28.9, 27.3, 19.5, 19.2, 17.7, and 15.7), 3 oxygenated methines (δ_C 84.3, 69.1, and 68.0), oxygenated quaternary carbon (δ_C 72.3), carbonyl carbon (δ_C 175.6) were observed, together with the sugar group (δ_C 95.5, 79.4, 78.9, 74.2, 71.2, and 62.2). From these data, compound **1** was deduced to be lupane-type triterpene glycoside (El-Gamal 2008). Comparison of the ^{1}H- and ^{13}C-NMR data of **1** with those of **2** indicated that the only difference was the presence of 2-hydroxy isopropyl moiety in **1**, instead of allylic methyl group in **2**. The HMBC correlations of Me-29/C-19, Me-29/C-20, Me-30/C-19, and Me-30/C-20 confirmed 2-hydroxy isopropyl moiety to be located at C-19 (Fig. 2). The relative configuration of aglycone was determined to be the same as compound **2** by NOESY cross-peaks of H-23/H-3, H-23/H-5, H-5/H-6, H-24/H-2, H-24/H-25, H-27/H-9, H-27/H-18, H-13/H-19, and H-13/H-26 (Adnyana et al. 2000) (Fig. 3). Acid hydrolysis of **1** afforded D-glucose, which was detected by co-TLC and identified by the sign of its specific rotation value (Kim et al. 2009a). The *J* coupling (d, *J* = 8.5 Hz) of the anomeric proton signal at δ_H 6.44 indicated *β*-orientation. Thus, the structure of compound **1** was determined as shown in Fig. 1 and named lagerindiside.

Cytotoxic activity

The cytotoxic activities of the isolates (**1**–**10**) were evaluated by determining their inhibitory effects on human tumor cell lines (A549, SK-OV-3, SK-MEL-2, and HCT-15) using the SRB bioassay (Skehan et al. 1990). Compounds **3** and **4** showed significant cytotoxicity on all tested tumor cell lines with IC_{50} values (Table 2). The linked acetoxyl moiety at C-3 in **4** significantly increased cytotoxic activity when compared to the compound **8**. Compounds **1**, **2**, and **5**, which combined the glucose at C-28 has a result that not show

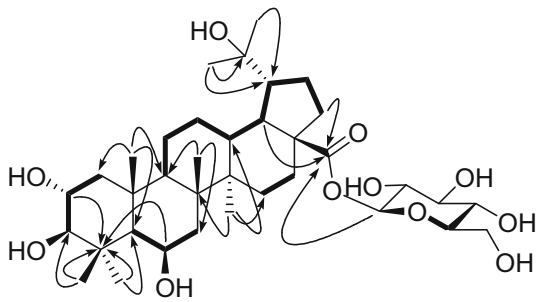

Fig. 2 COSY (*bold lines*) and HMBC (*arrow*) correlations of compound **1**

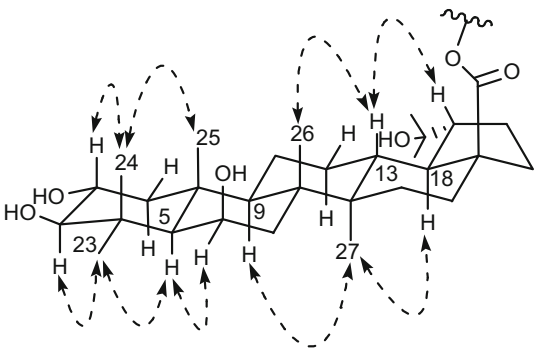

Fig. 3 Key NOE (*dashed arrow*) correlations of compound **1**

Table 2 Cytotoxic activities of compounds (**1**–**10**) isolated from *L. indica*

Compound	IC_{50} (μM)			
	A549[a]	SK-OV-3[a]	SK-MEL-2[a]	HCT-15[a]
1	>30	>30	>30	>30
2	>30	>30	>30	>30
3	5.41	5.85	5.66	6.07
4	3.38	6.29	4.72	5.81
5	>30	>30	>30	>30
6	27.54	>30	27.67	29.18
7	21.74	23.81	21.16	22.54
8	10.06	>30	11.96	23.17
9	25.76	>30	25.19	28.23
10	28.61	>30	27.63	28.75
Doxorubicin	0.0014	0.0217	0.0025	0.1077

[a] A549 (non-small cell lung adenocarcinoma), SK-OV-3 (ovarian cancer cells), SK-MEL-2 (skin melanoma), and HCT15 (colon cancer cells)

cytotoxic activity ($IC_{50} > 30.0$ μM) in comparison to that of aglycone (**3**, **4**, **6**–**10**).

Discussion

This study elucidated the structures of one new triterpene glycoside, lagerindiside (**1**) and nine known triterpene present in the stems of *L. indica* using bioactivity-guided isolation, spectroscopic data analysis, and chemical methods. To evaluate compounds **1**–**8** as cytotoxic agents, compounds **3** and **4** had significant cytotoxicity on all of the tested cell lines, with IC_{50} values ranging from 3.38 to 6.29 μM. These results implied that triterpene derivatives from *L. indica* could be used as candidates for the treatment of various cancer diseases.

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Compliance with ethical standards

Conflict of interest All authors have no conflict of interest to declare.

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