DOI: 10.31276/VJSTE.64(4).79-83

Triterpenoid saponins from the root of *Weigela florida*

Duc Hung Nguyen*, Quang Tan Tu, Hoang Mau Chu

University of Education, Thai Nguyen University

Received 3 December 2021; accepted 21 January 2022

Abstract:

Plants belonging to the *Weigela* genus are traditionally used to flourish gardens, homes, and other living areas. This genus contains ten species that are mainly found in Asia, but many cultivars have been cultivated around the world. Previous studies on some species of this genus revealed the occurrence of triterpenoid saponins, which possess interesting biological activities such as cytotoxicity, anti-complementary activity, and anti-inflammatory activity. Based on this suggestion, a phytochemical study on the roots of *W. florida* "Jean's Gold" was carried out using different chromatographic methods, spectroscopic analysis in 1D- and 2D-NMR, and mass spectroscopic method (HRESI-MS in negative mode). The isolation of three known oleanane-type triterpenoid saponins was revealed as olean-12-en-28-oic acid, 3-[(O-β-D-xylopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→4)-O-β-D-xylopyranosyl-(1→3)-O-6-deoxy-α-L-mannopyranosyl-(1→2)-β-D-xylopyranosyl)oxy]-, (3β)- (compound 1), olean-12-en-28-oic acid, 3-[(O-β-D-xylopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→4)-O-β-D-xylopyranosyl-(1→3)-O-6-deoxy-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl)oxy]-, (3β)- (compound 2), and olean-12-en-28-oic acid, 3-[(O-α-L-arabinopyranosyl-(1→3)-O-[α-L-xylopyranosyl-(1→4)]-O-β-D-glucopyranosyl-(1→4)-O-β-D-xylopyranosyl-(1→3)-O-6-deoxy-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl)oxy]-, (3β)- (compound 3). Full characterisation of the three saponins presented in this study gives a better understanding of the phytochemistry of ornamental plants, which can be used further for medicinal purposes.

Keywords: **Caprifoliaceae, NMR, ornamental shrub, triterpenoid saponin, *Weigela florida* "Jean's Gold".**

Classification number: **3.3**

Introduction

Ornamental plants can have many useful purposes. Although they possess beautiful flowering and a lovely attraction, they are being grown not only for environment beauty in workplaces, homes, universities, etc., but also for spreading freshness and eliminating toxic volatile substances inside homes, hospitals, and workplaces [1, 2]. Another interest in ornamental plants comes from their medicinal use as they possess many bioactive compounds like flavonoids, phenolic acids, and vitamins [3, 4]. Plants in the *Weigela* genus are traditionally used to flourish gardens, homes, and other living areas. This genus contains ten species that are mainly found in Asia, but many cultivars have been cultivated [5]. Among those, *W. florida* "Jean's Gold" is a medium-sized ornamental shrub with green leaves and red flowers. Previous studies on some species of this genus led to a revelation of the presence of triterpenoid saponins, which possess interesting biological activities such as cytotoxicity, anti-complementary activity, and anti-inflammatory activity [5-8]. Based on this suggestion, a phytochemical study on the roots of *W. florida* "Jean's Gold" was carried out using different chromatographic methods, analysis of 1D- and 2D-NMR, and mass spectroscopy using negative mode HRESI-MS. The isolation of three known oleanane-type triterpenoid saponins was further revealed. A suggestion for future phytochemical research on the Weigela genus were carried out together with the biological activities of components and specifically on oleanane-type triterpenoid saponins. This research will be useful to establish a relationship between the structure and activity of those saponins.

Materials and methods

Plant material

The ornamental shrub *W. florida* "Jean's Gold" (Fig. 1) was collected in 2016 from the flower boutique Botanic, Quetigny, France at 47°18'45.6"N, 5°05'41.4"E. A voucher specimen was stored in Laboratoire de Pharmacognosie EA 4267, Université de Bourgogne, Dijon, France.

Fig. 1. The ornamental shrub *W. florida* "Jean's Gold".

General experimental procedure

An automatic polarimeter AA-10R (Optical Activity®, England) was used to record optical rotation values. A Varian VNMR-S 600 MHz (Agilent Technologies®, USA) was used to record the 1D and 2D spectra including 1H, ^{13}C, HSQC, ROESY, HMBC, TOCSY, COSY NMR, and further recorded at the temperature of 35°C in pyridine-*d5* (C_5D_5N). HRESI-MS in the negative mode was done by a micrOTOF II mass spectrometer (Bruker®, Germany). A microwave apparatus

*Corresponding author: Email: hungnd@true.edu.vn

(MARS 6, CEM®, USA) was used to perform the extractions. The performance of VLC (Vacuum liquid chromatography) was carried out on normal phased silica gel 60 (60-200 µm, Merck®, Germany). MPLC (Medium-pressure liquid chromatography) was carried out on normal phased silica gel 60 (Merck®, Germany) with an M305 pump (Gilson®, USA). HPTLC (High-performance thin-layer chromatography, Merck®, Germany) and TLC (Thin layer chromatography, Silicycle®, Canada) were precoated silica gel plates 60F_{254}. A vanillin reagent prepared by dissolving 1% vanillin in a solvent of EtOH:H_2SO_4 (50:1, v/v) was used to reveal the saponins in the samples. The phytochemical study was carried out at the Laboratory of Pharmacognosy, University of Burgundy, Dijon, France. The spectroscopic data were recorded at the Faculty of Science and Technology, University of Siegen, Siegen, Germany.

Extraction and isolation of compounds

After drying the whole plant at room temperature, each part of *W. florida* "Jean's Gold" was separated individually for different purposes. The dried root (58 g) was ground and further presented to an extraction using the microwave-assisted method with a solvent of EtOH: H_2O (75:35) in the following conditions: 60°C, 200 W, 45 min, 400 ml each x 3 times. After removing solvent using a rotary evaporator at 53°C, 5.2 g of crude extract was collected and further submitted to a VLC reversed-phased silica gel RP-18 with each 500 ml of solvents including 100% H_2O, 50% EtOH/50% H_2O, and 100% EtOH yielding three fractions A-C. Fraction B (295.4 mg) was separated by an MPLC on normal-phased silica gel 60 using solvent $CHCl_3$/MeOH/H_2O 75/25/3, 70/30/5, 60/32/7 (v/v/v) to give 4 subfractions B1-B4. Subfraction B2 was fractionated again by a successive MPLC on normal-phased silica gel 60 ($CHCl_3$/MeOH/H_2O 70/30/5, v/v/v) resulting in compound **1** (wbr2314) (3.5 mg). Subfractions B3 was presented to a normal phased MPLC on silica gel 60 using solvent $CHCl_3$/MeOH/H_2O 70/30/5, 60/32/7 (v/v/v) affording compound **2** (wbr2311) (4.2 mg) and compound **3** (wbr2321) (3.5 mg) (Figs. 2-6).

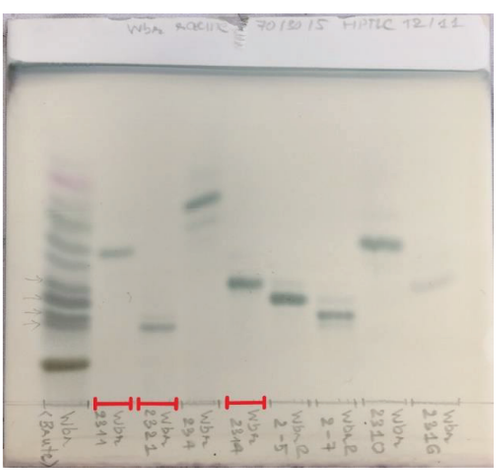

Fig. 2. HPTLC of isolated compounds (in red).

Compound **1**: Amorphous powder. ^{1}H-NMR (pyridine-d_5, 600 MHz) and ^{13}C-NMR (pyridine-d_5, 150 MHz), see Tables 1 and 2. HRESI-MS (negative mode) *m/z* 1160.4 [M-H]$^-$, $C_{57}H_{92}O_{24}$.

Compound **2**: Amorphous powder. ^{1}H-NMR (pyridine-d_5, 600 MHz) and ^{13}C-NMR (pyridine-d_5, 150 MHz), see Tables 1 and 2. HRESI-MS (negative mode) *m/z* 1160.1 [M-H]$^-$, $C_{57}H_{92}O_{24}$.

Compound **3**: Amorphous powder. ^{1}H-NMR (pyridine-d_5, 600 MHz) and ^{13}C-NMR (pyridine-d_5, 150 MHz), see Tables 1 and 2. HRESI-MS (negative mode) *m/z* 1322.1 [M-H]$^-$, $C_{63}H_{102}O_{29}$.

Results and discussion

The aqueous ethanolic crude extract of the roots of *W. florida* "Jean's Gold" was isolated and purified by various chromatographic methods running on normal phased silica gel affording compounds

Table 1. The spectroscopic data of the aglycone moiety of 1-3 (C_5D_5N, δ in ppm, *J* in Hz).

	1		2		3	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	39.0	0.94, 1.55	38.7	0.94, 1.52 m	39.1	0.96, 1.53
2	27.0	1.84, 2.12 m	26.5	1.83, 2.13	26.8	1.87, 2.12 *m*
3	88.6	3.33 *dd* (11.7, 4.0)	88.8	3.31 *dd* (12.1, 3.6)	89.0	3.31 *dd* (12.0, 3.9)
4	39.5	–	39.6	–	39.6	–
5	56.2	0.85 *d* (12.0)	56.0	0.84 *d* (12.0)	56.3	0.83
6	18.5	1.30, 1.55 *m*	18.4	1.27, 1.49 *m*	19.0	1.33, 1.50 *m*
7	33.3	1.24, 1.50 *m*	32.9	1.30, 1.46	33.5	1.26, 1.48 *m*
8	39.7	–	39.9	–	39.8	–
9	48.0	1.64 *dd* (15.2, 8.7)	48.1	1.65 *dd* (15.2, 8.8)	48.1	1.66
10	37.0	–	37.2	–	37.2	–
11	23.8	1.88, 1.94	23.6	1.89, 1.96	23.8	1.90, 1.95
12	122.5	5.45 *t-like* (3.7)	123.0	5.46 *t-like* (3.6)	123.1	5.45 *t-like* (3.8)
13	145.0	–	145.1	–	145.0	–
14	42.1	–	42.2	–	42.3	–
15	28.3	1.22 *m*, 2.12	27.9	1.22, 2.16	27.9	1.23, 2.16
16	24.0	1.96, 2.20	24.1	1.96, 2.15	24.0	1.97, 2.17
17	46.6	–	46.5	–	46.7	–
18	42.0	3.31 *dd* (11.8, 3.9)	42.0	3.33 *dd* (12.0, 4.0)	42.2	3.36 *dd* (11.8, 3.9)
19	46.5	1.33, 1.82	46.4	1.32, 1.82	46.6	1.30, 1.83
20	30.9	–	31.0	–	31.2	–
21	34.2	1.22 *m*, 1.50 *m*	34.4	1.24, 1.46	34.3	1.23, 1.46
22	33.4	1.82, 2.05 *m*	33.2	1.85, 2.10	34.0	1.85, 2.05
23	28.5	1.34 *s*	27.9	1.31 *s*	27.8	1.35 *s*
24	17.1	1.21 *s*	17.2	1.12 *s*	17.4	1.21 *s*
25	15.4	0.90 *s*	16.0	0.91 *s*	15.6	0.90 *s*
26	17.3	0.98 *s*	17.5	0.99 *s*	17.4	1.01 *s*
27	26.2	1.31 *s*	26.2	1.33 *s*	26.3	1.33 *s*
28	180.0	–	180.2	–	180.3	–
29	33.5	0.96 *s*	33.5	0.95 *s*	33.6	0.97 *s*
30	23.5	1.02 *s*	23.7	1.05 *s*	23.5	1.05 *s*

Table 2. The spectroscopic data of the sugar moieties of 1-3 (C5D5N, δ in ppm, J in Hz).

	1		2		3	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
Ara-1			105.1	4.89 *d* (6.0)		
2			75.5	4.56 *t* (6.0)		
3			74.4	4.24		
4			69.2	4.23 *m*		
5			65.3	3.81,4.33 *dd* (11.6, 3.6)		
Rha-1	101.5	6.49 *br s*	101.4	6.19 *br s*	101.5	6.51 *br s*
2	71.6	4.92 *br s*	71.8	4.86 *br s*	71.8	4.97 *br s*
3	83.3	4.71 *dd* (9.2, 2.8)	83.1	4.65 *dd* (9.6, 2.8)	83.4	4.75 *dd* (9.5, 3.5)
4	72.7	4.46 *dd* (9.6, 9.2)	72.8	4.47 *dd* (9.6, 9.2)	72.7	4.47 *dd* (9.5, 9.3)
5	69.5	4.77 *dq* (9.6, 6.4)	69.6	4.62 *dq* (9.2, 6.0)	69.5	4.77 *dq* (9.3, 5.9)
6	18.3	1.64 *d* (6.4)	18.4	1.55 *d* (6.0)	18.5	1.66 *d* (5.9)
Xyl-1	106.1	4.84 *d* (7.3)	106.9	5.27 *d* (7.5)	105.9	4.84 *d* (7.5)
2	77.7	4.24	75.4	4.05	77.7	4.19
3	76.3	4.15	76.1	4.17	79.8	4.15
4	71.6	4.17	77.7	4.28	71.7	4.17
5	67.2	3.71, 4.35	64.8	3.64, 4.39 *dd* (11.3, 5.1)	67.3	3.75 *t* (11.1), 4.32 *dd* (11.1, 4.9)
Xyl II-1	107.4	5.27 *d* (7.5)	105.5	5.07 *d* (7.5)	107.0	5.27 *d* (7.5)
2	75.4	4.05	74.5	4.01	75.5	4.06
3	76.3	4.14	78.4	4.13	76.3	4.12
4	77.8	4.26	70.9	4.16	77.7	4.21
5	64.6	3.67, 4.36	67.5	3.67 *t* (10.6), 4.26	64.8	3.64 *t* (11.1), 4.38 *dd* (11.1, 4.8)
Xyl III-1	105.6	5.05 *d* (7.6)			102.8	5.47 *d* (6.0)
2	74.7	3.99			73.8	4.16
3	78.4	4.09			76.3	4.18
4	70.6	4.14			70.5	4.24
5	67.5	3.66, 4.26			66.3	3.73 *t* (11.3), 4.63 *dd* (11.3, 4.1)
Glc I-1	103.3	4.99 *d* (8.0)	103.2	4.98 *d* (7.6)	102.7	4.92 *d* (7.6)
2	74.0	4.03	74.3	4.03	74.4	4.02
3	76.1	4.24	76.2	4.21	82.4	4.44
4	80.8	4.26	80.7	4.25	74.3	4.56 *dd* (9.4, 9.1)
5	76.5	3.90	76.7	3.93 *m*	77.6	3.83 *m*
6	61.4	4.47, 4.49	61.6	4.45, 4.49	61.4	4.34, 4.37
Glc II-1					104.6	5.51 *d* (8.0)
2					75.4	4.05
3					71.4	4.16
4					78.2	4.19
5					78.6	3.93 *m*
6					62.6	4.26 *dd* (11.9, 5.3), 4.42

1, **2,** and **3**. The structural determination of these compounds was carried out by spectroscopic methods (1D- and 2D-NMR) and mass spectroscopy (HRESI-MS in negative mode). The sugar moieties were determined by 2D-NMR analyses including HMBC, COSY, HSQC, ROESY, and TOCSY as three xylopyranosyl, one rhamnopyranosyl, one glucopyranosyl for compound **1**, two xylopyranosyl, one arabinopyranosyl, one rhamnopyranosyl, one glucopyranosyl for compound **2**, and three xylopyranosyl, one rhamnopyranosyl, two glucopyranosyl for compound **3**. Their absolute configurations were further determined to be D for xylose (Xyl) and glucose (Glc) and L for rhamnose (Rha) and arabinose (Ara) according to previous methods of acid hydrolysis and absolute configuration determination [9]. The relatively large values of Ara, Xyl, and Glc from 7.2 to 8.0 Hz revealed a β anomeric orientation for Xyl and Glc, and the α for Ara. The relatively large $J_{C\text{-}1,H\text{-}1}$ value of Rha from 165 to 167 Hz indicated an α-equatorial pyranoid form for this anomeric proton.

Compound **1** displayed a pseudo-molecular ion peak $[M\text{-}H]^-$ at 1160 compatible with a molecular formula of $C_{57}H_{92}O_{24}$ (see Fig. 6 in Supplementary data). Seven angular methyl groups were showed on the aglycone of the HSQC spectrum of compound **1** at $\boldsymbol{\delta}_H/\boldsymbol{\delta}_C$ 1.34 (*s*)/28.5 (23-Me), 1.21 (*s*)/17.1 (24-Me), 0.90 (*s*)/15.4 (25-Me), 0.98 (*s*)/17.3 (26-Me), 1.31 (*s*)/26.2 (27-Me), 0.96 (*s*)/33.5 (29-Me) and 1.02 (*s*)/23.5 (30-Me) (Table 1). In addition, the observation of the characteristic signal was reached as one olefinic proton at $\boldsymbol{\delta}_H/\boldsymbol{\delta}_C$ 5.45 (*br t*, *J*=3.6 Hz)/122.5 (C-12). The positions of those signals were assigned by HMBC cross-peaks (Fig. 3). The configurations of H-3, H-5, H-9, H-12 and H-18 were determined by analysing ROESY spectra at $\boldsymbol{\delta}_H/\boldsymbol{\delta}_H$ 1.34 (23-Me α-equatorial)/3.33 (H-3 α-axial), 1.34 (23-Me α-equatorial)/0.85 (H-5 α-axial), 1.31 (27-Me α-axial)/1.64 (H-9 α-axial), 1.02 (30-Me β-axial)/3.31 (H-18 β-axial), and 3.31 (H-18 β-axial)/5.45 (H-12 β-equatorial). Those of cross-peaks were validated the configuration of 3-OH as β-equatorial (Fig. 4). All the evidence above led to an assignment to the aglycone of compound **1** as oleanolic acid which was in agreement with literature data [8].

The sugar region of compound **1** displayed five signal protons at $\boldsymbol{\delta}_H$ 4.84 (*d*, *J*=7.3 Hz), 5.27 (*d*, *J*=7.5 Hz), 5.05 (*d*, *J*=7.6 Hz), 4.99 (*d*, *J*=8.0 Hz) and 6.49 (*br s*), further identified the correlations with five signal carbons through the HSQC spectrum at $\boldsymbol{\delta}_C$ 106.1, 107.4, 105.6, 103.3 and 101.5 respectively (Table 2). Analysing the extensive NMR spectra including ^1H, ^{13}C, HSQC, HMBC, TOCSY, ROESY and TOCSY led to an identification of three Xyl, one Glc and one Rha moiety. The establishment of the oligosaccharidic sequence of compound **1** was carried out using ROESY and HMBC spectra: the HMBC correlation at $\boldsymbol{\delta}_H/\boldsymbol{\delta}_C$ 4.84 (*d*, *J*=7.3 Hz)/88.6 (C-3) and the ROESY cross peak at $\boldsymbol{\delta}_H/\boldsymbol{\delta}_H$ 4.84 (*d*, *J*=7.3 Hz, Xyl I-1)/3.33 (*dd*, *J*=11.7, 4.0 Hz, 3-H) displayed the linkage between the Xyl I and the C-3 position of the aglycone.

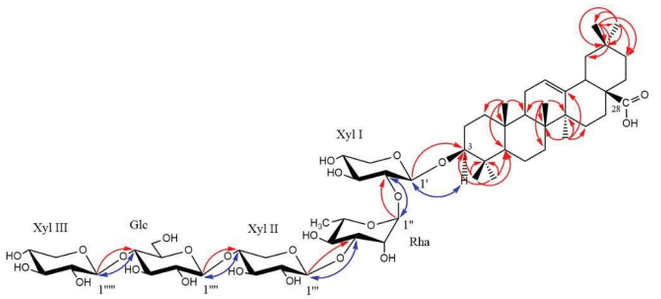

Fig. 3. Structure of compound 1 (HMBC correlations in red, ROESY correlations in blue).

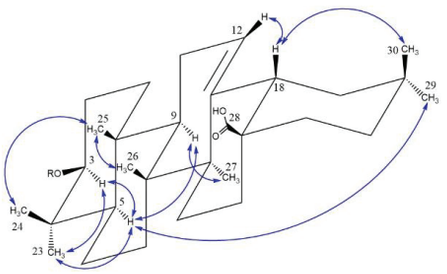

Fig. 4. ROESY correlations in the aglycone region of compound 1 (in blue).

The Rha was identified to be linked to the Xyl I by the HMBC correlation at δ_H/δ_C 6.49 (*br s*, Rha-1)/77.7 (Xyl I-2) together with the ROESY correlation at δ_H/δ_H 6.49 (*br s*, Rha-1)/4.24 (Xyl I-2). The (1→3) cross peak of Xyl II and Rha was determined by the HMBC correlation at δ_H/δ_C 5.27 (*d*, *J*=7.5 Hz, Xyl II-1)/83.3 (Rha-3) together with the ROESY cross peak at δ_H/δ_H 5.27 (*d*, *J*=7.5 Hz, Xyl II-1)/4.71 (*br s*, Rha-3). The linkage between the Glc and the Xyl II was identified by the HMBC cross peak at δ_H/δ_C 4.99 (*d*, *J*=8.0 Hz, Glc-1)/77.8 (Xyl II-4) and the ROESY cross peak at δ_H/δ_H 4.99 (*d*, *J*=8.0 Hz, Glc-1)/4.26 (Xyl II-4). Finally, the terminal sequence β-D-xylopyranosyl-(1→4)-β-D-glucopyranosyl was established by the HMBC cross peak at δ_H/δ_C 5.05 (*d*, *J*=7.6 Hz, Xyl III-1)/80.8 (Glc-4) and the ROESY correlation at δ_H/δ_H 5.05 (*d*, *J*=7.6 Hz, Xyl III-1)/4.26 (Glc-4).

Due to the results above, the structure as Olean-12-en-28-oic acid, 3-[(*O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→3)-*O*-6-deoxy-α-L-mannopyranosyl-(1→2)-β-D-xylopyranosyl)oxy]-, (3β)- was established for compound **1**. Comparison with literature data revealed that this compound was firstly isolated from *Pterocephalus hookeri* [10].

The similar HRESI-MS of compound **2** as compound **1** was shown with a pseudo-molecular ion peak $[M-H]^-$ at 1160 related to a molecular formula of $C_{57}H_{92}O_{24}$ (see Fig. 12 in Supplementary data). Analysing 1D and 2D NMR revealed that the aglycone of compound **2** was the same as compound **1** (Table 1). The difference between these compounds was only displayed in the sugar region which further identified that the Xyl I moiety in **1** was replaced by the Ara moiety in compound **2** (Table 2). This identification was confirmed by analysing mainly ROESY and HMBC spectra: the HMBC cross peak at δ_H/δ_C 4.89 (*d*, *J*=6.0 Hz, Ara-1)/88.8 (C-3), together with the ROESY cross peak at δ_H/δ_H 4.89 (*d*, *J*=6.0 Hz, Ara-1)/3.31 (*dd*, *J*=12.1, 3.6 Hz, 3-H) displayed a linkage of the Ara at the C-3 position of the aglycone. The Xyl I was determined to be linked to the Rha by the HMBC correlation at δ_H/δ_C 6.19 (*br s*, Rha-1)/75.5 (Ara-2) and the ROESY correlation at δ_H/δ_H 6.19 (*br s*, Rha-1)/4.56 (Ara-2). The oligosaccharidic sequence was further established as Xyl II-(1→4)-Glc-(1→4)-Xyl-(1→3)-Rha-(1→2)-Ara by comparison with those data of compound **1** (Fig. 5).

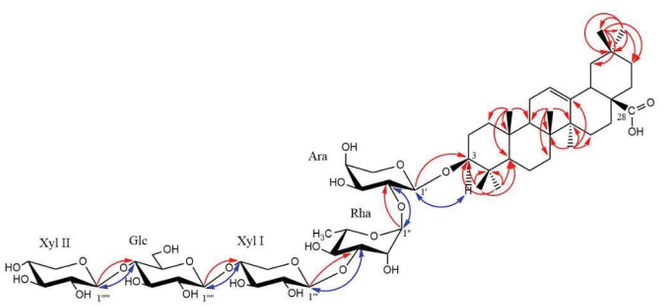

Fig. 5. Structure of compound 2 (HMBC correlations in red, ROESY correlations in blue).

According to all the evidence above, the structure as Olean-12-en-28-oic acid, 3-[(*O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→3)-*O*-6-deoxy-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl)oxy]-, (3β)- was elucidated for compound **2**. This saponin has been already isolated from *W*. x "Bristol ruby" [7].

The pseudo-molecular ion peak $[M-H]^-$ at *m/z* 1322 in the HRESI-MS led to an establishment of the molecular formula of compound **3** as $C_{63}H_{102}O_{29}$ (see Fig. 18 in Supplementary data). Comparison with signal data in the aglycone region of compound **1** gave an identification that the aglycone of compound **3** was the same as compounds **1** and **2** (Table 1). The molecular weight of compound **3** differs than compound **1** by 162 amu indicating the supplement of one additional sugar unit. Six anomeric cross-peaks in the HSQC spectrum at δ_H/δ_C 4.84 (*d*, *J*=7.5 Hz)/105.9, 5.27 (*d*, *J*=7.5 Hz)/107.0, 5.47 (*d*, *J*=6.0 Hz)/102.8, 4.92 (*d*, *J*=7.6 Hz)/102.7, 5.51 (*d*, *J*=8.0 Hz)/104.6 and 6.51 (*br s*)/101.5 were identified as three β-D-xylopyranosyl, one α-L-rhamnopyranosyl, and two β-D-glucopyranosyl.

The establishment of the oligosaccharidic chain and heterosidic linkage was further achieved by analysing the 2D NMR spectra (HSQC, COSY, ROESY, HMBC, TOCSY). The HMBC cross peak at δ_H/δ_C 4.84 (*d*, *J*=7.5 Hz)/89.0 together with the ROESY cross peak at δ_H/δ_H 4.84 (*d*, *J*=7.5 Hz)/3.31 (*dd*, *J*=12.0, 3.9) confirmed that the Xyl I was attached to the C-3 position of the

aglycone (Table 2). The oligosaccharidic sequence attached to the C-2 position of the Xyl I was confirmed by the HMBC cross peaks at δ_H/δ_C 6.51 (Rha-1)/77.7 (Xyl I-2), 5.27 (Xyl II-1)/83.4 (Rha-3), 4.92 (Glc I-1)/77.7 (Xyl II-4), 5.51 (Glc II-1)/82.4 (Glc I-3) and 5.47 (Xyl III-1)/74.3 (Glc I-4), in combination with the ROESY cross peaks at δ_H/δ_H 6.51 (Rha-1)/4.19 (Xyl I-2), 5.27 (Xyl II-1)/4.75 (Rha-3), 4.92 (Glc I-1)/4.21 (Xyl II-4), 5.51 (Glc II-1)/4.44 (Glc I-3) and 5.47 (Xyl III-1)/4.56 (Glc I-4). The conclusion was further reported as the (1→3) and (1→4) linkages between Glc II, Xyl III and Glc I, the (1→4) linkage between Glc I and Xyl II, the (1→3) linkage between Xyl II and Rha (Fig. 6). Based on above results, structural characterization of compound **3** was established as Olean-12-en-28-oic acid, 3-[(*O*- β-D-glucopyranosyl-(1→3) -*O*-[α-L-xylopyranosyl-(1→4)]-*O*-β-D-glucopyranosyl-(1→4) -*O*-β-D-xylopyranosyl-(1→3)-*O*-6-deoxy-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl)oxy]-, (3β)-. Literature revealed that this compound was firstly isolated from *W*. x "Bristol ruby" [7].

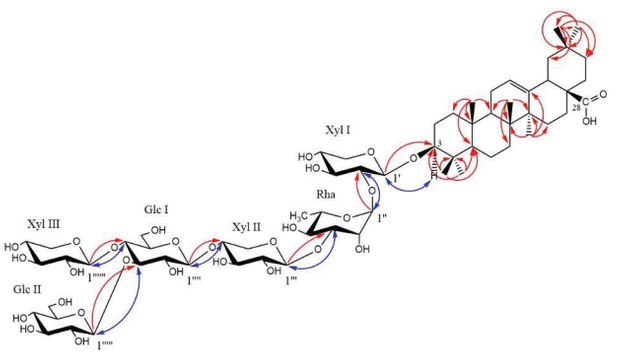

Fig. 6. Structure of compound 3 (HMBC correlations in red, ROESY correlations in blue).

Previous studies led to the conclusion that the oleanane-type triterpenoid saponins exhibit various biological activities including anti-tumour, anti-allergic, anti-inflammatory, immunological, anti-bacterial, and molluscicidal activities [11]. Specifically, various phytochemical and biological studies have been carried out on some species of the *Weigela* genus, which revealed that the oleanane-type triterpenoid saponins potentially possess biological activities. The investigations on cytotoxic activity against some cancer cell lines for the triterpenoid saponins isolated from *W. stelzneri* and *W*. x "Bristol ruby" were carried out against SW480, EMT6, and CT26 cancer cell lines that further exhibited a potent result for the oleanane-type triterpenoid saponins with high IC_{50} values [7, 8]. The immunological activity of oleanane-type triterpenoid saponins isolated from *W. florida* "rumba" (Bunge) A. DC was further evaluated by A.S.C. Tixier, et al. (2018) [12]. The first evaluation on toxicological properties in an *in vivo* zebrafish assay of those saponins revealed a significant result from the larvae in the range of 1 µM [5]. The characterisation of those saponins gives a better understanding of the phytochemistry and biological activities of the *Weigela* genus and suggests more evaluations of biological activity to be performed in order to establish structure activity related to these compounds.

Conclusions

Three oleanane-type triterpenoid saponins were characterised from the roots of *W. florida* "Jean's Gold" using various chromatographic methods. Their structures were further established by spectroscopic analysis of 1D- and 2D-NMR and mass spectroscopy (HRESI-MS in negative mode) in combination with data from the literature. Full structural characterisation of these three compounds together with structure activity discussion based on facts from the literature afford us a better understanding of the *Weigela* genus phytochemistry and putative biological activities.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

[1] M.W. Iersel, M.R. Chappell, P.A. Thomas (2016), "Optimizing growth, quality, and profits through precision irrigation in ornamental plant production", *Acta Horticulturae*, **1131**, pp.57-64.

[2] X. Wei, et al. (2017), "Phylloremediation of air pollutants: Exploiting the potential of plant leaves and leaf-associated microbes", *Frontiers in Plant Science*, **8**, DOI: 10.3389/fpls.2017.01318.

[3] M. Goleniowski, et al. (2013), "Phenolic acids", *Natural Products*, **3**, pp.1951-1973.

[4] K. Jakubczyk, et al. (2018), "Garden nasturtium (*Tropaeolum majus* L.) - A source of mineral elements and bioactive compounds", *Rocz. Państwowego Zakładu Hig.*, **69(2)**, pp.119-126.

[5] N. Andriamisaina, et al. (2018), "Phytochemistry of *Weigela* x "kosteriana variegata" (Caprifoliaceae)", *Nat. Prod. Commun.*, **13(4)**, pp.403-406.

[6] P.T. Thuong, et al. (2006), "Anti-complementary activity of ursane-type triterpenoids from *Weigela subsessilis*", *Biol. Pharm. Bull.*, **29(4)**, pp.830-833.

[7] D.H. Nguyen, et al. (2019), "Cytotoxic glycosides from the roots of *Weigela* x "Bristol Ruby", *Fitoterapia*, **137**, DOI: 10.1016/j.fitote.2019.104242.

[8] A. Rezgui, et al. (2016), "Oleanolic acid and hederagenin glycosides from *Weigela stelzneri*", *Phytochemistry*, **123**, pp.40-47.

[9] D.H. Nguyen, et al. (2021), "Steroidal glycosides from the Vietnamese cultivar *Cordyline fruticosa* "Fairchild red", *Phytochemistry*, **192**, DOI: 10.1016/j.phytochem.2021.112966.

[10] T. Jun, et al. (1993), "Triterpenoid saponins from *Pterocephalus hookeri*", *Phytochemistry*, **32(6)**, pp.1535-1538.

[11] Z. Fang, et al. (2020), "A review: The triterpenoid saponins and biological activities of *Lonicera Linn*", *Molecules*, **25(17)**, DOI: 10.3390/molecules25173773.

[12] A.S.C. Tixier, et al. (2018), "Oleanane-type glycosides from the roots of *Weigela florida* "rumba" and evaluation of their antibody recognition", *Fitoterapia*, **128**, pp.198-203.