FLAVONOIDS FROM THE LEAVES OF *ARALIA HIEPIANA*

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ABSTRACT

Aralia hiepiana J.Wen & Lowry (Araliaceae), a new species from southern Vietnam, is described and illustrated by J. Wen & Lowry in 2002. Up to now, there are not any chemical data from this endemic species. By various chromatography methods, fiveflavonoids namely kaempferitrin (**1**), kaempferol 3-O-β-D-glucopyranosyl-7-O-α-L-rhamnopyranoside(**2**), kaempferol (**3**), quercetin (**4**),andapigetrin (**5**) were isolated from the methanol extract of leaves of *Aralia hiepiana*collected in Da Lat, Lam Dong province. Their structures were elucidated using 1-D and 2-D NMR techniques and by comparison with the literature data. This is the first time to isolate these compounds from *Aralia hiepiana*.

*Keywords:Aralia hiepiana,*Araliaceae, kaempferitrin, kaempferol3-O-β-D-glucopyranosyl-7-O-α-L-rhamnopyranoside, kaempferol, quercetin, apigetrin.

1. INTRODUCTION

Aralia genus belonging to Araliaceae family, comprises of 74 species over the world [1] and15 species in Vietnam [2]. Several species have been used as in traditional herbal medicine for the treatment of gastric ulcer, hepatitis, rheumatic, arthritis, cancer and other diseases [2]. There have been many publications on the chemical compositions and biological activity of the *Aralia* species such as *Aralia elata* [3, 4], *A. taibaiensis* [5, 6], and *A. armata* [7]. Compounds mainly found in this genus were triterpenoid saponin and flavonoid glycosides [3-7].

Aralia hiepiana J.Wen & Lowry (Araliaceae) is widely distributed in the west highland of Vietnam. No phytochemical work, however, has been performed on this plant to date.This paper mainly describes the isolation andstructural identification of five flavonoids compounds, namely kaempferitrin (**1**), kaempferol 3-O-β-D-glucopyranosyl-7-O-α-L-rhamnopyranoside (**2**), kaempferol (**3**), quercetin (**4**), and apigetrin (**5**) from the methanol extract of the leaves of *A.hiepiana.*

2. EXPERIMENT

2.1. General experimental procedures

^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectrawere measured on a Bruker AM500 FT-NMR spectrometer. The Electrospray Ionization – Mass Spectroscopy (ESI-MS) spectra were obtained from an Agilent 1260 series single quadrupole LC/MS system. Column chromatography (CC) was carried out on silica gel (Si 60, 230-400 mesh, Merck) and RP-C_{18} column. All solvents were redistilled before use. Thin-layer chromatography was performed on silica gel 60 F_{254} sheets (Merck) and RP-18 F_{254S} plates (Merck).Compounds were visualized under UV radiations (254, 365 nm) and by spraying plates with 10% H_2SO_4 followed by heating.

2.2. Plant material

The leaves of *Aralia hiepiana* J.Wen & Lowry were collected in April 2017 at DaLat, Lam Dong province and were identified by Dr. Nong Van Duy from Tay Nguyen Institute for Scientific Research, VAST. A voucher specimen (No.TN3/129) is deposited at Tay Nguyen Institute for Scientific Research, VAST.

2.3. Extraction and isolation

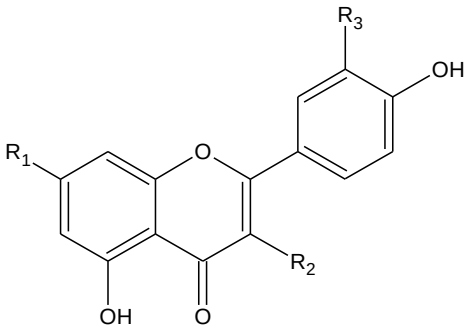
The powdered leaves of *A.hiepiana* (4.5 kg) were extracted with methanol at 40^{o}C three times at room temperature. The solvent was evaporated and concentrated under low pressure to give the crude extract (619.0g).

The crude extract was dissolved in methanol:water (50:50, v/v) and then partitioned with hexane, chloroform, ethyl acetateto provide the corresponding extracts of: hexane (187.6g), $CHCl_3$(17.6g), EtOAc (52.3g) and a water layer.

The chloroform extract was fractionated by silica gel column chromatography using a mixture of chloroform-methanol (100:0 - 0:100, v/v)to yield twelve fractions (C1 - C12). Fraction C10 (177mg) was applied to a silica gel CC and eluted with ethylacetate-methanol (6:1, v/v) to give three sub-fractions (C10.1 - C10.3). The sub-fraction C10.2 (132mg) was chromatographed on RP-C18, eluted with methanol-water (1:4, v/v) to obtain compound **1**. Fraction C11 was separated on an RP-18CC using the mobile phase of methanol-water (1:3 - 1:0, v/v) to obtain six sub-fractions (C11.1 - C11.6). Compound **2** was purified from sub-fraction C11.5 (39 mg) by silica gel CC eluting with chloroform-methanol (5:1, v/v).

The ethyl acetate extract was separated on a silica gel CC, eluted with chloroform-methanol (100:0 - 0:100, v/v) to yield five fractions. Fraction E4 (23.6g) was chromatographed on a silica gel CC using a mixture of dichloromethane-acetone-methanol (10:1:1 - 5:1:1, v/v/v) to give twenty sub-fractions (E4.1 – E4.20). The sub-fraction E4.7 (1.7g) was subjected on a Sephadex LH-20 column using a gradient of methanol-water (1:3 – 1:0, v/v) to obtain sixsub-fractions (E4.7.1 – E4.7.6). The sub-fraction E4.7.6 (30 mg) was further purified by solid-phase extraction (SPE) to obtain compound **3**. Fraction E5 (30.3g) was chromatographed over a silica gel CC, eluted with chloroform-methanol (100:0 - 0:100, v/v) to afford fourteenth sub-fractions (E5.1-E5.14). Compounds **4** and **5** were purified from sub-fraction E5.8 (596 mg) by SPE eluting with methanol-water (0:1-1:0, v/v).

Kaempferitrin (**1**): yellow crystals; ^{1}H-NMR (500 MHz, CD_3OD-d_4) and ^{13}C-NMR (125 MHz, CD_3OD-d_4) see Table 1. ESI-MS (*m/z*): 579 $[M+H]^+$ ($C_{27}H_{30}O_{14}$, M = 578).



(1) $R_1 = R_2$ = O-Rha, R_3 = H
(2) R_1= O-Rha, R_2 = O-Glu, R_3 = H
(3) $R_1 = R_2$ = OH, R_3 = H
(4) $R_1 = R_2 = R_3$ = OH
(5) R_1 = O-Glc, $R_2 = R_3$ = H

Figure 1.Structures of compounds **1**-**5**.

Kaempferol 3-*O*-β-D-glucopyranosyl-7-*O*-α-L-rhamnopyranoside (**2**): yellow amorphous powder; ^{1}H-NMR (500 MHz, DMSO-d_6) and ^{13}C-NMR (125 MHz, DMSO-d_6), see Table 1; ESI-MS: *m/z* 617 $[M+Na]^+$, 593 $[M-H]^-$ ($C_{27}H_{30}O_{15}$, M = 594).

Kaempferol (**3**): yellow powder; ^{1}H-NMR (500MHz, CD_3OD-d_4) δ_H (ppm): 8.10 (2H, d, *J* = 8.5 Hz, H-2', H-6'), 6.93 (2H, d, *J* = 8.5 Hz, H-3', H-5'), 6.20 (1H, d, *J* = 2.0 Hz, H-6), 6.42 (1H, d, *J* = 2.0 Hz, H-8); ^{13}C-NMR (125 MHz, CD_3OD-d_4) see Table 2; ESI-MS *m/z:* 285 $[M-H]^-$, 287 $[M+H]^+$ ($C_{15}H_{10}O_6$, M = 286).

Quercetin (**4**): yellow amorphous powder**;** ^{1}H-NMR (500 MHz, CD_3OD-d_4) δ_H (ppm): 7.76 (1H, s, H-2'), 7.66 (2H, d, *J* = 8.5 Hz, H-6'), 6.91 (2H, d, *J* = 8.5 Hz, H-5'), 6.41 (1H, s, H-8), 6.21 (1H, s, H-6); ^{13}C-NMR (125 MHz, CD_3OD-d_4) see table 2; ESI-MS *m/z:* 301 $[M-H]^-$ ($C_{15}H_{10}O_7$, M = 302).

Apigetrin (**5**): yellow amorphous powder; ^{1}H-NMR (500 MHz, DMSO-d_6)7.96 (2H, d, *J* = 8.5 Hz, H-2', H-6'), 6.96 (2H, d, *J* = 8.5 Hz, H-3', H-5'), 6.45 (1H, d, *J* = 2.0 Hz, H-6), 6.84 (1H, d, *J* = 2.0 Hz, H-8), 6.86 (1H, s, H-3), 5.07 (1H, d, *J* = 7.5 Hz, H-1''), 3.49-3.17 (4H, H-2'', H-3'', H-4'', H-5''), 3.72 (1H, m, H-6''), 3.48 (1H, m, H-6''); ^{13}C-NMR (125 MHz, DMSO-d_6) see Table 2; ESI-MS *m/z:* 431 $[M-H]^-$ ($C_{21}H_{20}O_{10}$, M = 432).

3. RESULTS AND DISCUSSION

Compound **1** was obtained as yellow crystals. The ^{1}H- and ^{13}C-NMR spectra (Table 1)showed the aromatic proton signals characteristic for the 1,4-disubstituted ring B of a flavonoid glycoside, at δ_H 7.81 (2H, d, *J* = 8.5 Hz, H-2', H-6') and 6.96 (2H, d, *J* = 8.5 Hz, H-3', H-5'). Two doublet signals observed at δ_H 6.46 (1H, d, *J* = 2.0 Hz) and 6.75 (1H, d, *J* = 2.0 Hz) were assigned for protons H-6 and H-8, respectively. Compound **1** displayed two anomeric proton signals at δ_H 5.42 (d, *J* = 2.0 Hz, rha-H-1'') and 5.58 (d, *J* = 2.0 Hz, rha-H-1''') in the ^{1}H NMR spectrum, and also exhibited two anomeric carbon signals at δ_C 103.53 (rha-C-1'') and 99.88 (rha-C-1''') in the ^{13}C-NMR spectrum. The sugar linkages were determined based on HMBC spectrum. A long-range correlation was observed between a proton signal at δ_H 5.42 (rha-H-1'') and a carbon signal at δ_C 136.50 (C-3) of the aglycone moiety, while the other anomeric proton signal at δ_H 5.58 (rha-H-1''') showed a correlation with a carbon signal at (δ_C 163.57 (C-7). These data and other NMR data thus allowed us to identify compound **1** as kaempferitrin (Figure 1)[8]. Kaempferitrin exerts immunostimulatory effects on immune responses mediated by splenocytes, macrophages, PBMC and NK cells [9].

Compound **2** was isolated as a yellow amorphous powder. The ^{1}H- and ^{13}C-NMR spectra of **2** were very similar to those of **1** (Table 1), except for one sugar unit signals. The addition of a carbon signal of the sugar residue at δ_C 60.87 (C-6")and the chemical shift value of the carbon signals at δ_C100.78 (C-1"), 74.22 (C-2"), 76.44 (C-3"), 69.93 (C-4"), and 77.55 (C-5") showed the presence of a glucose at **2** instead of rhamnose at **1**. This β-D-glucopyranosyl unit was determined to attach on carbon C-3 of the flavonoid ring system by the HMBC correlations of anomeric proton signal [δ_H5.47 (d, *J* = 7.0 Hz, H-1")] to carbon C-3 (δ_C 133.48). From the spectroscopic data, the structure of compound **2** was determined as kaempferol 3-O-β-D-glucopyranosyl-7-O-α-L-rhamnopyranoside, isolated also from *Brugmansiasuaveolens*[10] (Figure 1).

*Table 1.*The ^{1}H and ^{13}C-NMR spectroscopic data of compounds **1** and **2**.

	1			**2**		
Position	[a]δ_C	δ_C	δ_H (*J* in Hz)	[b]δ_C	δ_C	δ_H (*J* in Hz)
2	159.90	159.82		156.84	156.79	
3	136.60	136.50		133.52	133.48	
4	179.90	179.80		177.68	177.64	
5	163.10	163.04		160.91	160.88	
6	100.56	100.56	6.46, d, 2.0	99.42	99.39	6.44, d, 2.0
7	163.60	163.57		161.63	161.59	
8	95.70	95.61	6.75, d, 2.0	94.54	94.5	6.82, d, 2.0
9	158.20	158.11		156.03	155.99	
10	107.60	107.58		105.71	105.68	
1′	122.50	122.42		120.75	120.70	
2′	132.00	132.00	7.81, d, 8.5	131.03	131	8.07, d, 9.0
3′	116.60	116.59	6.96, d, 8.5	115.21	115.18	6.89, d, 9.0
4′	161.80	161.78		160.23	160.24	
5′	116.60	116.59	6.96, d, 8.5	115.21	115.18	6.89, d, 9.0
6′	132.00	132.00	7.81, d, 8.5	131.03	131	8.07, d, 9.0
1″	103.53	103.53	5.42, d, 2.0	100.82	100.78	5.47, d, 7.0
2″	71.92	71.92	4.24, dd, 2.0, 3.5	74.24	74.22	3.20, m
3″	72.20	72.13	3.74, dd, 3.5, 9.0	76.46	76.44	3.22, m
4″	73.20	73.19	3.38, m	69.94	69.93	3.08, m
5″	72.00	72.00	3.38, m	77.57	77.55	3.08, m
6″	17.70	17.66	1.28, d, 6.0	60.87	60.87	3.56, m; 3.32, m
1‴	99.90	99.88	5.58, d, 2.0	98.42	98.4	5.55, s
2‴	71.70	71.70	4.04, m	70.09	70.07	3.43, m
3‴	72.14	72.09	3.85, m	70.30	70.26	3.63, m
4‴	73.60	73.61	3.50, t, 9.5	71.66	71.62	3.30, m
5‴	71.30	71.30	3.63, m	69.84	69.82	3.84, m
6‴	18.10	18.07	0.96, d, 6.0	17.94	17.91	1.11, d, 6.0

[a]δ_C of kaempferitrin (125 MHz. CD_3OD-d_4) [8]

[b]δ_C of kaempferol 3-O-β-D-glucopyranosyl-7-O-α-L-rhamnopyranoside (125 MHz.CD_3OD-d_4) [10].

Compound **3** was isolated as the yellow powder. The ^{1}H-NMR spectrum of compound **3** displayed pattern of flavonol, with characteristic signals of two doublet signals in ring A at δ_H 6.20 (1H, d, *J* = 2.0 Hz, H-6) and 6.42 (1H, d, *J* = 2.0 Hz, H-8), and signals of an AA'BB' spin system (ring B) at δ_H 8.10 (2H, d, *J* = 8.5 Hz, H-2', H-6'), 6.93 (2H, d, *J* = 8.5 Hz, H-3', H-5'). The ^{13}C-NMR spectrum of compound **3** contained 15 carbon signals, including 8 quaternary

aromatic carbons, a carbonyl group, and 6 methine aromatic carbons (Table 2). By comparison of the spectroscopic data with those in published literature [11], compound **3** was identified as kaempferol (Figure 1), which is commonly found in many plant species.

*Table 2.*The ^{13}C-NMR data of compounds (**3-5**).

	3		**4**		**5**	
Position	$^{c}\delta_C$	δ_C	$^{d}\delta_C$	δ_C	$^{e}\delta_C$	δ_C
2	148.1	148.08	147.9	148.03	164.9	164.31
3	137.1	137.13	137.2	137.20	103.8	103.09
4	177.3	177.39	177.3	177.35	182.7	182.00
5	162.4	162.52	162.5	162.51	162.1	161.10
6	99.3	99.29	99.3	99.25	100.2	99.54
7	165.5	165.59	165.7	165.57	163.6	162.97
8	94.5	94.48	94.4	94.42	95.5	94.87
9	158.2	158.28	158.2	158.25	157.6	156.95
10	104.5	104.56	104.4	104.53	106.0	105.34
1′	123.7	123.75	124.1	124.17	121.7	120.99
2′	130.7	130.69	116.0	116.02	129.3	128.61
3′	116.3	116.32	146.2	146.23	116.7	116.03
4′	160.5	160.56	148.7	148.03	161.7	161.42
5′	116.3	116.32	116.2	116.24	116.7	116.03
6′	130.7	130.69	121.6	121.69	129.3	128.61
1″					100.6	99.93
2″					73.8	73.11
3″					77.8	77.17
4″					70.2	69.58
5″					77.1	76.44
6″					61.3	60.61

$^{c}\delta_C$ of kaempferol (125 MHz, CD_3OD-d_4) [11], $^{d}\delta_C$ of quercetin (75 MHz, CD_3OD-d_4) [12], $^{e}\delta_C$ of apigetrin (100 MHz, DMSO-d_6) [13].

Compound **4** was obtained as a yellow amorphous powder. The ^{1}H and ^{13}C NMR spectral data of **4** were similar to those of **3** (Table 2), except for the appearance of an ABX system at δ_H 7.76 (1H, br s, H-2'), 7.66 (1H, d, *J* = 8.5 Hz, H-6'), and 6.91 (1H, d, *J* = 8.5 Hz, H-5') in **4** (Figure 1). Based on the NMR data and comparison of the data given in theliterature, compound **4** was determined as quercetin (Figure 1) [12].

Compound **5** was isolated as a yellow amorphous powder. The ^{1}H NMR spectra displayed pattern of flavone glycoside, with characteristic signals of two doublet signals in ring A at δ_H 6.45 (1H, d, *J* = 2.0 Hz, H-6) and 6.84 (1H, d, *J* = 2.0 Hz, H-8), signals of 1,4-disubstituted ring B of a flavonoid at δ_H 7.96 (2H, d, *J* = 8.5 Hz, H-2', H-6'), 6.96 (2H, d, *J* = 8.5 Hz, H-3', H-5'), and a singlet signal at δ_H 6.86 (H-3). Compound **5** also displayed an anomeric proton signal at δ_H 5.07 (d, *J* = 7.5 Hz, glc-H-1") in the ^{1}H-NMR spectrum, and exhibited an anomeric carbon signal at δ_C 99.93 (glc-C-1") in the ^{13}C-NMR spectrum. This β-D-glucopyranosyl residue was determined to attach on carbon C-7 of the flavonoid ring system by the HMBC correlations of anomeric proton signal [δ_H 5.47 (H-1")] to carbon C-7 (δ_C 162.97). Based on the NMR data and comparison of the data given in the literature, the structure of compound **5** was identifed as apigetrin (Figure 1) [13]. This compound has antioxidant properties as well as immunomodulating effects upon splenocytes, NK and CTL cells, and macrophages [14].

4. CONCLUSION

From the methanol extract of leaves of *Aralia hiepina*, five flavonoids namely kaempferitrin (**1**), kaempferol 3-O-β-D-glucopyranosyl-7-O-α-L-rhamnopyranoside (**2**), kaempferol (**3**), quercetin (**4**), andapigetrin (**5**) were isolated. Their structures were elucidated by 1D- and 2D-NMR spectral data as well as comparison with those reports. This is the first report on the chemical constituents of this plant.

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