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Isolation and characterization of triterpenoid saponins from leaves of *Aralia nudicaulis* L

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ABSTRACT

Three new oleanolic glycosides (**1**–**3**), along with seven known saponins from various plants (**4**–**10**) were isolated for the first time from the leaves of *Aralia nudicaulis*. Their structures were elucidated on the basis of spectroscopic evidence, including 1D and 2D NMR, and HRESIMS. Nudicauloside A and B (**1**–**2**) have shown moderate anti-inflammatory activity, as demonstrated by inhibition of LPS-induced NO production in raw 264.7 murine macrophages (IC_{50} = 74–101 μM).

1. Introduction

Saponins are ubiquitous in plants. Some families have been studied for centuries for their rich saponin composition, like Caryophyllaceae, Leguminosae and Araliaceae (Sparg et al., 2004). The latter comprise species of the genus *Panax* (ginseng) which were shown to contain several bioactive saponins (Jia and Zhao, 2009). Several species of the genus *Aralia*, especially *A. elata* (Table 1) which is an adaptogen (Panossian et al., 2021), also contain bioactive saponins explaining their broad usage as an herbal remedy in Russia, Korea, Japan and China (Shikov et al., 2016). In the province of Québec in Canada, three species of *Aralia* are indigenous: *A. racemosa*, *A. hispida* and *A. nudicaulis*. The latter was used by aboriginal peoples of Québec as root infusion or decoction to treat several health problems such as stomach pain, kidney disorder, cold, and cough (Moerman, 1998). More recently, polyacetylenes with bioactivity against *Mycobacterium tuberculosis* were isolated from the roots of *A. nudicaulis* (Li et al., 2012). However, the saponin content of *A. nudicaulis* was never reported.

In the last 15 years, we dedicated some of our research effort toward the synthesis of glycosides with molecular scaffold obtained from plant material (Gauthier et al., 2009; Piochon et al., 2009). For this reason, we have a strong interest for plants containing glycosides. In this study, we report the isolation and structural elucidation of three new saponins, nudicauloside A–C (**1**–**3**), from an *Aralia nudicaulis* leaf extract. Furthermore, their anticancer, antimicrobial, antioxidant, and anti-inflammatory activities were evaluated *in vitro*.

2. Results and discussion

2.1. Chemical investigation of the A. nudicaulis leaves extract

The methanolic extract from leaves of *Aralia nudicaulis* was subjected to a number of MS-guided separation steps using Diaion HP-20, silica gel and reversed phase C_{18}. The final purification of the saponin compounds was realized using semi-preparative HPLC, yielding three new (**1**–**3**) and seven known compounds (**4**–**10**) identified for the first time on this specie (Fig. 1). By comparison of NMR spectroscopic data with literature values, the known compounds were identified as hemsgiganoside B (**4**) (Chen et al., 2003), guaiacin B (**5**) (Shaker et al., 1999), araliasaponin I (**6**) (Song et al., 2000), 3-*O*-[β-D-glucopyranosyl-(1→2)]-[β-D-glucopyranosyl-(1→3)]-- α-L-arabinopyranosyl hederagenin (**7**) (also known as prosapogenin) (Chen et al., 1997), araliasaponin XII (**8**) (Ahmad et al., 1990), araliasaponin IX (**9**) (Song et al., 2001), and araliasaponin III (**10**) (Song et al., 2000).

2.2. Compound 1

Compound **1** was isolated as a white amorphous powder with a molecular formula determined as $C_{53}H_{86}O_{22}$ based on a HRESIMS

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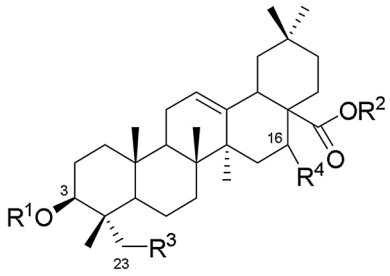
Table 1
Species of *Aralia* known for their saponin content.

Species	Part	Reference
A. elata	leaf	Zhang et al. (2013)
	root bark	Song et al. (2001), Lee et al. (2005)
	bark	Nhiem et al. (2011)
A. cordata	aerial	Kawai et al. (1989), Ik et al. (2006)
A. spinifolia	root	Yu et al. (1994)
A. armata	root bark	Hu et al. (1995)
A. continentalis	aerial	Kim and Kang (1998)
A. taibaiensis	root bark	Bi et al. (2012)
A. subcapitata	root	Zou et al. (2001)
A. dasyphylla	root bark	Xiao et al. (1999)
A. decaisneana	root	Miyase et al. (1996a)
	bark	Tian et al. (2006)
A. echinocaulis	root	Li et al. (2016)
A. chinensis	root	Miyase et al. (1996b)



	R1	R2	R3	R4
1	β-D-Glc(1→3)β-D-Glc(1→3)α-L-Arap	β-D-Glc	H	H
2	β-D-Glc(1→3)β-D-Glc(1→3)[β-D-Glc(1→2)]α-L-Arap	β-D-Glc	H	H
3	β-D-Glc(1→3)β-D-Glc(1→3)[β-D-Glc(1→2)]α-L-Arap	β-D-Glc	OH	H
4	β-D-GlcA	β-D-Glc(1→6)β-D-Glc	H	H
5	β-D-Glc(1→3)α-L-Arap	β-D-Glc	H	H
6	β-D-Glc(1→3)α-L-Arap	β-D-Glc	H	OH
7	β-D-Glc(1→3)[β-D-Glc(1→2)]α-L-Arap	H	OH	H
8	β-D-Glc(1→3)[β-D-Glc(1→2)]α-L-Arap	β-D-Glc	H	H
9	β-D-Glc(1→3)[β-D-Glc(1→2)]α-L-Arap	β-D-Glc	OH	H
10	β-D-Glc(1→3)β-D-Glc(1→3)α-L-Arap	β-D-Glc	H	H

Fig. 1. Structure of compounds1–10 from *Aralia nudicaulis*.

sodiated adduct at *m/z* 1097.5503. The ^{1}H NMR spectrum shows typical resonances for saponins with four anomeric protons at δ_H 5.38 (d, J =8.1 Hz, H-1^{IV}), 4.62 (d, J =7.7 Hz, H-1^{II}), 4.57 (d, J =7.8 Hz, H-1^{III}) and 4.28 (d, J =7.3 Hz, H-1^{I}), one olefinic proton at δ_H 5.25 (t, J =3.7 Hz, H-12), and seven methyl groups at δ_H 1.16 (s, H$_3$-27), 1.05 (s, H$_3$-23), 0.95 (s, H$_3$-25), 0.93 (s, H$_3$-30), 0.91 (s, H$_3$-29), 0.84 (s, H$_3$-24), 0.80 (s, H$_3$-26) (Table 2). The ^{13}C NMR spectrum indicated one carbonyl carbon at δ_C 178.0 (C-28) and a pair of sp^2 carbon at δ_C 144.9 (C-13) and 123.8 (C-12). After careful examination of COSY, HSQC and HMBC 2D NMR spectra, the aglycone of **1** could be identified as an oleanolic acid, typical of most saponins isolated from *Aralia* (Ma et al., 2013; Zhang et al., 2013). The remaining 23 carbon signals accounted for three hexoses and one pentose. The ^{1}H and ^{13}C chemical shifts were assigned for each sugar using HSQC, COSY, and a series of 1D TOCSY spectra obtained by selectively exciting the anomeric protons and using an increasing mixing time (Table 3). With the help of literature data (Agrawal, 1992), and considering that all anomeric signals were doublets with J values > 7.5 Hz, three β-glucopyranoside and one

Table 2
^{1}H and ^{13}C NMR spectroscopic data for the aglycone part of 1–3.[a]

position	1[b]		2[c]		3[c]	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	39.9 t	1.62 m 0.98 m	38.8 t	1.47 m 0.88 m	39.2 t	1.52 m 1.01 m
2	27.1 t	1.83 m 1.70 m	26.6 t	2.04 m 1.80 m	26.4 t	2.13 m 1.94 m
3	90.5 d	3.15 dd (11.7, 4.3)	89.0 d	3.23 m	82.7 d	4.16 m
4	40.2 s	–	39.7 s	–	43.9 s	
5	57.1 d	0.78 m	55.9 d	0.74 d (11.4)	48.2 d	1.60 m
6	19.3 t	1.54 m 1.41 m	18.5 t	1.48 m 1.31 m	18.5 t	1.68 m 1.36 m
7	34.0 t	1.48 m 1.32 m	33.2 t	1.43 m 1.32 m	33.2 t	1.57 m 1.30 m
8	40.7 s	–	39.9 s	–	40.3 s	
9	49.0 d	1.58 m	48.1 d	1.61 m	48.5 d	1.73 m
10	37.9 s	–	37.0 s	–	37.2 s	
11	24.6 t	1.89 m	23.8 t	1.88 m	24.2 t	1.91 m
12	123.8 d	5.25 t (3.7)	122.9 d	5.44 t (3.5)	123.3 d	5.42 br s
13	144.9 s	–	144.1 s	–	144.4 s	
14	42.9 s	–	42.1 s	–	42.5 s	
15	28.9 t	1.80 m 1.08 m	28.3 t	2.35 m 1.18 m	28.6 t	2.33 m 1.10 m
16	24.0 t	2.05 m 1.72 m	23.4 t	2.08 m 1.97 m	23.7 t	2.04 m 1.93 m
17	48.0 s	–	47.0 s	–	47.3 s	
18	42.6 d	2.85 dd (13.6, 4.8)	41.8 d	3.21 m	42.1 d	3.19 dd (13.9, 4.9)
19	47.2 t	1.70 m 1.15 m	46.2 t	1.77 m 1.26 m	46.5 t	1.73 m 1.24 m
20	31.5 s	–	30.8 s	–	31.1 s	
21	34.9 t	1.40 m 1.22 m	34.0 t	1.36 m 1.10 m	34.3 t	1.33 m 1.08 m
22	33.1 t	1.74 m 1.61 m	32.6 t	1.83 m 1.76 m	32.9 t	1.82 m 1.74 m 4.29 m
23	28.5 q	1.05 s	28.1 q	1.25 s	65.0 t	3.70 d (10.5)
24	17.0 q	0.84 s	16.7 q	1.08 s	13.7 q	1.06 s
25	16.0 q	0.95 s	15.6 q	0.83 s	16.5 q	0.93 s
26	17.7 q	0.80 s	17.5 q	1.10 s	17.9 q	1.12 s
27	26.3 q	1.16 s	26.1 q	1.25 s	26.4 q	1.18 s
28	178.0 s	–	176.4 s	–	176.8 s	
29	33.5 q	0.91 s	33.2 q	0.92 s	33.5 q	0.90 s
30	24.0 q	0.93 s	23.7 q	0.89 s	24.0 q	0.88 s

[a] Measured at 400 MHz for **1**–**2**, or 800 MHz for **3**.
[b] Recorded in CD_3OD.
[c] Recorded in pyridine-d_5.

α-arabinopyranoside could be identified. To further confirm this conclusion, an acid hydrolysis of **1** was performed and the sugars were acetylated and analyzed by chiral GC-FID. The retention time of the sugars matched those for D-glucose and L-arabinose. Finally, the linkage was elucidated with HMBC correlations from H-1^{I} to δ_C 90.5 (C-3), from H-1^{II} to δ_C 83.9 (C-3^{I}), from H-1^{III} to δ_C 87.4 (C-3^{II}), and from H-1^{IV} to δ_C 178.0 (C-28). From all the above evidence, compound **1**, named nudicauloside A, was identified as β-D-glucopyranosyl 3β-*O*-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl]-olean-12-en-28-oate.

2.3. Compound 2

Compound **2**, obtained as a white amorphous powder, exhibited a pseudo-molecular sodium adduct in its HRESIMS spectrum at *m/z* 1275.5978, indicative of the molecular formula $C_{59}H_{96}O_{27}$. The ^{1}H NMR spectra revealed only four anomeric proton signals at δ_H 6.37 (d, J =8.0 Hz, H-1^{IV}), 5.50 (d, J =7.7 Hz, H-1^{V}), 5.32 (d, J =7.8 Hz, H-1^{II}) and 5.20 (d, J =7.8 Hz, H-1^{III}), along with an olefinic proton at δ_H 5.44 (t, J =3.5 Hz, H-1). The HSQC spectrum showed a correlation at δ_H 4.77 (m, H-1^{I}) and δ_C 105.8 which was assigned to the anomeric proton of an additional sugar moiety. Analysis of the 2D NMR spectra revealed that the aglycone of **2** was also an oleanolic acid. Literature data (Agrawal et al., 1995) and acid hydrolysis allowed the identification of four D-glucopyranosyl and one L-arabinopyranoside moieties with linkage established from HMBC correlations between H-1^{I} and δ_C 89.3 (C-3), H-1^{II} and δ_C 83.6 (C-3^{I}), H-1^{III} and δ_C 88.3 (C-3^{II}), H-1^{IV} and δ_C 176.8 (C-28), and H-1^{V} and δ_C 77.6 (C-2^{I}). Therefore, compound **2**, named nudicauloside B, was identified as 3β-*O*-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl]-olean-12-en-28-oate.

Table 3
^{1}H and ^{13}C NMR spectroscopic data for the sugar moieties of 1–3.[a]

position	**1**[b]		**2**[c]		**3**[c]	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
3-*O*-α-L-Ara*p*						
1^{I}	107.1 d	4.28 d (7.3)	105.4 d	4.77 m	104.7 d	5.00 d (6.8)
2^{I}	72.1 d	3.71 m	77.3 d	4.74 m	77.7 d	4.70 t (7.8)
3^{I}	83.9 d	3.66 m	83.3 d	4.32 m	83.8 d	4.15 m
4^{I}	69.6 d	4.03 m	68.8 d	4.48 m	69.0 d	4.41 m
5^{I}	66.7 t	3.86 m 3.55 m	66.0 t	4.16 m 3.65 m	66.2 t	4.11 m 3.52 d (11.2)
β-D-Glc at *O*-3 of α-L-Ara*p*						
1^{II}	105.0 d	4.62 d (7.7)	104.6 d	5.32 d (7.8)	104.8 d	5.24 d (7.7)
2^{II}	74.8 d	3.51 m	73.9 d	4.01 m	74.3 d	4.01 m
3^{II}	87.4 d	3.59 m	87.9 d	4.22 m	88.2 d	4.25 m
4^{II}	69.7 d	3.47 m	69.7 d	4.11 m	70.0 d	4.11 m
5^{II}	78.2 d	3.32 m	78.1 d	3.90 m	78.4 d	3.90 m
6^{II}	62.3 t	3.85 m 3.69 m	62.2 t	4.41 m 4.24 m	62.5 t	4.41 m 4.25 m
β-D-Glc at *O*-3 of β-D-Glc						
1^{III}	105.3 d	4.57 d (7.8)	105.5 d	5.20 d (7.8)	105.9 d	5.21 d (7.9)
2^{III}	75.6 d	3.28 m	75.4 d	4.05 m	75.7 d	4.06 m
3^{III}	77.8 d	3.39 m	78.1 d	4.19 m	78.4 d	4.21 m
4^{III}	71.6 d	3.28 m	71.5 d	4.19 m	71.8 d	4.19 m
5^{III}	78.7 d	3.35 m	78.6 d	3.96 m	78.9 d	3.97 m
6^{III}	62.7 t	3.89 m 3.64 m	62.4 t	4.53 m 4.31 m	62.8 t	4.53 d (10.3) 4.31 m
28-*O*-β-D-Glc						
1^{IV}	95.7 d	5.38 d (8.1)	95.8 d	6.37 d (8.0)	96.1 d	6.34 d (8.0)
2^{IV}	73.9 d	3.32 m	74.2 d	4.22 m	74.5 d	4.21 m
3^{IV}	78.3 d	3.41 m	78.9 d	4.30 m	79.3 d	4.30 m
4^{IV}	71.1 d	3.37 m	71.1 d	4.38 m	71.4 d	4.38 m
5^{IV}	77.6 d	3.33 m	79.4 d	4.05 m	79.7 d	4.04 m
6^{IV}	62.4 t	3.82 m 3.68 m	62.2 t	4.48 m 4.42 m	62.5 t	4.48 d (10.2) 4.42 m
β-D-Glc at *O*-2 of α-L-Ara*p*						
1^{V}			104.3 d	5.50 d (7.7)	104.6 d	5.48 d (7.8)
2^{V}			76.2 d	4.06 m	76.5 d	4.08 m
3^{V}			78.6 d	4.19 m	78.9 d	4.18 m
4^{V}			72.4 d	4.18 m	72.3 d	4.18 m
5^{V}			77.4 d	3.70 m	77.8 d	3.67 m
6^{V}			63.2 t	4.36 m 4.30 m	63.3 t	4.36 m 4.29 m

[a] Measured at 400 MHz for **1**–**2**, or 800 MHz for **3**.
[b] Recorded in CD_3OD.
[c] Recorded in pyridine-d_5.

2.4. Compound 3

Compound **3** was obtained as a white amorphous solid, and its molecular formula was established as $C_{59}H_{96}O_{28}$ based on a pseudo-molecular sodiated ion in its HRESIMS spectrum at *m/z* 1275.5980. A close inspection of the 2D NMR spectra revealed the same sugar pattern as for **2**, with almost the same chemical shifts for ^{1}H and ^{13}C. This assertion was further confirmed by acid hydrolysis of **3** yielding the same saccharide species as for **2**. The largest difference was observed for the anomeric proton of arabinose, which was suspected of being linked to a different aglycone. The latter was identified as hederagenin, based on the following evidence: an additional primary alcohol at δ_H 4.29 (m, H_2-23) and 3.70 (d, J =10.5 Hz, H_2-23), together with the methyl at 1.06 (s, H_3-24), showed HMBC correlations to δ_C 82.5 (C-3), 43.3 (C-4) and 48.2 (C-5). Additionally, NOESY crosspeaks between H_2-23, H-6_{eq} and H-5, H-3 and H-5, H-5 and H-9, and H-9 and H_3-27 allowed to assign all these protons on the α face of the aglycone, while the protons of the β face were located from correlations between H_3-24 and H_3-25, H_3-25 and H_3-26, and H-18 and H_3-30. From all the above evidence, nudicauloside C (**3**) was identified as 3β-*O*-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl]-23-hydroxyolean-12-en-28-oate.

2.5. Evaluation of pharmacological activities

Nudicauloside A–C (**1**–**3**) were subjected to several biological assays to evaluate their pharmaceutical and cosmeceutical potential (Table 4). At first, we have evaluated the biological activities of isolated compounds (**1**–**10**) including cytotoxicity against human cancer cell lines A549, DLD-1 and healthy human skin fibroblasts (WS1); antimicrobial activities against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. The results show that all compounds tested are inactive at the highest concentration of 160 μM. Kuang et al. (2013) found cytotoxicity against A549 with analogs of **1**–**3** having a β-D-glucopyranosyl in place of the α-L-arabinopyranosyl at C-3, suggesting the arabinose moiety would be detrimental for this activity. The anti-inflammatory activity of *Aralia* extracts was already reported (Lee et al., 2009; Suh et al., 2007). For this reason, the inhibition of LPS-induced NO production by **1**–**3** in raw 264.7 murine macrophages was studied (Table 4). Moderate activities were obtained for **1** and **2** with IC_{50} values of 74 ± 28 μM and 101 ± 20 μM respectively.

3. Experimental

3.1. General experimental procedures

Styrene-divinylbenzene polymer (Diaion HP-20, Sigma-Aldrich) and silica gel (Silicycle, 40–63 μm) adsorbents were used for column chromatography. Low-pressure liquid chromatography was achieved using a Sepacore flash system (Buchi) equipped with two pumps, a UV detector and a fraction collector. Since saponins rarely have chromophore, the fraction collector was set in time mode. TLC analyses were performed on precoated silica gel plates (Kieselgel 60F_{254}, Merck) using a $CHCl_3$/

Table 4
Pharmacological activities of 1–3.

	1	2	3	Positive control[a]
Cytotoxicity IC_{50} (μM)				
A549	> 160	> 160	> 160	13 ± 2
DLD-1	> 160	> 160	> 160	6.2 ± 0.8
WS1	> 160	> 160	> 160	6 ± 2
Antimicrobial IC_{50} (μM)				
E. coli	> 160	N.D.	> 160	0.012 ± 0.001
S. aureus	> 160	N.D.	> 160	0.023 ± 0.005
C. albicans	> 160	N.D.	> 160	1.1 ± 0.1
Antioxidant				
ORAC (μmol TE/μmol)	N.D.	N.D.	0.35 ± 0.09	24 ± 3
Cell based antioxidant	N.D.	N.D.	93 ± 22	0.081 ± 0.005
Anti-inflammatory IC_{50} (μM)	74 ± 28	101 ± 20	> 130	

[a] Positive controls were etoposide for A549, DLD-1, and WS1; gentamycin for *E. coli* and *S. aureus*, amphotericin B for *C. albicans*, L-NAME for anti-inflammatory, and quercetin for antioxidant. N.D.: not determined.

$MeOH/H_2O$ (26:14:3) solvent systems. Spots were detected by spraying the plates with 20 % H_2SO_4 in MeOH, followed by heating at 110 °C for 10 min. Saponins content was monitored by LC–MS using an 1100 LC unit and a G1946 VL MS instrument (Agilent Technologies). A dual ESI and APCI ionization source ran in negative mode was used. A Kinetex XB-C18 HPLC column (Phenomenex, 250 × 4.6 mm, 5 μm particle size) was used with 1 mL/min of H_2O and MeCN, both acidified with 0.1 % HCOOH. Semi-preparative HPLC purifications were performed on an Agilent 1100 Series equipped with two pumps, an autosampler, a UV detector, and a fraction collector. The column used for separations was a Kinetex XB-C18 (Phenomenex, 250 × 21.2 mm, 5 μm particle size). Solutions of HCOOH 0.1 % in H_2O (solvent A) and HCOOH 0.1 % in MeCN (solvent B) were used as eluents with a flow rate of 20 mL/min. Optical rotations were measured in methanol at 589 nm using an Autopol IV polarimeter (Rudolph Research Analytical). FTIR spectra were recorded from a thin film of compounds deposited on a NaCl windows, and using a Cary 630 instrument (Agilent Technologies). Chiral separations of acetylated monosaccharides were performed on a 7890A GC-FID instrument (Agilent Technologies) using a Chiramix column (GL Sciences, 30 m × 0.25 mm ×0.25 μm), and helium as the carrier gas (flow 1 mL/min). The injector temperature was maintained at 230 °C, while the GC oven was kept at 120 °C for 1 min and raised at a rate of 4 °C/min up to 180 °C, which was held constant for 120 min. NMR spectra (^1H, ^{13}C, DEPT-135, DQF-COSY, edited-HSQC, HMBC and NOESY) were recorded on a 9.4 T instrument (400 MHz for ^1H and 100 MHz for ^{13}C), equipped with a QNP 5 mm probe (Bruker) or a 18.8 T instrument (800 MHz for ^1H and 200 MHz for ^{13}C), equipped with a 5 mm triple resonance broadband cryoprobe (Bruker). Spectra were acquired in pyridine-d_5 or methanol-d_4, and chemical shifts were reported in ppm (δ) relative to the residual solvent peaks (Gottlieb et al., 1997). All spectra were processed using MestReNova 12.0. High resolution mass spectrometry was conducted on a 6210 Q-TOF spectrometer (Agilent Technologies) equipped with an electrospray source operating in the positive ion mode.

3.2. Plant material

The leaves of *Aralia nudicaulis* L. were collected in June and July 2016, near Laterrière, Québec, Canada (48°23′07″ N, 71°25′07″ W), and dried in the shade. The plant was authenticated by Mr Patrick Nadeau (Département des sciences fondamentales, Université du Québec à Chicoutimi). A voucher specimen (No. QFA0297432) has been deposited at the Louis-Marie herbarium of Laval University (Québec).

3.3. Extraction and isolation

Dried and powdered leaves from *A. nudicaulis* (1.1 kg) were refluxed sequentially in DCM (3 × 5 L), and MeOH (3 × 5 L), each time for 90 min. The solutions were filtered and concentrated under vacuum yielding two fractions (DCM, 41.4 g; MeOH, 168 g). An aliquot from the MeOH extract (45.3 g) was suspended in 500 mL H_2O and partitioned using 3 L of EtOAc (A, 4.5 g), then 3 L of *n*-BuOH (B, 9.2 g). An aliquot of the BuOH extract (8.5 g) was separated by CC using Diaion HP-20 and a step gradient of H_2O/MeOH (1:0 → 0:1) yielding five fractions (B1–B5). Fraction B4 (3.0 g) was subjected to flash chromatography on silica gel and eluting isocratically with $CHCl_3/MeOH/H_2O$ 26:14:3 to afford five fractions (B4.1–B4.5). Fraction B4.2 was separated on CC using silica gel and $CHCl_3/MeOH/H_2O$ 50:15:1 as eluent yielding nine fractions (B4.2.1–B4.2.9). Fraction B4.2.4 was purified by HPLC with 40 % of eluent B to afford pure **5** (21.7 mg). Compounds **6** (17.2 mg) and **7** (5.8 mg) were obtained after purification of fraction B4.2.5 by HPLC using 40 % of eluent B. Fraction B4.2.6 was purified by HPLC using 40 % of eluent B to afford compound **1** (39.3 mg). Fraction B4.2.7 was subjected to HPLC purification with 40 % of eluent B yielding pure **10** (17.9 mg). Compound **8** (21.5 mg) was obtained from fraction B4.2.8 following HPLC purification with 40 % of eluent B. Fraction B4.4 was separated by CC on silica gel using $CHCl_3/MeOH/H_2O$ 48:20:3 as the eluent yielding five fractions (B4.4.1–B4.4.5), along with pure **4** (158.2 mg) and **3** (69.9 mg). Fraction B4.4.4 was subjected to HPLC separation with 36 % of eluent B to afford compounds **2** (25.7 mg) and **9** (21.7 mg). Fraction B4.5 was purified by HPLC using a gradient of 20 % B → 37 %B in 30 min to afford more **4** (83.6 mg)

3.3.1. Nudicauloside A (1)

White amorphous solid; $[\alpha]_D^{25}$ +25.7° (*c* 0.19, MeOH); IR (film) ν_{max}: 3307, 2942, 2831, 1449, 1416, 1113, 1021 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; HRESIMS *m/z* 1097.5560 $[M + Na]^+$ (calcd for $C_{53}H_{86}O_{22}Na^+$ 1097.5503).

3.3.2. Nudicauloside B (2)

White amorphous solid; $[\alpha]_D^{25}$ +16.4 (*c* 0.23, MeOH); IR (film) ν_{max} 3354, 2941, 2878, 1745, 1160, 1073, 1028 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; HRESIMS *m/z* 1259.6013 $[M + Na]^+$ (calcd for $C_{59}H_{96}O_{27}Na^+$ 1259.6031).

3.3.3. Nudicauloside C (3)

White amorphous solid; $[\alpha]_D^{25}$ +15.9° (*c* 0.41, MeOH); IR (film) ν_{max} 3370, 2942, 2361, 1747, 1625, 1074, 1030 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; HRESIMS *m/z* 1275.5978 $[M + Na]^+$ (calcd for $C_{59}H_{96}O_{28}Na^+$ 1275.5980).

3.4. Acid hydrolysis

Compounds **1**–**3** (3 mg of each) were refluxed in aqueous HCl (10 % v/v, 10 mL) during 4 h. Aglycons were recovered with $CHCl_3$ and the aqueous layers were neutralized with *N,N*-dioctylmethylamine (10 % in $CHCl_3$). The sugar residues were acetylated with pyridine/acetic anhydride (1:1, 2 mL) under agitation during 24 h. The monosaccharide acetates were extracted with EtOAc (3 mL), and the organic fraction was washed successively with sat. aqueous $NaHCO_3$ (3 × 2 mL), H_2SO_4 20 % (3 × 2 mL) and water (3 × 1 mL). The samples were subjected to chiral GC-FID analysis and compared to retention times of monosaccharide acetates synthesized from authentic samples.

3.5. Pharmacological assays

3.5.1. Cell culture

Human skin fibroblasts (WS1, ATCC CRL-1502), human lung carcinoma (A549, ATCC CCL-185), human colon adenocarcinoma (DLD-1,

ATCC CCL-221), and RAW 264.7 murine macrophage (ATCC TIB-71) were cultured in Dulbecco's minimum essential medium (DMEM) with Earle's salts and L-glutamine (Mediatech Cellgro), to which were added vitamins, sodium pyruvate and non-essential amino acids (all at a 1:100 *v/v* dilution of supplied solutions), 10 % fetal bovine serum (Hyclone), penicillin (100 IU/mL) and streptomycin (100 mg/mL). Cells were maintained at 37 °C in a humidified environment containing 5 % CO_2.

3.5.2. Cytotoxic assay

Exponentially growing cells (WS1, A549 and DLD-1) were transferred in 96-well microplates (Costar, Corning Inc.) at a density of 5×10^3 cells/well in 100 µL of culture medium and were allowed to adhere for 16 h before treatment. Increasing concentrations of each compound in DMSO were diluted with culture medium and added to the wells (100 µL/well). The final concentration of DMSO in the culture medium was maintained at 0.5 % to avoid solvent toxicity. Cytotoxicity was assessed after incubation for 48 h and using resazurin (λ_{ex} 530 nm, λ_{em} 590 nm) (O'Brien et al., 2000) on an automated 96-well Fluoroskan Ascent F1 plate reader (Labsystems). Fluorescence was proportional to the cellular metabolic activity in each well. Survival percentages were defined as the fluorescence in experimental wells compared to that in control wells after subtraction of blank values. Cytotoxicity results were expressed as means ± standard deviation and represent the concentration inhibiting 50 % of cell growth (IC_{50}). The values were compared to etoposide, used as a positive control.

3.5.3. Anti-inflammatory activity

Exponentially growing RAW 264.7 murine macrophages were transferred in 96-well microplates (Falcon, BD) at a density of 7.5×10^4 cells/well in 100 µL of culture medium and were allowed to adhere overnight. Cells were then incubated for 24 h with either culture medium (blank), L-NAME (positive control, 250 µM) or increasing concentrations of **1**–**3** dissolved in DMSO. The final concentration of solvent in the culture medium was maintained at 0.25 %. Cells were then stimulated with 100 ng/mL LPS and incubated for 24 h. Cell-free supernatants were collected, and the NO concentration was immediately determined using the Griess reaction (Green et al., 1990) with minor modifications (Legault et al., 2010).

3.5.4. Antioxidant activity

The procedure was modified from the method described by Ou et al. (2001). Briefly, the ORAC assay was carried out with a Fluoroskan Ascent Fl™ plate reader (Labsystems). Trolox was used as a control standard. The experiment was conducted at 37.5 °C and pH 7.4, with a blank sample in parallel. The fluorimeter was programed to record the fluorescence of fluorescein every 1 min after addition of 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH). The final results were calculated by comparing the net areas under the fluorescein decay curves between the blank and the samples. ORAC values were expressed in micromoles of Trolox equivalents (TE) per milligram (µmol TE/mg). The cell-based assay was evaluated using the DCFH-DA, as described by Girard-Lalancette (Coté et al., 2017; Girard-Lalancette et al., 2009). Human skin fibroblasts WS1 were incubated with compounds dissolved in DMSO at different concentrations. Quercetin was used as a positive control and expressed with the IC_{50} values.

3.5.5. Antibacterial and antifungal activities

Antibacterial and antifungal tests were performed following the same method described by Banfi with some modifications (Banfi et al., 2003; Georges et al., 2012). The compounds were incubated with different strains depending on the kind of test. Antibacterial activity was measured comparing *E. coli* and *S. aureus* results with gentamycin as reference. Instead of the antifungal activity, which one is evaluated on *C. albicans* and compared to amphotericin B. Results are all expressed at the IC_{50}.

4. Conclusion

This research has allowed the identification of ten saponins, including three new ones. Their chemical structures have been established from 1D and 2D NMR spectroscopy, HRESIMS, and chemical degradation. The isolated compounds were not found active in cytotoxicity and antimicrobial assays, but two of them (**1** and **2**) showed moderate anti-inflammatory activities, as demonstrated by inhibition of LPS-induced NO production in raw 264.7 murine macrophages.

Authors' contributions

JP, DR and QL carried out this study. SL, VM, JL and AP designed the experiments and supervised the work. SL, JP, JL and AP wrote the manuscript. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.phytol.2021.04.004.

References

Agrawal, P.K., 1992. NMR Spectroscopy in the structural elucidation of oligosaccharides and glycosides. Phytochemistry 31, 3307–3330.
Agrawal, P.K., Jain, D.C., Pathak, A.K., 1995. NMR Spectroscopy of steroidal sapogenins and steroidal saponins: an update. Magn. Reson. Chem. 33, 923–953.
Ahmad, V.U., Perveen, S., Bano, S., 1990. Saponins from the leaves of Guaiacum officinale. Phytochemistry 29, 3287–3290.
Banfi, E., Scialino, G., Monti-Bragadin, C., 2003. Development of a microdilution method to evaluate *Mycobacterium tuberculosis* drug susceptibility. J. Antimicrob. Chemother. 52, 796–800.
Bi, L., Tian, X., Dou, F., Hong, L., Tang, H., Wang, S., 2012. New antioxidant and antiglycation active triterpenoid saponins from the root bark of Aralia taibaiensis. Fitoterapia 83, 234–240.
Chen, M., Wu, W.W., Nanz, D., Sticher, O., 1997. Leonticins D-H, five triterpene saponins from Leontice kiangnanensis. Phytochemistry 44, 497–504.
Chen, Y., Chiu, M.H., Gu, K., Li, Z.R., 2003. Cucurbitacin and triterpenoid glycosides from Hemsleya giganthy. Chin. Chem. Lett. 14, 475–478.
Coté, H., Boucher, M.A., Pichette, A., Legault, J., 2017. Anti-inflammatory, antioxidant, antibiotic, and cytotoxic activities of Tanacetum vulgare L. essential oil and its constituents. Medicines 4.
Gauthier, C., Legault, J., Rondeau, S., Pichette, A., 2009. Synthesis of betulinic acid acyl glucuronide for application in anticancer prodrug monotherapy. Tetrahedron Lett. 50, 988–991.
Georges, P., Legault, J., Lavoie, S., Grenon, C., Pichette, A., 2012. Diterpenoids from the buds of *Pinus banksiana* lamb. Molecules 17, 9716–9727.
Girard-Lalancette, K., Pichette, A., Legault, J., 2009. Sensitive cell-based assay using DCFH oxidation for the determination of pro- and antioxidant properties of compounds and mixtures: analysis of fruit and vegetable juices. Food Chem. 115, 720–726.
Gottlieb, H.E., Kotlyar, V., Nudelman, A., 1997. NMR chemical shifts of common laboratory solvents as trace impurities. J. Org. Chem. 62, 7512–7515.
Green, S.J., Meltzer, M.S., Hibbs Jr, J.B., Nacy, C.A., 1990. Activated macrophages destroy intracellular Leishmania major amastigotes by an L-arginine-dependent killing mechanism. J. Immunol. 144, 278–283.
Hu, M., Ogawa, K., Sashida, Y., Xiao, P.-G., 1995. Triterpenoid glucuronide saponins from root bark of Aralia armata. Phytochemistry 39, 179–184.
Ik, S.L., Jin, W., Zhang, X., Tran, M.H., Kyung, S.S., Yeon, H.S., Bae, K., 2006. Cytotoxic and COX-2 inhibitory constituents from the aerial parts of Aralia cordata. Arch. Pharmacal Res. 29, 548–555.

Jia, L., Zhao, Y., 2009. Current evaluation of the millennium phytomedicine- ginseng (I): etymology, pharmacognosy, phytochemistry, market and regulations. Curr. Med. Chem. 16, 2475–2484.
Kawai, H., Nishida, M., Tashiro, Y., Kuroyanagi, M., Ueno, A., Satake, M., 1989. Studies on the structures of udosaponins A,B,C,D,E, and F from Aralia cordata Thunb. Chem. Pharm. Bull. 37, 2318–2321.
Kim, J.S., Kang, S.S., 1998. Saponins from the aerial parts of Aralia continentalis. News Physiol. Sci. 4, 45–50.
Kuang, H.X., Wang, Z.B., Wang, Q.H., Yang, B.Y., Xiao, H.B., Okada, Y., Okuyama, T., 2013. Triterpene glucosides from the leaves of aralia elata and their cytotoxic activities. Chem. Biodivers. 10, 703–710.
Lee, E.B., Kim, O.J., Kang, S.S., Jeong, C., 2005. Araloside A, an antiulcer constituent from the root bark of Aralia elata. Biol. Pharm. Bull. 28, 523–526.
Lee, J.H., Ha, Y.W., Jeong, C.S., Kim, Y.S., Park, Y., 2009. Isolation and tandem mass fragmentations of an anti-inflammatory compound from aralia elata. Arch. Pharmacal Res. 32, 831–840.
Legault, J., Girard-Lalancette, K., Grenon, C., Dussault, C., Pichette, A., 2010. Antioxidant activity, inhibition of nitric oxide overproduction, and in vitro antiproliferative effect of maple sap and syrup from Acer saccharum. J. Med. Food 13, 460–468.
Li, H., O'Neill, T., Webster, D., Johnson, J.A., Gray, C.A., 2012. Anti-mycobacterial diynes from the Canadian medicinal plant Aralia nudicaulis. J. Ethnopharmacol. 140, 141–144.
Li, Y., Yue, R., Liu, R., Zhang, L., Wang, M., 2016. Secondary metabolites from the root of Aralia echinocaulis Hand. -Mazz. Rec. Nat. Prod. 10, 639–644.
Ma, Z.Q., Zhang, Y., Cai, C.K., Li, Q., Ni, J., 2013. Two new triterpenoid saponins from the leaves of Aralia elata. J. Asian Nat. Prod. Res. 15, 849–854.
Miyase, T., Shiokawa, K.-I., Zhang, D.M., Ueno, A., 1996a. Araliasaponins I-IX, triterpene saponins from the roos of *Aralia decaisneana*. Phytochemistry 41, 1411–1418.
Miyase, T., Sutoh, N., Zhang, D.M., Ueno, A., 1996b. Araliasaponins XII-XVIII, triterpene saponins from the roots of Aralia chinensis. Phytochemistry 42, 1123–1130.
Moerman, D.E., 1998. Native American Ethnobotany. Timber Press Inc.
Nhiem, N.X., Lim, H.Y., Kiem, P.V., Minh, C.V., Thu, V.K., Tai, B.H., Quang, T.H., Song, S.B., Kim, Y.H., 2011. Oleanane-type triterpene saponins from the bark of Aralia elata and their NF-κB inhibition and PPAR activation signal pathway. Bioorg. Med. Chem. Lett. 21, 6143–6147.
O'Brien, J., Wilson, I., Orton, T., Pognan, F., 2000. Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity. Eur. J. Biochem. 267, 5421–5426.
Ou, B., Hampsch-Woodill, M., Prior, R.L., 2001. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. J. Agric. Food Chem. 49, 4619–4626.
Panossian, A.G., Efferth, T., Shikov, A.N., Pozharitskaya, O.N., Kuchta, K., Mukherjee, P. K., Banerjee, S., Heinrich, M., Wu, W., Guo, D., Wagner, H., 2021. Evolution of the adaptogenic concept from traditional use to medical systems: pharmacology of stress- and aging-related diseases. Med. Res. Rev. 41, 630–703.
Piochon, M., Legault, J., Gauthier, C., Pichette, A., 2009. Synthesis and cytotoxicity evaluation of natural α-bisabolol β-d-fucopyranoside and analogues. Phytochemistry 70, 228–236.
Shaker, K.H., Bernhardt, M., Elgamal, M.H.A., Seifert, K., 1999. Triterpenoid saponins from Fagonia indica. Phytochemistry 51, 1049–1053.
Shikov, A.N., Pozharitskaya, O.N., Makarov, V.G., 2016. Aralia elata var. mandshurica (Rupr. & Maxim.) J.Wen: an overview of pharmacological studies. Phytomedicine 23, 1409–1421.
Song, S.J., Nakamura, N., Ma, C.M., Hattori, M., Xu, S.X., 2000. Four new saponins from the root bark of Aralia elata. Chem. Pharm. Bull. 48, 838–842.
Song, S.J., Nakamura, N., Ma, C.M., Hattori, M., Xu, S.X., 2001. Five saponins from the root bark of Aralia elata. Phytochemistry 56, 491–497.
Sparg, S.G., Light, M.E., Van Staden, J., 2004. Biological activities and distribution of plant saponins. J. Ethnopharmacol. 94, 219–243.
Suh, S.-J., Jin, U.-H., Kim, K.-W., Son, J.-K., Lee, S.H., Son, K.-H., Chang, H.W., Lee, Y.-C., Kim, C.-H., 2007. Triterpenoid saponin, oleanolic acid 3-O-β-D-glucopyranosyl(1 → 3)-α-L-rhamnopyranosyl(1 → 2)-α-L-arabinopyranoside (OA) from Aralia elata inhibits LPS-induced nitric oxide production by down-regulated NF-κB in raw 264.7 cells. Arch. Biochem. Biophys. 467, 227–233.
Tian, Z., Lin, G., Zheng, R.X., Huang, F., Yang, M.S., Xiao, P.G., 2006. Anti-hepatoma activity and mechanism of ursolic acid and its derivatives isolates from Aralia decaisneana. World J. Gastroenterol. 12, 874–879.
Xiao, K., Yi, Y.H., Wang, Z.Z., Tang, H.F., Li, Y.Q., Lin, H.W., 1999. A cytotoxic triterpene saponin from the root bark of Aralia dasyphylla. J. Nat. Prod. 62, 1030–1032.
Yu, S.-S., Yu, D.-Q., Liang, X.-T., 1994. Triterpenoid saponins from the roots of Aralia spinifolia. J. Nat. Prod. 57, 978–982.
Zhang, Y., Peng, Y., Li, L., Zhao, L., Hu, Y., Hu, C., Song, S., 2013. Studies on cytotoxic triterpene saponins from the leaves of Aralia elata. Food Chem. 138, 208–213.
Zou, M.-L., Mao, S.-L., Sang, S.-M., Xia, Z.-H., Lao, A.-N., 2001. Two new triterpenoid saponins from Aralia subcapitata. Nat. Prod. Lett. 15, 157–161.