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Iridoid glucosides from *Wendlandia ligustroides* (Boiss. &Hohen.) Blakelock

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ABSTRACT

Eight iridoid glucosides were reported from the aerial parts of *Wendlandia ligustroides.* 10-deoxygeniposidic acid (**1**), 7-deoxygardoside (**2**), geniposidic acid (**3**), 7-deoxy-8-epi-loganic acid (**4**), deacetyl-daphylloside (**5**), scandoside methyl ester (**6**), 6-*O*-methyl-deacetyl-daphylloside (**7**), 6-*O*-methyl-scandoside methyl ester (**8**). Compounds **3** – **8** were isolated as a pure form while **1** and **2** as a mixture. The structures of the compounds **1 – 8** were established by spectroscopic methods including 1D-NMR (^{1}H NMR, ^{13}C NMR, DEPT-135), 2D-NMR (COSY, NOESY, HSQC, HMBC) and HRMS.

1. Introduction

Wendlandia Bartl. ex DC. (Rubiaceae) is a genus distributed in paleotropical region with about 70 species. *Wendlandia ligustroides* (Boiss. &Hohen.) Blakelock is naturally grows at North Iraq and it was reported fort he first time in flora of Turkey by one of us (Dönmez, 2002). The species is closely allied to *W. arabica* Deflers which is South Arabian species. The plant is a small chasmophytic bush with fragrant smelling and is growing on rocky limestone cliffs at Hakkari in Zap George. *W. ligustroides* is a small erect shrub (30 – 40 cm) with puberulent branches. Leaves deciduous and coriaceous with stipules. Inflorescence terminal thyrses with many smaller. Calyx teeth as long as ovary. Petals white and fragrant. Fruit globose.

Prevous studies performed on *Wendlandia* species have focused on the isolation of iridoids. 10-*O*-veratroyleranthemoside, 5-dehydro-8-epi-adoxosidic acid, 5-dehydro-8-epimussaenoside, 10-*O*-dihydroferuloyldeacetyldaphylloside, wendoside, 8-epi-mussaenoside, 8-*O*-caffeoylmussaenosidic acid, ixoside have been reported from the roots of *Wendlandia tinctoria* (Dinda et al., 2006, 2011a, 2011b). Tarennoside, gardenoside, geniposidic acid, 10-*O*-caffeoylscandoside methyl ester, scandoside methyl ester, 6-*O*-methylscandoside methyl ester, methyl deacetylasperulosidate, 10-*O*-caffeoyldaphylloside have been reported from the leaves of *Wendlandia formosana* (Raju et al., 2004), and scandoside methyl ester from the wood of *Wendlandia bicuspidata* (De Silva et al., 1987). Among the *Wendlandia* species studied, *W. tinctoria* has been reported to use as an antidote of snake-bite by local people living in the sub-Himalayan region of India (Dinda et al., 2006).

As a part of our studies on the iridoid containing plants, *Wendlandia ligustroides* was selected for this study as one of the member of Rubiaceae recently recorded for Flora of Turkey. No previous phytochemical and pharmacological and ethnopharmacological studies have been carried out before on it.

2. Material and methods

2.1. General experimental procedures

Classical column chromatography and a gradient Medium Pressure Liquid Chromatography (Büchi MPLC equipped by Pump Modules C-601 & C-605 with a pump Controller C-610 and pump manager C-605) and Büchi Fraction Collector C-615 were used for the isolation process. Silica gel (0.063 – 200 mμ, Merck),

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LiChroprep C-18 (0.063 – 200 mm, Merck) and Sephadex LH-20 were used as stationary phases throughout chromatographical studies. Silica gel alumina plates (Silica Gel 60 F_{254}, Merck) were used for Thin Layer Chromatography. Optical rotations were measured on a Schmidt + Haensch Polartronic MHZ-8 polarimeter. NMR measurements in CD_3OD were performed on Bruker DRX 500 spectrometers operating at 500 MHz for ^1H and 125 MHz for ^{13}C, respectively, using the XWIN NMR software package for the data acquisition and processing. Negative- and positive-mode HRMS were recorded on a Finnigan TSQ 7000 and HR-Mass Spectrometer and an UPLC-Quadrupole Orbitrap instruments. For lyophlization a CHRIST Alpha 1–4 LD Plus was used. Throughout the study Büchi R-210 and Heidolph 4001 rotary evaporators were used.

2.2. Plant material

Wendlandia ligustroides (Boiss. & Hohen.) Blakelock was collected from Hakkari: 5.4 km from Şırnak-Çukurca road junction to Hakkari, Geçimli village, limestone rock crevicies, 908 m, 37°21′186″ N, 038°30′375″E, 29.06.2009. Voucher specimen (Ali A. Dönmez 15,490 is deposited at the HUB (Herbarium of Hacettepe University).

2.3. Extraction and isolation (Aerial Parts)

The air-dried, ground, aerial parts (stems, leaves, and flowers, 290 g) of *W. ligustroides* were extracted with MeOH (4 L × 2) at 50 °C. The combined methanolic extracts were concentrated in vacuo at 40 °C, diluted with 200 mL of water and extracted with dichloromethane (200 mL × 5). The water phase was concentrated and then lyophilized to give 47.13 g crude extract (yield 16.25%). An aliquot (45 g) of the crude extract was dissolved in water (70 mL) and subjected to vacuum liquid chromatography

(VLC) using reversed-phase material (LiChroprep C18, 100 g) employing H_2O (500 mL), H_2O – MeOH (95:5, 200 mL; 90:10, 200 mL) and increasing amount of 5% MeOH at each 100 mL of H_2O – MeOH mixture. The volume of the fractions was 100 mL. This stepwise gradient elution yielded 22 fractions. TLC was used to monitor to content of the fractions and combined into the eleven fractions (frs.: A – K; A. 3.94 g; B, 26.2 g; C, 2.52 g; D, 947 mg; E, 723 mg; F, 3.2 g; G, 709 mg; H, 829 mg; I, 1,23 g; J, 1.57 g; and K, 460 mg).

An aliquot of fraction B (13 g) was reapplied to vacuum liquid chromatography (VLC) using reversed-phase material (LiChroprep C18, 100 g) employing H_2O (100 mL), and increasing amount of 3% MeOH in H_2O at each 100 mL as eluent until 30% of MeOH in H_2O. 22 fractions were collected (fraction volume 50 mL). The column was then eluted using same mixture with increasing amount of 10% MeOH at each of 100 mL of eluent. Additional 8 fractions were collected. By the help of TLC monitoring, these 30 fractions were combined into thirteen fractions (Frs. B1 – B14: B1, 930 mg; B2, 1070 mg; B3, 1038 mg; B4, 459 mg; B5, 146 mg; B6, 334 mg; B7, 189 mg; B8, 145 mg; B9, 46 mg; B10, 98 mg; B11, 146 mg; B12, 480 mg; B13, 223 mg).

Fraction B2 (1070 mg) was subjected to a Si gel (55 g) column using DCM-MeOH-H_2O

mixtures (80:20:2, 250 mL, 75:25:2.5, 200 mL; 70:30:3, 200 mL; 60:40:4,200 mL) to give geniposidic acid (**3**, 90 mg) and deacetyl-daphylloside (**5**, 48 mg).

Fraction B6 (334 mg) was subjected to a Si gel (70 g) column using DCM-MeOH-H_2O

mixtures (80:20:2, 350 mL, 75:25:2.5, 100 mL; 70:30:3, 200 mL; 50:50:5,600 mL) to give 6-*O*-methyl-scandoside methyl ester (**8**, 25 mg) and scandoside methyl ester (**6**, 65 mg).

Fraction B8 (145 mg) was subjected to a Si gel (25 g) column using DCM-MeOH-H_2O

mixtures (80:20:2, 250 mL, and 70:30:3, 100 mL) to give 6-*O*-methyl-deacetyl-daphylloside (**7**, 22 mg).

Fraction G (709 mg) was subjected to a Si gel (70 g) column eluting with DCM-MeOH-H_2O

mixtures (80:20:2, 600 mL, 75:25:2.5, 100 mL; 70:30:3, 200 mL; 60:40:4, 200 mL) to give a mixture of 7-deoxygeniposidic acid (**1**) and 7-deoxygardoside (**2**).

Fraction I (1.145 g of 1.23 g) rich in colored material was subjected to a polyamide column (50 g). Water has been used in the column preparation and also for the first elutions. Subsequent elutions have been performed using H_2O-EtOH mixtures with increasing amount of EtOH. Fractions eluted with 60% EtOH yielded pure 7-deoxy-8-*epi*-loganic acid (**4**, 30 mg).

Further separations performed on the fraction F (3.2 g) resulted in the isolation of geniposidic acid (**3**, 172 mg) and additional 7-deoxy-8-*epi*-loganic acid (**4**, 32 mg).

10-Deoxygeniposidic acid (**1**): ^1H NMR (400 MHz, CD_3OD): δ 7.45 s (H-3), 5.47 br s (H-7), 5.25 d (J = 8.0 Hz, H-1), 4.71 d (J = 8.0 Hz, H-1′), 3.88 dd (J = 2.0 and 12.0 Hz, H-6′a), 3.66 dd (J = 6.0 and 12.0 Hz, H-6′b), 3.37 t (J = 9.0 Hz, H-3′), 3.30 m (H-5′), 3.28 t (J = 9.0 Hz, H-4′); 3.22 dd (J = 8.0 and 9.0 Hz, H-2′), 3.14 dq like (J = 8.0 and 0.5 Hz, H-5), 2.73 m (H-6a), 2.61 t (J = Hz, H-9), 2.08 m (H-6b), 1.81 br s (H_3-10) (Inoue et al. 1992, Takeda et al., 1996).

7-Deoxygardoside (**2**): ^1H NMR (400 MHz, CD_3OD): δ 7.42 s (H-3), 5.45 d (J = 4.0 Hz, H-1), 5.12 and 5.07 (each 1H, br d, J = 2.0 Hz, H_2-10), 4.68 d (J = 8.0 Hz, H-1′), 3.88 dd (J = 2.0 and 12.0 Hz, H-6′a), 3.66 dd (J = 6.0 and 12.0 Hz, H-6′b), 3.34 t (J = 9.0 Hz, H-3′), 3.30 m (H-5′), 3.28 t (J = 9.0 Hz, H-4′); 3.22 dd (J = 8.0 and 9.0 Hz, H-2′), 3.02 m (H-9), 2.86 m (H-5), 2.30 – 1.95 (4H, H_2-6 and H_2-7) (Bianco et al. 1986).

Geniposidic acid (**3**): $[\alpha]_D^{20}$ + 13.3° (c 0.5, MeOH); ^1H NMR (500 MHz, CD_3OD): δ 7.40 s (H-3), 5.81 br s (H-7), 5.13 d (J = 7.6 Hz, H-1), 4.74 d (J = 7.9 Hz, H-1′), 4.33 d and 4.21 d (AB system, J_{AB} = 14.1 Hz, H_2-10), 3.88 dd (J = 2.0 and 12.0 Hz, H-6′a), 3.66 dd (J = 6.0 and 12.0 Hz, H-6′b), 3.37 t (J = 9.0 Hz, H-3′), 3.40–3.30 (2H, H-4′ and H-5′); 3.20 dd (J = 8.0 and 9.0 Hz, H-2′), 3.17 m (H-5), 2.86 m (H-9), 2.72 t (J = 7.6 Hz, H-9), 2.10 m (H-6b); ^{13}C NMR (125 MHz, CD_3OD): δ 172.0 (C, C-11), 151.2 (CH, C-3), 144.7 (C, C-8), 128.5 (CH, C-7), 115.7 (C, C-4), 99.3 (CH, C-1′), 98.1 (CH, C-1), 78.3 (CH, C-5′), 77.8 (CH, C-3′), 74.9 (CH, C-2′), 71.2 (CH, C-4′), 62.7 (CH_2, C-6′), 61.6 (CH_2, C-10), 47.2 (CH, C-9), 39.9 (CH_2, C-6), 37.2 (CH, C-5); Negative-ion HRMS m/z 373.1127 [M−H], (calc. for $C_{16}H_{22}O_{10}$, Mol. Wt. 374.12 (Akdemir & Çalış, 1991; Tzakou et al., 2007).

8-*epi*-deoxyloganic acid (**4**): $[\alpha]_D^{20}$ −76.7° (c 0.5, MeOH); ^1H NMR and ^{13}C NMR (500 and 125 MHz, resp., CD_3OD): δ 7.44 s (H-3), 5.48 d (J = 4.5 Hz, H-1), 4.71 d (J = 8.0 Hz, H-1′), 3.93 dd (J = 12.0 and 2.0 Hz, H-6′a), 3.67 dd (J = 12.0 and 6.0 Hz, H-6′b), 3.40 t (J = 9.0 Hz, H-3′), 3.32 m (H-5′), 3.27 t (J = 9.0 Hz, H-4′), 3.22 dd (J = 8.0 and 9.0 Hz, H-2′), 2.92 dd (J = 14.2 and 7.6 Hz, H-5), 2.28 m (H-8), 2.27 m (H-9), 2.09 m (H-6a), 1.81 m (H-7a), 1.61 m (H-6b), 1.38 m (H-7b), 1.11 d (J = 6.7 Hz, H_3-10); ^{13}C NMR (125 MHz, CD_3OD): δ 171.0 (C, C-11), 152.7 (CH, C-3), 113.4 (C, C-4), 99.8 (CH, C-1′), 96.2 (CH, C-1), 78.4 (CH, C-5′), 77.0 (CH, C-3′), 74.8 (CH, C-2′), 71.8 (CH, C-4′), 63.0 (CH_2, C-6′), 44.4 (CH, C-9), 37.6 (CH, C-8), 34.6 (CH, C-5), 33.3 (CH_2, C-7), 32.4 (CH_2, C-6), 16.8 (CH_3, C-10); Positive-ion HRMS: m/z 383.1279 $[M+Na]^+$, Negative-ion HRMS m/z 359.1336 $[M-H]^-$, (calc. for $C_{16}H_{24}O_9$, Mol. Wt. 360.14) (Nakamura et al., 2000: Murai et al., 1984: Teng et al., 2005).

Deacetyl-daphylloside (**5**): $[\alpha]_D^{20}$ −14.4° (c 0.5, MeOH); ^1H NMR and ^{13}C NMR (500 and 125 MHz, resp., CD_3OD): Table 1; Positive-ion HRMS: m/z 427.1175 [M+Na]+ (calc. for $C_{17}H_{24}O_{11}$, Mol. Wt. 404.13) (Tzakou et al. 2007).

Table 1
^{1}H and ^{13}C NMR data of Compounds deacetyl-daphylloside (**5**) and scandoside methyl ester (**6**) ($CDCl_3$; δ_H 500 MHz; δ_C 125 MHz).

C/H	DEPT	5		6	
		δ_C ppm	δ_H ppm, *J* (Hz)	δ_C ppm	δ_H ppm, *J* (Hz)
1	CH	100.5	5.06 d (9.0)	98.3	5.22 d (6.3)
3	CH	155.5	7.66 d (1.2)	153.9	7.53 s
4	C	108.3	–	110.8	–
5	CH	45.9	3.02 ddd (7.3, 5.8, 1.2)	45.6	3.02 ddd (7.2, 4.5, 0.8)
6	CH	75.4	4.80 dd (1.7, 5.8)	82.3	4.57 br s
7	CH	129.8	6.03 d (1.7)	130.1	5.83 br s
8	C	151.5	–	147.6	–
9	CH	42.7	2.57 t (9.0, 7.3)	47.1	3.06 dd (6.3, 7.2)
10	CH_2	62.8	4.46 d (16.0) 4.21 d (16.0)	62.7	4.36 d (15.3) 4.21 d (15.3)
11	C	169.1	–	170.4	–
$COO\underline{C}H_3$	CH_3	51.9	3.75 s	52.2	3.78 s
Glucosyl					
1′	CH	101.6	4.72 d (8.0)	100.3	4.70 d (8.0)
2′	CH	75.0	3.25 dd (8.0, 9.0)	74.8	3.24 dd (8.0, 9.0)
3′	CH	77.8	3.40 t (9.0)	77.9	3.40 t (9.0)
4′	CH	71.6	3.30 – 3.26†	71.5	3.30†
5′	CH	78.5	3.30 * 3.26†	78.4	3.32†
6′	CH_2	61.7	3.85 dd (12.0, 1.2) 3.63 dd (11.7, 6.0)	61.1	3.89 dd (11.7, 1.2) 3.66 dd (11.7, 5.3)

† Signal pattern unclear due to overlapping.

Scandoside methyl ester (**6**): $[\alpha]_D^{20}$ −42.7° (*c* 0.5, MeOH); ^{1}H NMR and ^{13}C NMR (500 and 125 MHz, resp., CD_3OD): Table 1; Positive-ion HRMS: *m/z* 427.1174 $[M+Na]^+$, Negative-ion HRMS *m/z* 403.1234 $[M-H]^-$, (calc. for $C_{17}H_{24}O_{11}$, Mol. Wt. 404.13 (Moreira et al. 2010).

6-*O*-methyl-deacetyl-daphylloside (**7**): $[\alpha]_D^{20}$ −84° (*c* 0.5, MeOH);

^{1}H NMR and ^{13}C NMR (500 and 125 MHz, resp., CD_3OD): Table 2; Positive-ion HRMS: *m/z* 441.1324 [M+Na]+, Negative-ion HRMS *m/z* 417.1387 $[M-H]^-$, (calc. for $C_{18}H_{26}O_{11}$, Mol. Wt. 418.15) (Machida et al. 2003).

6-*O*-methyl-scandoside methyl ester (**8**): $[\alpha]_D^{20}$ + 32° (*c* 0.5, MeOH); ^{1}H NMR and ^{13}C NMR (500 and 125 MHz, resp., CD_3OD): Table 2; Positive-ion HRMS: *m/z* 441.1334 [M+Na]+, Negative-ion HRMS *m/z* 417.1391 $[M-H]^-$, (calc. for $C_{18}H_{26}O_{11}$, Mol. Wt. 418.15) (Machida et al. 2003).

3. Results and discussion

The methanolic extract of overground parts of *W. ligustroides* resulted in the isolation of eight iridoid glucosides (**1 – 8**). The one proton singlet observed between 7.40 and 7.66 assigned to H-3 for the compounds in the ^{1}H NMR spectra showed that all isolated compounds were C-4 substitued carboxylic iridoids. The proton signals observed as doublets between 4.62 and 4.74 ppm with 8.0 Hz coupling constants arising from a *trans*-diaxial interaction were indicative for their mono glycosidic structures. The other protons found in the same spin system of the anomeric protons and the corresponding carbon resonances were established by COSY and HSQC experiments, respectively. The coupling constants ($J_{1,2}$ = 8 Hz, $J_{2,3}$ = $J_{3,4}$ = $J_{4,5}$ = 9 Hz, $J_{5,6a}$ = 2 Hz, $J_{5,6b}$ = 6 Hz, and $J_{6a,6b}$ = 12 Hz) of the sugar protons and the chemical shift values observed at ca. δ 100.0, 74.0, 78.0, 71.0, 79.0 and 62.0 assigned to

Table 2
^{1}H and ^{13}C NMR data of 6-*O*-methyl-deacetyl-daphylloside (**7**), 6-*O*-methyl-scandoside methyl ester (**8**) ($CDCl_3$; δ_H 500 MHz; δ_C 125 MHz).

C/H	DEPT	7		8	
		δ_C ppm	δ_H ppm, *J* (Hz)	δ_C ppm	δ_H ppm, *J* (Hz)
1	CH	101.84	4.98 d (8.8)	95.20	5.64 d (2.8)
3	CH	155.15	7.64 br s	153.65	7.43 s
4	C	108.16	–	110.48	–
5	CH	42.08	3.11 t (6.3)	39.08	3.25†
6	CH	84.99	4.39 br d (6.0)	90.05	4.19 br s
7	CH	127.61	6.20 s	127.37	5.85 br s
8	C	152.87	–	149.71	–
9	CH	46.00	2.56 t (8.0)	47.50	3.30†
10	CH_2	61.74	4.50 d (15.0) 4.22 d (15.0)	60.45	4.30 d (15.0) 4.21 d (15.0)
11	C	169.50	–	169.18	–
$COO\underline{C}H_3$	CH_3	51.93	3.77 s	51.82	3.74 s
OCH_3	CH_3	57.45	3.25 s	57.12	3.45 s
Glucosyl					
1′	CH	100.78	4.74 d (7.9)	99.98	4.62 d (7.9)
2′	CH	74.93	3.27†	74.64	3.21 dd (7,9, 9.0)
3′	CH	77.81	3.43 t (9.0)	77.92	3.38 t (9.0)
4′	CH	71.37	3.37 t (9.0)	71.56	3.30†
5′	CH	78.26	3.29†	78.35	3.33†
6′	CH_2	62.51	3.84 brd (12,1, 2.0) 3.70 br d (12,1, 5.3)	62.76	3.91 dd (11.9, 1.2) 3.68 dd (11.0, 5.9)

† Signal pattern unclear due to overlapping.

the carbon resonances of the sugar units proved the presence of a β-glucopyranosyl moiety for **1 – 8** (*see* Experimental).

The ^{1}H NMR spectrum of **1** exhibited a methyl resonance at δ 1.81 as a broad singlet which was assigned as H_3-10. The downfield shift of the methyl signal was consistent with the presence of a double bond between C-7 and C-8. An olefinic proton signal at δ 5.47 (br s, H-7) was further evidence for this proposal. In addition to the protons assigned to H-5 (δ 3.14) and H-9 (δ 2.61), a set of methylene protons at δ 2.73 (H-6a) and 2.08 (H-6b) were observed. Based upon these observations the structure of the compound **1** was identified as 10-deoxygeniposidic acid. The ^{1}H NMR data of **1** suggested the presence of a structure similar to those of nepetanudoside B isolated from a *Nepeta nuda* ssp. *albiflora* (Takeda et al. 1996). As shown by these authors, the characteristic H-1′ shift of the Nepeta-compounds is δ_C 104.5, while the 'normal' shift for this carbon is δ_C 99–100. Thus, the aglycone of nepetanudoside B is an enantiomer of 10-deoxy-geniposidic acid (**1**) (Inoue et al., 1992).

Compound **2** was isolated in admixture with compound **1** due to their similar polarities. Compound **1** was the major one in the mixture. The major differences were arising in their cyclopentane rings. In the ^{1}H NMR spectrum of the mixture, the methyl resonance and the olefinic H-7 proton were missing for the minor compound, **2**. Instead of a methyl resonance and olefinic H-7 proton, the two protons of the isolated exocyclic methylene protons are found at δ 5.12 and 5.07 as broadened singlets due to allylic coupling (each 1H, br d, J = 2.0 Hz, H_2-10). The remaining signals between 2.30 and 1.95 ppm were evident for the presence of two methylene protons for cyclopentane ring (see Experimental). Based upon these observations the structure of compound **2** was determined as 10-deoxygardoside. The assignments of all protons were in good agreement with those of reported for 7-deoxygardoside confirming this deduction (Bianco et al., 1986).

Compound **3** was obtained as an amorphous colourless powder. The molecular formula was found as $C_{16}H_{22}O_{10}$ by negative-ion HRMS (m/z 373.1127 $[M-H]^-$ and NMR data: Molecular weight: 374,12). The ^{1}H NMR spectrum was characteristic for C-4 substituted iridoids (loganin-type). H-3 was observed at 7.40 ppm as a singlet. Additionaly, an olefinic proton signal at 5.81 assigned as H-7 indicated a structure similar to that of 10-deoxygeniposidic acid (**1**). However, a pair of protons observed at δ 4.33 and 4.21 as an AB system (J_{AB} = 14.1 Hz) showed the presence of a hydoxymethylene functionality instead of a methyl gruoup at C-8. Thus the structure of compound **3** was determined as geniposidic acid (Akdemir&Çalış, 1991; Tzaoku et al., 2007). All proton and carbon resonances were in good accordance with those of reported for geniposidic acid confirming the proposed structure (*see* Experimental).

Compound **4** was obtained as an amorphous powder. The molecular weight was established as 360,14 indicating a molecular formula of $C_{16}H_{24}O_9$ by negative-ion HRMS (m/z 373.1127 $[M-H]^-$) and NMR data (*see* Experimental). The ^{1}H NMR spectrum of 4 showed the signals at highfield region arising three methines (H-5, H-9 and H-8), two methylene groups (H_2-6 and H_2-7) for cyclopentane moiety in addition to H-1 and olefinic H-3 protons of pyrane moiety. Except H-3, all of these protons were observed as a part of a single spin system in COSY experiment which clearly assigned all protons including the locations of two methylene protons to be at C-6 and C-7. The stereochemistry of the secondary methyl resonance was based on a NOESY experiment which shows a NOE correlation between H-1 (δ 5.48 d, J = 4.5 Hz) and Me-10 (δ 1.11 d, J = 6.7 Hz). Furthermore, the protons on the α- and β-sites of the cyclopentan-pyrane ring system were also established by the help of NOESY experiment (*see* Experimental). Especially, NOE correlations observed between H-5, H-9 and H-8 supported this argument. The stereochemistry at C-8 for **4** was determined by the ^{13}C NMR spectrum. Thus, the shift for C-9 and C-10 (δ_C 44.4 and 16.8, respectively) is characteristic for 7-deoxy-8-*epi*-loganic acid, as opposed to the other epimer which would be found at δ_C 48.5 and 20, respectively (Damtoft et al., 1981). Based on these observations together with the comparison of the NMR data with those of reported, the structure of compound 4 was determined as 8-*epi*-deoxyloganic acid (Murai et al., 1984; Tasdemir et al., 1999; Teng et al., 2005).

The molecular formula of the compounds **5** and **6** were established by HRMS (m/z 403.3549 $[M-H]^-$ and m/z 427.3519 $[M+Na]^+$ for both). These results supported the molecular weight for both compounds to be 404,3629 calculated for $C_{17}H_{24}O_{11}$. Their ^{1}H and ^{13}C NMR data (Table 2) showed the presence of an iridoid mono-glucoside having loganin-type skeletons with similar substitutients and functionalities in the two compounds. These were a $COOCH_3$, an OH group, an olefinic proton and a CH_2OH group. The ^{13}C NMR resonances (δ 129.8 and 151.5 and 130.1 and 147,6, respectively) assigned for carbons C-7 and C-8 showed the location of double bond. The major differences between **5** and **6** were observed for chemical shifts and the coupling constants of the H-1 and C-1 as well as H-6 and C-6. Therefore, the structural difference between 5 and 6 was arising the stereochemistry of OH group located at C-6. The ^{1}H and ^{13}C NMR data of **5** and **6** were in accordance to those of 6α-hydroxy-geniposide (=deacetylasperulosidic acid methyl ester, deacetyldaphylloside; Tzakou et al., 2007) and 6β-hydroxy-geniposide (=scandoside methyl ester; Moreira et al., 2010), respectively.

The molecular weights of the compounds **7** and **8** were also found to be the same and 14 Da bigger than those of **5** and **6** by HRMS. The negative ion HRMS od **7** and **8** showed the quasi molecular ion peaks at m/z 417.3583 $[M-H]^-$ while positive ion HRMS at m/z 441.3557 $[M+Na]^+$ showing the molecular weight of both compounds to be 418,3896 giving the molecular composition $C_{18}H_{26}O_{11}$. These observation was supported by the presence of an additional methoxyl signals in the ^{1}H NMR spectra of **7** and **8**. (δ_H: 3.25 s and 3.45 s, resp., OC***H***$_3$) as well as in ^{13}H NMR spectra (δ_C: 57.45 and 57.12, resp., O**C**H_3) (Table 2). All protons and carbon resonances of the compounds **7** and **8** were assigned based on the 2D-NMR experiments (COSY, HSQC and HMBC). Moreover, the same similarities were observed between **5** and **7** and between **6** and **8** for the chemical shifts of all proton and carbon resonances as well as for the coupling constants strongly supporting that the 7 and 8 were the 6-*O*-methoxy derivatives of **5** and **6**. These deductions were supported by the long-range ^{13}C, ^{1}H correlations between C-6 [δ_C 84.99 and 90.05; C-6 of **7** and **8**, resp.)and methoxy signals (δ_H 3.25 and 3.45; C(6)OCH_3 of **7** and **8**, resp.] for both compounds. Consequently, based on the NMR data presented in Table 3 the structures of **7** and **8** were established as 6-*O*-methyl deacetylasperulosidic acid methyl ester and 6-*O*-methyl deacetylscandoside methyl ester, resp. (Machida et al., 2003). Furthermore, the optical rotations of **7** ($[\alpha]_D^{20}$ −84°) and **8** ($[\alpha]_D^{20}$ + 32°) were in good agreement to those of reported in the same study confirming this deduction.

The coupling constants ($J_{1\text{-}9}$) observed for H-1 of the aglycone moieties in compounds **5 – 8** (J = 9.0 and 8.8 Hz for **5** and **7**; J = 6.3 and 2.8 Hz for **6** and **8**) are caused by a change in the conformations of cyclopentan-pyrane ring junctions. These effects are best explained by the *O*-substitution at C-6 as reported (Damtoft et al.,1981).

4. Conclusion

Wendlandia ligustroides (Rubiaceae) have been investigated chemically for the first time. The iridoid glucosides isolated in the present work are common in parts of the Rubiaceae family. None of them have to our knowledge any particular reported phar-

macological activities. However, ester derivatives of scandoside and daphylloside from *Wendlandia formosana* are known to show strong radical scavenging activity (Dinda, 2019).

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