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New oleanane-type saponins from the leaves of *Derris trifoliata*

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Phytochemical investigation of the polar fraction of the leaves of *Derris trifoliata* led to the isolation of five new oleanane-type saponins, named derristrifosides A–E (**1–5**). Their chemical structures were elucidated through comprehensive analyses of HR-ESI-MS and one-dimensional and two-dimensional NMR spectra. These new saponins possess a cantoniensistriol-type sapogenin framework, which is rarely found in vascular plants. Compounds **1–5** exhibited weak inhibitory activity against nitric oxide (NO) production in LPS-stimulated RAW 264.7 cells and weak antiproliferative activity against SK-LU-1 and Hep-G2 human cancer cell lines.

Introduction

Plants of the genus *Derris*, such as *D. elliptica*, *D. montana*, and *D. trifoliata*, have long been used as natural piscicides and insecticides.[1] Previous phytochemical studies on these species have indicated that the *Derris* genus is a rich source of rotenoids and prenylated flavonoids, including flavanones, flavones, isoflavones, pterocarpans, and chalcones.[2–5] To date, hundreds of such compounds have been isolated from *Derris* species.[6] Rotenone and its derivatives are known for their piscicidal and insecticidal properties.[7] In addition, rotenoids and prenylated flavonoids have been reported to exhibit various biological activities, such as cytotoxic, anti-inflammatory, antimicrobial, and α-glucosidase inhibitory effects.[2,8–10]

Derris trifoliata, a climbing plant, is widely distributed in tropical mangrove forests. Its fresh roots are traditionally pounded and mixed with water for use in fishing and as an insect repellent.[11,12] In folk medicine, *D. trifoliata* has been used to enhance the immune system and to treat inflammation, coughs, and diarrhea.[13] Our previous phytochemical studies revealed several rotenoids and prenylated flavonoids from the ethyl acetate fraction of *D. trifoliata* leaves. Some isolated compounds, such as 6*aα*,12*aα*-12ahydroxyelliptone, (2*S*)-lonchocarpol D, lonchocarpol C_1, and lonchocarpol C_2, exhibited cytotoxic effects against SK-LU-1 and Hep-G2 human cancer cell lines, with IC_{50} values ranging from 7.11 to 34.85 μM.[13] As rotenoids are relatively non-polar substances, most previous studies have focused on less polar fractions for compound isolation and purification.[11–15] However, traditional medicinal preparations are usually made as decoctions, which preferentially extract polar constituents. Therefore, we selected the polar fraction of *D. trifoliata* for further phytochemical investigation. Herein, we report the identification of five new oleanane-type saponins from the leaves of *D. trifoliata* and their antiproliferative and nitric oxide production inhibitory activities.

Experimental

General experimental procedures

The optical rotations were measured using a Jasco P2000 polarimeter. The 1D and 2D NMR spectra were recorded on a Bruker Avance NEO 600 MHz spectrometer. HR-ESI-MS data were acquired using an Agilent 6530 Accurate-Mass QTOF LC/MS system. Thin-layer chromatography (TLC) was performed on pre-coated silica gel or reversed-phase C18 (RP18) plates. Column chromatography was carried out using silica gel (40–63 μm) or RP18 (150 μm) as adsorbents. Semi-preparative HPLC was performed on an Agilent 1260 Infinity II system equipped with a binary pump, autosampler, and DAD detector, using a YMC J'sphere ODS-H80 (20 × 250 mm, 4 μm) column. The mobile phases consisted of isocratic systems of methanol/water or acetonitrile/water at a flow rate of 3 mL min^{-1}.

Plant material

The leaves of *Derris trifoliata* Lour. (Fabaceae family) were collected at Xuan Thuy National Park, Nam Dinh provine, Vietnam, in May 2024 and taxonomically identified by botanist

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Table 1 ^{1}H-NMR and ^{13}C-NMR spectral data for **1–3** in CD_3OD

	1		2		3	
No.	δ_C	δ_H (mult., J in Hz)	δ_C	δ_H (mult., J in Hz)	δ_C	δ_H (mult., J in Hz)
1	40.0	1.05 (m)/1.64 (m)	40.0	1.04 (m)/1.64 (m)	40.0	1.01 (m)/1.64 (m)
2	27.1	1.04 (m)/1.92 (m)	27.1	1.04 (m)/1.92 (m)	27.1	1.04 (m)/1.95 (m)
3	91.1	3.24 (dd, 11.4, 4.2)	91.3	3.26 (dd, 11.4, 4.2)	92.0	3.20 (dd, 11.4, 4.2)
4	40.5	—	40.5	—	40.5	—
5	57.0	0.80 (br d, 11.4)	57.0	0.81 (br d, 11.4)	57.1	0.79 (br d, 11.4)
6	19.3	1.47 (br d, 11,4)/1.60 (m)	19.3	1.47 (br d, 11.4)/1.60 (m)	19.3	1.44 (br d, 11.4)/1.58 (m)
7	33.7	1.38 (m)/1.60 (m)	33.7	1.38 (m)/1.59 (m)	33.8	1.37 (m)/1.58 (m)
8	41.2	—	41.3	—	41.3	—
9	49.0	1.63 (m)	49.0	1.62 (m)	48.9	1.61 (m)
10	37.7	—	37.8	—	37.8	—
11	24.7	1.92 (m)/1.94 (m)	24.7	1.90 (m)/1.95 (m)	24.6	1.90 (m)/1.95 (m)
12	123.9	5.26 (t, 3.0)	123.8	5.26 (t, 3.0)	123.9	5.26 (t, 3.0)
13	145.0	—	145.0	—	145.0	—
14	43.0	—	43.0	—	43.0	—
15	27.0	1.70 (m)/2.05 (br d, 13.2)	27.0	1.71 (m)/2.04 (br d, 13.2)	27.0	1.76 (m)/2.00 (br d, 13.2)
16	28.2	1.03 (m)/1.92 (m)	28.2	1.05 (m)/1.95 (m)	28.2	1.05 (m)/1.95 (m)
17	40.0	—	40.0	—	39.9	—
18	44.4	2.27 (dd, 13.2, 3.6)	44.5	2.27 (dd, 13.2, 3.6)	44.5	2.27 (dd, 13.2, 3.6)
19	47.9	1.18 (dd, 13.2, 4.2)/1.97 (m)	47.9	1.17 (dd, 13.2, 3.6)/1.96 (m)	47.9	1.17 (br d, 13.2)/1.96 (m)
20	37.3	—	37.3	—	37.3	—
21	85.6	3.53 (d, 3.0)	85.5	3.53 (d, 3.0)	85.8	3.54 (d, 3.0)
22	79.8	3.44 (d, 3.0)	79.7	3.44 (d, 3.0)	79.7	3.46 (d, 3.0)
23	28.5	1.09 (s)	28.4	1.09 (s)	28.8	1.13 (s)
24	17.0	0.90 (s)	16.9	0.91 (s)	16.9	0.91 (s)
25	16.1	1.00 (s)	16.1	1.00 (s)	16.0	1.00 (s)
26	17.5	1.04 (s)	17.5	1.04 (s)	17.5	1.04 (s)
27	26.9	1.20 (s)	26.9	1.20 (s)	26.9	1.20 (s)
28	22.0	0.98 (s)	22.1	0.98 (s)	22.0	0.98 (s)
29	31.3	0.96 (s)	31.3	0.96 (s)	31.3	0.96 (s)
30	21.8	1.08 (s)	21.8	1.08 (s)	21.7	1.08 (s)
21-*O*-Rha			21-*O*-Rha		21-*O*-Rha	
1′	104.7	4.80 (d, 1.8)	104.7	4.80 (br s)	104.4	4.83 (d, 1.2)
2′	72.3	3.94 (dd, 4.8, 1.8)	72.3	3.93 (br s)	72.2	3.97 (br s)
3′	72.3	3.80 (m)	72.3	3.79 (m)	72.3	3.80 (m)
4′	74.0	3.41 (m)	74.0	3.41 (m)	74.2	3.41 (m)
5′	70.4	3.76 (m)	70.4	3.76 (m)	70.4	3.78 (m)
6′	18.0	1.27 (d, 7.0)	18.0	1.27 (d, 7.0)	17.9	1.27 (d, 7.2)
3-*O*-Glu			3-*O*-Glu		3-*O*-Glu	
1″	105.2	4.48 (d, 7.8)	105.3	4.49 (d, 7.8)	105.4	4.45 (d, 7.8)
2″	83.7	3.51 (m)	82.0	3.57 (m)	79.1	3.72 (m)
3″	78.0	3.75 (m)	78.0	3.73 (m)	78.9	3.68 (m)
4″	72.9	3.52 (m)	73.1	3.52 (m)	73.9	3.48 (m)
5″	76.6	3.58 (d, 9.6)	76.6	3.58 (d, 9.0)	76.7	3.57 (d, 9.0)
6″	176.8	—	177.0	—	176.7	—
2″-*O*-Gal			2″-*O*-Glc		2″-*O*-Gal	
1‴	104.4	4.74 (d, 7.8)	103.3	4.87 (d, 7.8)	102.8	4.85 (d, 7.8)
2‴	83.5	3.79 (m)	85.4	3.42 (dd, 7.8, 9.6)	77.3	3.70 (m)
3‴	74.8	3.71 (m)	76.7	3.58 (m)	76.0	3.65 (m)
4‴	69.7	3.89 (br s)	72.0	3.22 (m)	71.0	3.80 (br s)
5‴	76.5	3.48 (m)	77.5	3.58 (m)	76.7	3.48 (m)
6‴	62.1	3.69[a]/3.75[a]	63.3	3.61[a]/3.84 (dd, 12.0, 2.4)	62.8	3.67[a]/3.81[a]
2‴-*O*-Glc			2‴-*O*-Glc		2‴-*O*-Rha	
1⁗	106.2	4.64 (d, 7.8)	106.1	4.61 (d, 7.8)	101.9	5.22 (d, 1.2)
2⁗	76.4	3.30 (d, 9.0)	76.4	3.30 (m)	72.2	3.96 (m)
3⁗	78.9	3.38 (m)	78.9	3.38 (m)	72.4	3.80 (m)
4⁗	71.0	3.39 (m)	71.0	3.38 (m)	74.0	3.45 (m)
5⁗	77.5	3.42 (m)	77.5	3.41 (m)	69.8	4.16 (m)
6⁗	62.3	3.77[a]/3.95 (br d, 10.8)	62.3	3.77[a]/3.95 (br d, 10.8)	18.2	1.26 (d, 7.2)

[a] Overlapped signal.

Nguyen The Cuong, Institute of Ecology and Biological Resources, VAST. Voucher specimens (number: NCCT-P127) were kept at the Institute of Chemistry, VAST and the Basic Science Department, University of Transport and Communications.

Extraction and isolation

The dried powdered leaves of *Derris trifoliata* (7.2 kg) were macerated with methanol (MeOH) in an ultrasonic bath (three times: 20 L MeOH at room temperature for 1 h each) to afford a methanolic residue (289 g). The methanol extract was suspended in water (4.0 L) and successively partitioned with dichloromethane and ethyl acetate to yield the corresponding dichloromethane-soluble, ethyl acetate-soluble, and aqueous fractions. The aqueous layer was applied to a Diaion HP-20 column, washed with water, and eluted with methanol to give the polar fraction, designated DFW (44 g). The DFW fraction was loaded on a silica gel column chromatography and eluted with dichloromethane/methanol/water (3/1/0.1, v/v/v) to give three fractions, DFW1–DFW3. Fraction DFW2 was separated on an RP18 column using methanol/water (1/4, v/v) as the eluent to give five fractions, DFW2A–DFW2E. Fraction DFW2C was purified by semi-preparative HPLC using 20% acetonitrile in water as the mobile phase to give compounds **1** (15.8 mg, t_R 34.1 min) and **2** (20.7 mg, t_R 36.0 min). Fraction DFW2D was separated on a silica gel column, eluting with dichloromethane/methanol/water (5/1/0.1, v/v/v) to give three fractions, DFW2D1–DFW2D3. Fraction DFW2D1 was purified by semi-preparative HPLC using 20% acetonitrile in water to give compound **3** (22.4 mg, t_R 32.9 min). Fraction DFW2D2 was purified by semi-preparative HPLC using 20% acetonitrile in water to give compounds **4** (20.8 mg, t_R 37.6 min) and **5** (18.5 mg, t_R 41.1 min).

Derristrifoside A (1). White amorphous powder; $[\alpha]_D^{28}$: −30.3 (*c* 0.1, MeOH); UV: none significant maxima absorption in the range of 200–400 nm; HR-ESI-MS *m*/*z*: 1103.5641 $[M\text{-}H]^-$, (calcd. for $[C_{54}H_{87}O_{23}]^-$, 1103.5644, $\Delta = -0.3$ ppm); ^{1}H- and ^{13}C-NMR data are shown in the Table 1.

Derristrifoside B (2). White amorphous powder; $[\alpha]_D^{28}$: −23.9 (*c* 0.1, MeOH); UV: none significant maxima absorption in the range of 200–400 nm; HR-ESI-MS *m*/*z*: 1103.5624 $[M\text{-}H]^-$, (calcd. for $[C_{54}H_{87}O_{23}]^-$, 1103.5644, $\Delta = -1.3$ ppm); ^{1}H- and ^{13}C-NMR data are shown in the Table 1.

Table 2 ^{1}H-NMR and ^{13}C-NMR spectral data for **4–5** in DMSO-d_6

	4 (aglycon)		5 (aglycon)			4 (sugar moieties)		5 (sugar moieties)	
No.	δ_C	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	No.	δ_C	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)
1	38.2	0.87 (m)/1.50 (m)	38.2	0.87 (m)/1.50 (m)		21-*O*-Rha		21-*O*-Rha	
2	25.5	0.90 (m)/1.65 (m)	25.6	0.90 (m)/1.65 (m)	1′	102.8	4.61 (br s)	102.8	4.61(br s)
3	89.4	3.24 (m)	89.0	3.17 (m)	2′	70.6	3.68 (m)	70.5	3.68 (m)
4	43.0	—	43.2	—	3′	70.6	3.59 (m)	70.5	3.59 (m)
5	55.3	0.85 (m)	55.4	0.83 (m)	4′	72.2	3.16 (m)	72.1	3.16 (m)
6	18.1	1.35 (m)/1.53 (m)	18.2	1.33 (m)/1.55 (m)	5′	68.7	3.62 (m)	68.7	3.62 (m)
7	32.3	1.25 (m)/1.45 (m)	32.3	1.25 (m)/1.45 (m)	6′	17.8	1.10 (m)	17.8	1.09 (m)
8	40.4	—	40.4	—		3-*O*-Glu		3-*O*-Glu	
9	46.9	1.50 (m)	46.9	1.49 (m)	1″	103.1	4.34 (d, 7.8)	103.1	4.31 (d, 7.8)
10	35.9	—	35.9	—	2″	78.4	3.37 (m)	79.0	3.26 (m)
11	23.3	1.80 (m)	23.3	1.80 (m)	3″	82.1	3.50 (m)	83.8	3.17 (m)
12	121.7	5.16 (brs)	121.7	5.16 (brs)	4″	71.8	3.20 (m)	71.8	3.17 (m)
13	143.7	—	143.7	—	5″	76.5	3.55[a]	76.5	3.54[a]
14	41.4	—	41.3	—	6″	173.4	—	173.4	—
15	25.6	1.63 (m)/1.97 (m)	25.4	1.63 (m)/1.97 (m)		2″-*O*-Gal		2″-*O*-Xyl	
16	26.6	0.93 (m)/1.73 (m)	26.6	0.93 (m)/1.73 (m)	1‴	100.7	4.81 (d, 7.8)	101.2	4.78 (d, 7.8)
17	38.4	—	38.3	—	2‴	72.6	3.50 (m)	72.1	3.18 (m)
18	42.7	2.13 (br d, 11.4)	42.7	2.12 (br d, 11.4)	3‴	74.3	3.34 (m)	75.4	3.28 (m)
19	46.2	1.02 (m)/1.84 (m)	46.2	1.03 (m)/1.84 (m)	4‴	68.3	3.65 (br s)	69.2	3.27 (m)
20	35.9	—	35.9	—	5‴	74.8	3.37 (m)	65.2	2.97 (dd, 10.2, 11.4)
21	82.5	3.40 (br s)	82.5	3.40 (br s)	6‴	60.2	3.52[a]/3.48[a]		3.63[a]
22	77.3	3.25 (br s)	77.3	3.25 (br s)		3″-*O*-Glc		3″-*O*-Glc	
23	22.2	1.08 (s)	22.1	1.08 (s)	1⁗	104.6	4.38 (d, 7.8)	104.7	4.36 (d, 7.8)
24	62.4	3.14 (m)	61.7	3.12 (m)	2⁗	74.8	3.03 (m)	75.0	3.03 (m)
		3.88 (d, 9.0)		3.89 (d, 9.0)	3⁗	76.2	3.17 (m)	76.0	3.17 (m)
25	15.3	0.81 (s)	15.1	0.81 (s)	4⁗	69.9	3.10 (m)	69.7	3.10 (m)
26	16.4	0.90 (s)	16.4	0.91 (s)	5⁗	77.2	3.12 (m)	77.3	3.13 (m)
27	26.1	1.11 (s)	26.1	1.11 (s)	6⁗	61.0	3.73 (dd, 10.2, 4.2)	60.9	3.72[a]
28	21.4	0.85 (s)	21.3	0.85 (s)			3.50[a]		3.49[a]
29	30.4	0.85 (s)	30.4	0.85 (s)					
30	21.5	0.97 (s)	21.5	0.97 (s)					

[a] Overlapped signal.

Derristrifoside C (3). White amorphous powder; $[\alpha]_D^{28}$: −18.6 (*c* 0.1, MeOH); UV: none significant maxima absorption in the range of 200–400 nm; HR-ESI-MS *m*/*z*: 1087.5692 $[M\text{-}H]^-$, (calcd. for $[C_{54}H_{87}O_{22}]^-$, 1087.5694, Δ = −0.2 ppm); ^{1}H- and ^{13}C-NMR data are shown in the Table 1.

Derristrifoside D (4). White amorphous powder; $[\alpha]_D^{28}$: −35.5 (*c* 0.1, MeOH); UV: none significant maxima absorption in the range of 200–400 nm; HR-ESI-MS *m*/*z*: 1119.5570 $[M\text{-}H]^-$, (calcd. for $[C_{54}H_{87}O_{24}]^-$, 1119.5593, Δ = −2.1 ppm); ^{1}H- and ^{13}C-NMR data are shown in the Table 2.

Derristrifoside E (5). White amorphous powder; $[\alpha]_D^{28}$: −39.4 (*c* 0.1, MeOH); UV: none significant maxima absorption in the range of 200–400 nm; HR-ESI-MS *m*/*z*: 1089.5485 $[M\text{-}H]^-$, (calcd. for $[C_{53}H_{85}O_{23}]^-$, 1089.5487, Δ = −0.2 ppm); ^{1}H- and ^{13}C-NMR data are shown in the Table 2.

Cytotoxic assay

Cytotoxic effects againts SK-LU-1 and Hep-G2 cell lines were determined using Sulforhodamine B (SRB) assay as previously described and referred to SI.[13,16]

Nitric oxide assay

Nitric oxide assay was performed as previously described and referred to SI.[17]

Results and discussion

The methanol extract from the leaves of *D. trifoliata* was thus successively fractionated with dichloromethane and ethyl acetate, followed by purification of the aqueous fraction through flash column chromatography and HPLC, to yield five oleanane-type saponins (**1–5**).

Compound **1** (Fig. 1) was isolated as a white amorphous powder. The negative-ion HR-ESI-MS spectrum of **1** showed a deprotonated molecular ion corresponding to the molecular formula $C_{54}H_{88}O_{23}$ (*m*/*z* 1103.5641 $[M\text{-}H]^-$; calcd. for $[C_{54}H_{87}O_{23}]^-$, 1103.5638), indicating 11 indices of hydrogen deficiency. The ^{1}H-NMR and HSQC spectra of **1** exhibited signals corresponding to eight methyl group [δ_H 1.20, 1.09, 1.08, 1.04, 1.00, 0.98, 0.96, and 0.90 (each 3H, s)], one olefinic proton [δ_H 5.26 (1H, t, J = 3.0 Hz)], and four anomeric protons [δ_H 4.80 (1H, d, J = 1.8 Hz), 4.74 (1H, d, J = 7.8 Hz), 4.64 (1H, d, J = 7.8 Hz), and 4.48 (1H, d, J = 7.8 Hz)]. The ^{13}C-NMR spectrum of **1** revealed signals of 54 carbons. Of these, 30 carbons were assigned to a triterpene skeleton and the remaining 24 carbons to four hexose-type monosaccharide units. The presence of eight singlet methyl groups, a C=C double bond (δ_C 145.0 and 123.9), and splitting pattern of the H-18 signal [δ_H 2.27 (dd, J = 13.2 and 3.6 Hz)] indicated that compound **1** was a derivative of an olean-12-ene triterpene. The COSY cross peaks between H-18 (δ_H 2.27) and H_2-19 (δ_H 1.18 and 1.97), the HMBC correlations between H_3-29 (δ_H 0.96)/H_3-30 (δ_H 1.08) and C-19 (δ_C 47.9)/C-20 (δ_C 37.3)/C-21 (δ_C 85.6), and the HMBC correlation between an anomeric proton (δ_H 4.80) and C-21 (δ_C 85.6) suggested *O*-glycosylation at C-21. Moreover, the small coupling constant of this anomeric proton (δ_H 4.80, J = 1.8 Hz), the consecutive COSY correlations among H-1′ (δ_H 4.80)/H-2′ (δ_H 3.94)/H-3′ (δ_H 3.80)/H-4′ (δ_H 3.41)/H-5′ (δ_H 3.76)/H_3-6′ (δ_H 1.27), and the corresponding carbon signals [C-1′ (δ_C 104.7)/C-2′ (δ_C 72.3)/C-3′ (δ_C 72.3)/C-4′ (δ_C 74.0)/C-5′ (δ_C 70.4)/C-6′ (δ_C 18.0)] illustrated an *O*-α-rhamnopyranosyl group attached at C-21. A COSY correlation between H-21 (δ_H 3.53) and H-22 (δ_H 3.44), together with the chemical shift of C-22 (δ_C 79.8), suggested the presence of a hydroxyl group at C-22. In addition, HMBC correlations between H_3-23 (δ_H 1.09)/H_3-24 (δ_H 0.90) and C-3 (δ_C 91.1)/C-4 (δ_C 40.5)/C-5 (δ_C 57.0), along with an HMBC correlation between an anomeric proton (δ_H 4.48) and C-3 (δ_C 91.1), indicated another *O*-glycosylation at C-3. A large coupling constant of this anomeric proton (δ_H 4.48, J = 7.8 Hz), the COSY correlations among H-1″ (δ_H 4.48)/H-2″ (δ_H 3.51)/H-3″ (δ_H 3.75)/H-4″ (δ_H

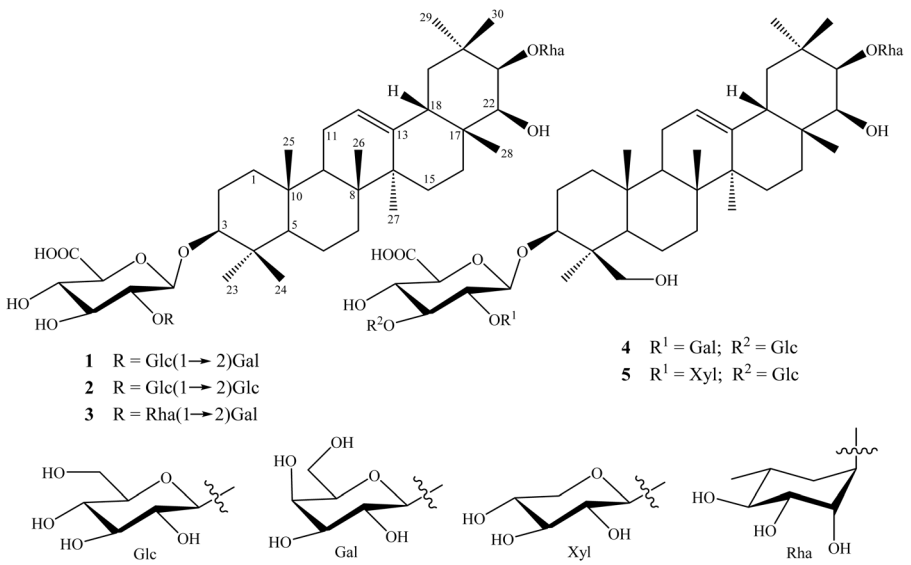

Fig. 1 New oleanane-type saponins **1–5** isolated from the leaves of *D. trifoliata*.

3.52)/H-5″ (δ_H 3.58), and the HMBC correlation between H-5″ (δ_H 3.58) and C-6″ (δ_C 176.8) suggested an *O*-β-glucuronopyranosyl group attached at C-3. Subsequently, an HMBC correlation between an anomeric proton (δ_H 4.74) and C-2″ (δ_C 83.7), COSY correlations among H-1‴ (δ_H 4.74)/H-2‴ (δ_H 3.79)/H-3‴ (δ_H 3.71)/H-4‴ (δ_H 3.89)/H-5‴ (δ_H 3.48)/H_2-6‴ (δ_H 3.69 and 3.75), and the broad singlet of H-4‴ (δ_H 3.89) demonstrated the presence of an *O*-β-galactopyranosyl group linked to the glucuronic acid moiety at C-2″. The final sugar unit was identified as a *β*-glucopyranosyl group, based on the chemical shift of the carbinol carbons (δ_C 106.2, 76.4, 78.9, 71.0, 77.5, 62.3) and the coupling constant of its anomeric proton (δ_H 4.64, J = 7.8 Hz). The HMBC correlation between Glc H-1⁗ (δ_H 4.64) and C-2‴ (δ_C 83.5) indicated that the O-β-glucopyranosyl group was linked to C-2‴ of the galactopyranosyl unit (Fig. 2). The presence of D-glucuronic acid, D-galactose, D-glucose, and L-rhamnose was confirmed by acid hydrolysis of 1, followed by TLC comparison with authentic standards and verification of optical rotation signs (SI).[18,19] The stereochemistry of the aglycone moiety was established through analysis of the NOESY spectrum and the coupling constants between H_{axial}–H_{axial} ($J_{ax/ax}$) and H_{axial}–$H_{equatorial}$ ($J_{ax/eq}$) protons (Fig. 3). The NOESY correlation between H-5 (δ_H 0.80) and H-3 (δ_H 3.24), together with $J_{ax/ax}$ = 11.4 Hz for H-3 (δ_H 3.24), indicated axial-alpha orientation for both H-3 and H-5. Similarly, the NOESY correlation between H-18 (δ_H 2.27) and H_3-30 (δ_H 1.08), and $J_{ax/ax}$ = 13.2 Hz for H-18 (δ_H 2.27) indicated *axial-beta* orientation for H-18 and methyl group C-30. The NOESY correlation of H_3-29 (δ_H 0.96)/H-21 (δ_H 3.53), along with $J_{ax/eq}$ = 3.0 Hz for H-21/H-22, suggested axial-alpha orientation for H-21 and equatoria-alpha orientation for H-22. The alpha orientation of H-22 was further validated by NOESY correlation between H-22 (δ_H 3.44) and $H_{equatorial}$-16 (δ_H 1.03) as well as the identical chemical shifts of C-21 (δ_C 85.6) and C-22 (δ_C 79.8) with those previously reported for similar compound ($\delta_{C\text{-}21}$ 85.5 and $\delta_{C\text{-}22}$ 79.7).[20] Thus, aglycone of **1** was identified as cantoniensistriol, an uncommon sapogenin found in vascular plants.[21,22] Consequently, compound **1** was elucidated as 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl cantoniensistriol 21-*O*-α-L-rhamnopyranoside, a new compound named as derristrifoside A.

Compound **2** (Fig. 1) was isolated as a white amorphous powder. The molecular formular of **2** was determined to be identical with that of **1**, $C_{54}H_{88}O_{23}$, as indicated by a deprotonated molecular ion in the HR-ESI-MS spectrum (*m*/*z* 1103.5624 $[M\text{-}H]^-$; calcd. for $[C_{54}H_{87}O_{23}]^-$, 1103.5638). Moreover, the 1D and 2D NMR spectral data (Table 1 and Fig. 2) of **2** were similar to those of **1**, except that the signals corresponding to a β-galactopyranosyl group (δ_C 104.4, 83.5, 74.8, 69.7, 76.5, and 62.1 in **1**) were replaced by those of an additional β-glucopyranosyl group (δ_C 103.3, 85.4, 76.7, 72.0, 77.5, and 63.3 in **2**). The structural difference in sugar moiety of **2** was elucidated through analysis of the HSQC, HMBC, and COSY spectra. The COSY cross-peak between Glu H-1″ (δ_H 4.49) and Glu H-2″ (δ_H 3.57), the HSQC correlation between Glu H-2″ (δ_H 3.57) and Glu C-2″ (δ_C 82.0), and the HBMC correlation between Glc H-1‴ (δ_H 4.87) and Glu C-2″ (δ_C 82.0) established a structural fragment of a glucopyranosyl-(1→2)-glucuronopyranosyl unit. Similarly, the

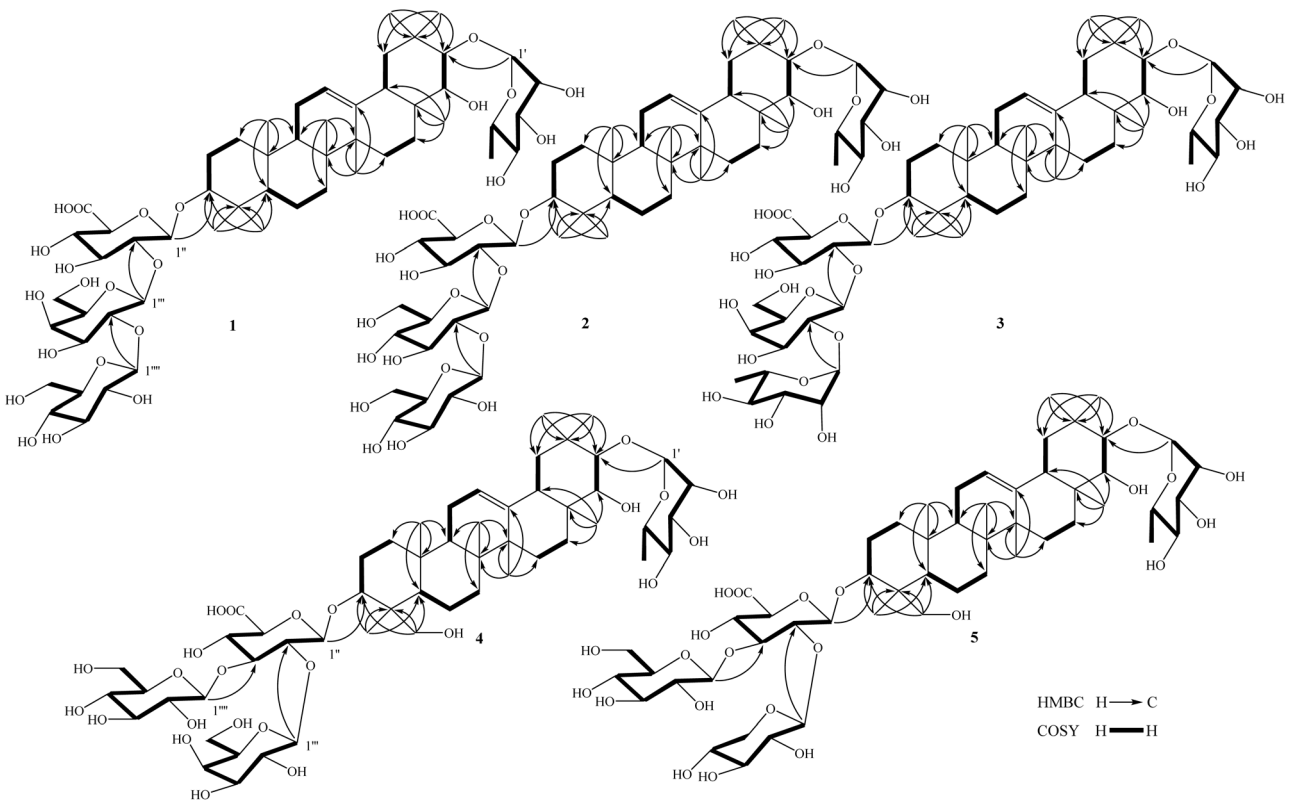

Fig. 2 Key HMBC and COSY correlations of compounds **1**–**5**.

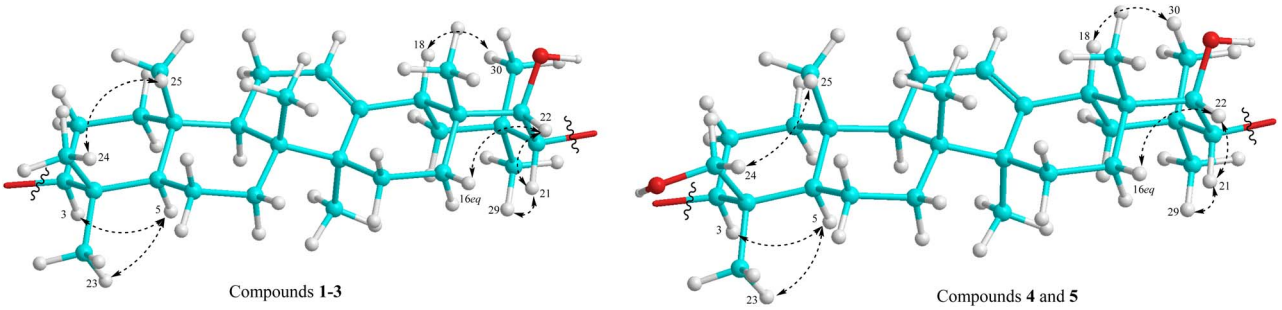

Fig. 3 Important NOESY correlations at aglycone moiety of compounds 1–5.

COSY cross-peak between Glc H-1‴ (δ_H 4.87) and Glc H-2‴ (δ_H 3.42), the HSQC correlation between Glc H-2‴ (δ_H 3.42) and Glc C-2‴ (δ_C 85.4), the HBMC correlation between Glc H-1⁗ (δ_H 4.61) and Glc C-2‴ (δ_C 85.4) demonstrated that a second glucopyranosyl group was attached at C-2‴ of the first glucopyranosyl unit, forming a trisaccharide moiety: glucopyranosyl-(1→2)-glucopyranosyl-(1→2)-glucopyranose. This trisaccharide moiety was linked to C-3 of the aglycone, as confirmed by an HMBC correlation between Glu H-1″ (δ_H 4.49) and C-3 (91.3). Acid hydrolysis of **2** yielded D-glucuronic acid, D-glucose, and L-rhamnose, which were identified by comparison with standard monosaccharides using TLC and confirmed by the positive signs of optical rotations (SI).[18,19] Consequently, compound **2** was identified as 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranosyl cantoniensistriol 21-*O*-α-L-rhamnopyranoside, a new compound named derristrifoside B (Fig. 3).

Compound **3** (Fig. 1) was isolated as a white amorphous powder. The molecular formular of **3** was determined to be $C_{54}H_{88}O_{22}$ based on its deprotonated molecular ion in the HR-ESI-MS spectrum (*m*/*z* 1087.5692 [M-H]⁻; calcd. for $[C_{54}H_{87}O_{22}]^-$, 1087.5694]. The NMR data indicated that compound **3** differed from both **1** and **2** by the sugar moiety attached at C-3 of the aglycone. The ^{1}H-NMR spectrum of **3** displayed a broad singlet carbinol proton (δ_H 3.80, Gal H-4‴), suggesting the presence of galactopyranosyl group similar to that in **1** (δ_H 3.89, Gal H-4‴). In contrast to **1** and **2**, the appearance of an anomeric proton [δ_H 5.22 (d, J = 1.2 Hz)] and additional doublet methyl signals [δ_H 1.26 (d, J = 7.2 Hz)] in the ^{1}H-NMR spectrum suggested replacement of a β-glucopyranosyl group (in **1** and **2**) by an α-rhamnopyranosyl group (in **3**). The COSY cross-peak between Glu H-1″ (δ_H 4.45) and Glu H-2″ (δ_H 3.72), the HSQC correlation between Glu H-2″ (δ_H 3.72) and Glu C-2″ (δ_C 79.1), and the HBMC correlation between Gal H-1‴ (δ_H 4.85) and Glu C-2″ (δ_C 79.1) established a structural fragment of a galactopyranosyl-(1→2)-glucuronopyranosyl group. Similarly, the COSY cross-peak between Gal H-1‴ (δ_H 4.85) and Gal H-2‴ (δ_H 3.70), the HSQC correlation between Gal H-2‴ (δ_H 3.70) and Gal C-2‴ (δ_C 77.3), and the HBMC correlation of Rha H-1⁗ (δ_H 5.22) and Gal C-2‴ (δ_C 77.3) demonstrated that an α-rhamnopyranosyl group was attached at C-2‴ of the galactopyranosyl to form a trisaccharide: rhamnopyranosyl-(1→2)-galactopyranosyl-(1→2)-glucopyranose. This trisaccharide moiety was linked to C-3 of the aglycone, confirmed by an HMBC correlation between Glu H-1″ (δ_H 4.45) and C-3 (δ_C 92.0). Acid hydrolysis of **3** yielded D-glucuronic acid, D-galactose, and L-rhamnose, identified by TLC comparison with authentic standards and optical rotation data. (SI).[18,19] Consequently, compound **3** was characterized as 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl cantoniensistriol 21-*O*-α-L-rhamnopyranoside, a new compound named derristrifoside C.

Compound **4** (Fig. 1) was isolated as a white amorphous powder. The negative-ion HR-ESI-MS spectrum of **4** showed a deprotonated molecular ion corresponding to $C_{54}H_{88}O_{24}$ (*m*/*z* 1119.5570 [M-H]⁻; calcd. for $[C_{54}H_{87}O_{24}]^-$, 1119.5593), indicating 11 indices of hydrogen deficiency. Comparison of the ^{1}H- and ^{13}C-NMR data of **4** with those of **1–3** suggested that **4** was also an olean-12-ene type glycoside. The NMR data showed seven singlet methyl groups [δ_H 1.11 (3H), 1.08 (3H), 0.97 (3H), 0.90 (3H), 0.85 (6H), and 0.81 (3H)] and one oxygenated methylene group (δ_C 62.4/δ_H 3.88 and 3.14), suggesting hydroxylation of one of the eight tertiary methyl groups in the olean-12-ene backbone. The HMBC correlations between H_3-23 (δ_H 1.08) and C-3 (δ_C 89.4)/C-4 (δ_C 43.0)/C-5 (δ_C 55.3)/C-24 (δ_C 62.4) confirmed hydroxylation at C-24. Two other oxygenated groups in the aglycone, an *O*-α-rhamnopyranosyl group at C-21 and a hydroxy group at C-22, were identified similarly to those in **1–3** by COSY, HMBC, and analysis of *J*-coupling patterns. A NOESY correlation between H-5 (δ_H 0.85) and H-3 (δ_H 3.24) indicated axial-alpha orientation of both protons. NOESY correlations between H-3 (δ_H 3.24)/H-5 (δ_H 0.85) and H_3-23 (δ_H 1.08), and between H_2-24 (δ_H 3.88) and H_3-25 (δ_H 0.81) confirmed axial-beta orientation of hydroxymethylene group (C-24). The NOESY correlation between H-18 (δ_H 2.13) and H_3-30 (δ_H 0.97), along with $J_{ax/ax}$ = 11.4 Hz for H-18 (δ_H 2.13) indicated axial-beta orientation for both H-18 and methyl group C-30. The NOESY correlation between H_3-29 (δ_H 0.85) and H-21 (δ_H 3.40), together with broad singlets for both H-21 and H-22 ($J_{ax/eq}$ coupling), suggested alpha-axial and alpha-equatorial orientations for H-21 and H-22, respectively. Therefore, aglycone of **4** was established as 24-hydroxycantoniensistriol. Besides the *O*-α-rhamnopyranosyl group at C-21, the presence of three anomeric protons [δ_H 4.81 (d, J = 7.8 Hz), 4.38 (d, J = 7.8 Hz), and 4.34 (d, J = 7.8 Hz)], a carboxylic carbon (δ_C 173.4), and a broad singlet carbinol proton (δ_H 3.65) indicated that the second sugar moiety was a trisaccharide composed of β-glucopyranose, β-galactopyranosyl, and β-glucopyranosyl units. The COSY cross-

peak between Glu H-1″ (δ_H 4.34) and Glu H-2″ (δ_H 3.37), the HSQC correlation between Glu H-2″ (δ_H 3.37) and Glu C-2″ (δ_C 78.4), and the HBMC correlation between Gal H-1‴ (δ_H 4.81) and Glu C-2″ (δ_C 78.4) established a β-galactopyranosyl-(1→2)-β-glucuronopyranosyl fragment. Additionally, COSY cross-peak between Glu H-2″ (δ_H 3.37) and Glu H-3″ (δ_H 3.50), the HSQC correlation between Glu H-3″ (δ_H 3.50) and Glu C-3″ (δ_C 82.1), and the HBMC correlation between Glc H-1⁗ (δ_H 4.38) and Glu C-3″ (δ_C 82.1) demonstrated that a β-glucopyranosyl group was attached at C-3″ of the glucopyranose units, forming a trisaccharide: β-glucopyranosyl-(1→3)-[β-galactopyranosyl-(1→2)]-β-glucopyranose. This trisaccharide was linked to C-3 of aglycone, as confirmed by an HMBC correlation between Glu H-1″ (δ_H 4.34) and C-3 (δ_C 89.4). Acid hydrolysis of **4** yielded D-glucuronic acid, D-galactose, D-glucose, and L-rhamnose, identified by TLC comparison with standards and optical rotation data (SI).[18,19] Consequently, compound **4** was elucidated as 3-*O*-β-D-glucopyranosyl-(1→3)-[β-D-glalacopyranosyl-(1→2)]-β-D-glucuronopyranosyl-24-hydroxycantoniensistriol 21-*O*-α-L-rhamnopyranoside, a new compound named derristrifoside D.

Compound **5** (Fig. 1) was isolated as a white amorphous powder. The molecular formular of **5** was determined as $C_{53}H_{86}O_{23}$ based on its deprotonated molecular ion in the HR-ESI-MS spectrum (*m*/*z* 1089.5485 $[M\text{-}H]^-$; calcd. for $[C_{53}H_{85}O_{23}]^-$, 1089.5487). Comparison of the NMR data between **4** and **5** indicated that **5** differed from **4** in the trisaccharide moiety attached at C-3 of the aglycone. Specifically, the β-galactopyranosyl group (a hexose) in **4** was replaced by a β-xylopyranosyl group (a pentose) in **5**. The presence of the β-xylopyranosyl group in **5** was supported by the signals of an anomeric carbon/proton [δ_C 101.2/δ_H 4.78 (d, J = 7.8 Hz)], three carbinol carbons (δ_C 72.1, 75.4, and 69.2), and one oxygenated methylene group [δ_C 65.2/δ_H 3.63 and 2.97 (dd, J = 10.2 and 11.4 Hz)]. The COSY cross-peak between Glu H-1″ (δ_H 4.31) and Glu H-2″ (δ_H 3.26), the HSQC correlation between Glu H-2″ (δ_H 3.26) and Glu C-2″ (δ_C 79.0), and the HBMC correlation between Xyl H-1‴ (δ_H 4.78) and Glu C-2″ (δ_C 79.0) established a β-xylopyranosyl-(1→2)-β-glucuronopyranosyl fragment. The COSY cross-peak between Glu H-2″ (δ_H 3.26) and Glu H-3″ (δ_H 3.17), the HSQC correlation between Glu H-3″ (δ_H 3.17) and Glu C-3″ (δ_C 83.8), and the HBMC correlation between Glc H-1⁗ (δ_H 4.36) and Glu C-3″ (δ_C 83.8) demonstrated that a β-glucopyranosyl group was attached at C-3″ of the glucopyranose units, forming a trisaccharide: β-glucopyranosyl-(1→3)-[β-xylopyranosyl-(1→2)]-β-glucopyranose. This trisaccharide was linked to C-3 of the aglycone, as confirmed by an HMBC correlation between Glu H-1″ (δ_H 4.31) and C-3 (δ_C 89.0). Acid hydrolysis of **5** yielded D-glucuronic acid, D-glucose, D-xylose, and L-rhamnose, which were identified by TLC comparison with authentic standards and confirmed by optical rotations (SI).[18,19] Thus, compound **5** was identified as 3-*O*-β-D-glucopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→2)]-β-D-glucuronopyranosyl-24-hydroxy cantoniensistriol 21-*O*-α-L-rhamnopyranoside, a new compound named derristrifoside E.

Compounds **1–5** were evaluated for their effects on nitric oxide (NO) production in LPS-activated RAW 264.7 cells and for cytotoxic activity against SK-LU-1 and Hep-G2 human cancer cell lines (Table 3, SI). Unfortunately, at a concentration of 20 μM, all tested compounds exhibited only weak inhibitory activities toward NO production (inhibitory rates in ranging from of 12.17 ± 1.22% to 17.09 ± 1.16%) and weak cytotoxic effects (cytotoxic rates in ranging from 8.02 ± 0.84% to 23.31 ± 0.56%). Although these compounds showed weak activity in both cytotoxicity and NO inhibition, saponins possessing a cantoniensistriol-type sapogenin framework are rarely found in vascular plants. Therefore, this study represents the first report of the cytotoxic and NO inhibitory activities of cantoniensistriol-type saponins.

Table 3 NO inhibition and cytotoxic effects of compounds **1–5** at a concentration of 20 μM

		Cytotoxicity (%)	
Comp.	NO inhibition (%)	SK-LU-1	Hep-G2
1	12.17 ± 1.22	21.23 ± 1.91	15.15 ± 1.05
2	15.07 ± 0.84	14.68 ± 1.07	8.02 ± 0.84
3	16.67 ± 0.92	23.31 ± 0.56	17.77 ± 1.15
4	16.33 ± 1.03	12.22 ± 1.02	13.40 ± 1.11
5	17.09 ± 1.16	17.59 ± 0.96	12.17 ± 1.07
Dex[a]	54.23 ± 1.17	—	—
Ell[b]	—	78.62 ± 1.25	79.85 ± 1.78

[a] Dexamethasone (20 μM) and. [b] Ellipticine (2.0 μg mL^{-1}) were used as positive control. Data are expressed as mean ± SD of triplicate experiments (n = 3). Statistical significances (P < 0.05) are determined by one-way ANOVA compare to vehicle group.

Conclusions

In conclusion, phytochemical investigation of the leaves of *Derris trifoliata* led to the isolation of five new oleanane-type saponins possessing a cantoniensistriol framework. Their structures were determined based on HR-ESI-MS and extensive NMR analyses. Five isolated compounds exhibited weak inhibitory effects on NO production in LPS-activated RAW 264.7 cells and weak antiproliferative activity against SK-LU-1 and Hep-G2 human cancer cell lines. Our results highlight that the leaves of *Derris trifoliata* contain unusual cantoniensistriol-type saponins. This is also the first report of the cytotoxic and NO production inhibitory activities of these saponins.

Author contributions

BTN Trang, BTM Anh, NT Mai, TT Nga contributed to research idea, isolation and prepare samples for bioassay; PV Kiem, BH Tai, BTT Trang contributed to structure elucidation; BTT Trang, NT Mai, BH Tai, PV Kiem contributed to writing and editing.

Conflicts of interest

The authors declare no conflicts of interest.

Data availability

Supplementary information (SI): HR-ESI-MS and NMR spectra of all new compounds would be found along with manuscript at the Journal home page. See DOI: https://doi.org/10.1039/d5ra08300a.

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