ISOLATION OF FLAVONOIDS FROM LEAVES OF *CARALLIA BRACHIATA*

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Trinh Thi Thanh Van, Nguyen Hoang Nam, Tran Van Hieu, Vu Van Nam, Le Nguyen Thanh, Tran Hong Quang, Pham Van Cuong, Nguyen Quoc Vuong*
Institute of Marine Biochemistry, Vietnam Academy of Science and Technology, Ha Noi, Viet Nam
* Email: nguyenvuong@imbc.vast.vn

TÓM TẮT

PHÂN LẬP MỘT SỐ HỢP CHẤT FLAVONOID TỪ LÁ LOÀI XĂNG MÃ (*CARALLIA BRACHIATA* MERR.)

*Từ dịch chiết ethyl acetate của lá loài Xăng mã (Carallia brachiata Merr.), một số hợp chất flavonoid gồm (+) - catechin (**1**), (-) - catechin (**2**), catechin 3-O-α-L-rhamnopyranoside (**3**), chrysoeriol (**4**) và engeletin (**5**) đã được phân lập. Cấu trúc của chúng đã được xác định dựa trên sự phân tích phổ cộng hưởng từ hạt nhân (NMR) và phổ khối (ESI-MS) và so sánh với tài liệu. Đây là lần đầu tiên những flavnoid (**2**-**5**) trên được phân lập từ chi Carallia cũng như từ loài Xăng mã.*

Keywords: *Flavonoid, catechin, chrysoeriol, engeletin, Xăng mã (Carrallia brachiata Merr.).*

1. INTRODUCTION

Carallia brachiata Merr. is a flower plant (Vietnamese name is "Xang ma, Truc tiet") belonging to the family Rhizophoraceae. This plant has been used as a folk medicinal plant in Vietnam for treatment of tongue sores, mouth ulcers, malaria and sore throats [1,2]. In the world, chemical investigations of this plant have shown megastigmanes, aromatic compounds, condensed tannins, flavonoids, and glyceroglycolipids from it [3]. Bioactives of this plant were also reported as anti-inflamatory [4] and antimicrobial activity [5]. In Vietnam, chemical studies on this plant have been still limited. Therefore, in our project, we recently reported, from leaves of *C. brachiata*, two new catechin derivatives (carabrachiatanins A and B) and seven known proanthocyanidins were isolated and their anti-inflammatory activity was evaluated by inhibition of NO production in RAW264.7 cells [6]. As it was known, the proanthocyanidins were oligomeric flavonoids, many of which were oligomers of flavan-3-ol compounds such as catechin and epicatechin. Despite low oral bioavailability, most polyphenols proved significant biological effects [7]. They were evaluated as affected biochemical and antioxidant agents used to treat various diseases such as cardiovascular, neurodegenerative and cancer [8, 9]. In this paper, we reported the isolation and structure resolution of five flavonoids including three flavan-3-ol compounds, (+)-catechin (**1**), (-)- catechin (**2**), and catechin 3-O-α-L-rhamnopyranoside (**3**), one flavone, chrysoeriol (**4**) and one flavononol, engeletin (**5**) from leaves of *C. brachiata* Merr.

2. RESEARCH MATERIALS AND METHODS

2.1. General experimental procedures

^{1}H-NMR, ^{13}C-NMR, and 2D-NMR spectra were recorded on a Bruker AM500 FT-NMR spectrometer using TMS as an internal standard. The electrospray ionization mass spectra (ESI-MS) were measured on an Agilent 1100 Series

LC/MSD Trap SL. Column chromatography (CC) was performed using silica gel 60 (230 - 400 mesh, Merck) or RP-18 resin (30 - 50 µm, Fuji Silysia Chemical Ltd., Aichi, Japan). Semi-preparative high-performance liquid chromatography (HPLC) were performed on an Agilent 1260 system including binary pump, autosampler, DAD detector, and semi-preparative HPLC column YMC J'sphere ODS-H80 (4 µm, 20 × 250 mm) with isocratic mobile phase at the flow rate of 2.5 mL/min used in Semi-prep-HPLC. The compound was monitored at wavelengths of 205, 230, 254, and 280 nm. Optical rotations were recorded on a JASCO P-2000 Polarimeter. Thin layer chromatography used precoated silica gel plates (Merck 60 F254).

2.2. Plant materials

Leaves and branches of *Carallia brachiata* Merr. were collected in Trieu Son, Thanh Hoa in March 2021 and identified by Dr Do Van Hai at Institute of Ecology and Biological Resources, VAST. The specimen (VAST.04.02/22-23) was kept at Institute of Marine Biochemistry, VAST.

2.3. Extraction and isolation

The *C. brachiata* (2.5 kg of the dried leaves) was pulverized then extracted with 85% MeOH (10 L × 4) by sonification at 50 °C for each time 2 h. The extracts were combined, and MeOH were removed in reduced pressure to give a crude MeOH extract (1.2 L). The MeOH extract was suspended with water (1.2 L) and successively partitioned with *n*-hexane and ethyl acetate (EtOAc) gave *n*-hexane (CH, 21.5 g) and ethyl acetate (CE, 72.0 g) residues and a water layer (CW, 1.5 L).

The AFE residue was chromatographed on a silica gel CC eluting with EtOAc to give five fractions (fr. CE1–CE5).

Fraction CE1 was chromatographed on a silica gel column eluting with *n*-hexane/EtOAc (1/1, v/v) to give five sub-fractions (CE1.1–CE1.5). The sub-fraction CE1.2 was chromatographed on a silica gel CC eluting with CH_2Cl_2/acetone (10/1, /v) to afford four fractions (CE1.2.1–CE1.2.4). Fraction CE1.2.1 was further chromatographed on sephadex LH-20 CC eluting with MeOH to give four fractions (CE1.2.1.1- CE1.2.1.4). Fraction CE1.2.1.2 was purified by HPLC with MeOH/H_2O (1/2, v/v) to give compound **1** (15 mg). Fraction CE1.2.3 was further purified by sephadex LH-20 CC eluting with MeOH to give five sub-fractions (CE1.2.3.1- CE1.2.3.5). Fraction CE1.2.3.2 was purified by HPLC with MeOH/H_2O (1/2, v/v) to give compound **2** (7.5 mg). Fraction CE1.2.3.4 was chromatographed by RP-18 CC eluting with MeOH/H_2O (1/2) to yield compound to give compound **3** (2.7 mg).

The fraction CE4 was chromatographed by RP-18 CC eluting with MeOH/H2O (1/4) to give five fractions (CE4.1-CE4.5). Fraction CE4.2 (27 g) was further purified by sephadex LH-20 CC eluting with MeOH to give compounds **4** (9.3 mg) and 5 (5.6 mg).

(+)-Catechin (**1**): yellow solid, $[\alpha]_D^{25}$ + 17 (c 0.1, MeOH). Negative ESI-MS: m/z 290 $[M]^-$. ^{1}H-NMR (CD_3OD, 500 MHz) and ^{13}C-NMR (CD_3OD, 125 MHz) see Table 1.

(−)-Catechin (**2**): yellow solid, $[\alpha]_D^{25}$ − 49 (c 0.1, MeOH). Negative ESI-MS: m/z 290 $[M]^-$. ^{1}H-NMR (CD_3OD, 500 MHz) and ^{13}C-NMR (CD_3OD, 125 MHz) see Table 1.

Catechin 3-*O*-α-L-rhamnopyranoside (**3**) yellow solid, $[\alpha]_D^{25}$ − 21 (c 0.1, MeOH). Negative ESI-MS: m/z 435 $[M]^-$. ^{1}H-NMR (CD_3OD, 500 MHz) and ^{13}C-NMR (CD_3OD, 125 MHz) see Table 1.

Chrysoeriol (**4**): yellow solid, $[\alpha]_D^{25}$ − 23 (MeOH; c 0,1). ESI-MS: m/z 301 $[M+H]^+$. ^{1}H-NMR (CD_3OD, 500 MHz) and ^{13}C-NMR (CD_3OD, 125 MHz) see Table 2.

Engeletin (**5**): yellow solid. ESI-MS: m/z 435 $[M]^-$. ^{1}H-NMR (CD_3OD, 500 MHz) and 13C-NMR (CD_3OD, 125 MHz) see Table 2.

3. RESULTS AND DISCUSSION

The structure of isolated compounds was described in Figure 1.

Compound **1** was isolated as yellow solid. The ^{1}H-NMR spectrum of **1** (Table 1) showed signals of two doublets of two meta coupling aromatic protons of the ring A at δ_H 5.93 (d, J = 2.2 Hz, H-

6) and δ_H 5.84 (d, *J* = 2.2 Hz, H-8; three ABX system aromatic protons of ring B at δ_H 6.82 (d, J = 1.6 Hz, H-2′), 6.75 (d, *J* = 8.0 Hz, H-5′), and 6.74 (dd, *J* = 1.6, 8.0 Hz, H-6′); and signals of C ring were observed with two oxymethine groups at δ_H 4.55 (d, *J* = 7.5 Hz, H-2) and 3.96 (m, H-3), and a methylene group at δ_H 2.84 (dd, *J* = 5.6, 16.0 Hz, H-4eq) and 2.49 (dd, *J* = 8.0, 16.0 Hz, H-4ax). The ^{13}C-NMR (Table 1) and DEPT spectra showed signals of fifteen carbons including seven non-protonated carbons at δ_C 157.6 (C-5), 157.8 (C-7), 156.9 (C-9), 146.2 (C-3′, C-4′), 100.8 (C-10) and 132.2 (C-1′); five methines at δ_C 96.2 (C-6), 95.4 (C-8), 115.2 (C-2′), 116.0 (C-5′) and 120.0 (C-6′); two oxygenated aliphatic methines at δ_C 82.9 (C-2) and 68.8 (C-3); and one aliphatic methylene at δ_C 28.6 (C-4). The HMBC correlations (Figure 2) between H-2 and C-4/C-3/C-1′/C-9/C-2′/C-6′; H-3 and C-1′/C-10; H-4ax and C-3/C-2/C-10/C-9/C-5, H-4eq and C-3/C-2/C-10 indicated that these protons were located at C-2, C-3, and C-4, respectively. Furthermore, the correlations between H-6 and C-5/C-8, H-8 and C-6/C-9/C-10, presented that two protons are located at C-6 and C-8, respectively. Above 1D and 2D NMR spectroscopic analysis of **1** and comparison with literature [10] identified compound **1** to be a catechin. The large coupling constant (*J* = 7.5 Hz) of H-2 with H-3 and the resonance position of C-2 at δ_C 82.8 ppm showed the 2,3-*trans* configuration of **1**. The optical rotation of **1** was determined to be $[\alpha]_D^{25}$ +17 (*c* 0.1, MeOH), establishing **1** to be (+)-catechin.

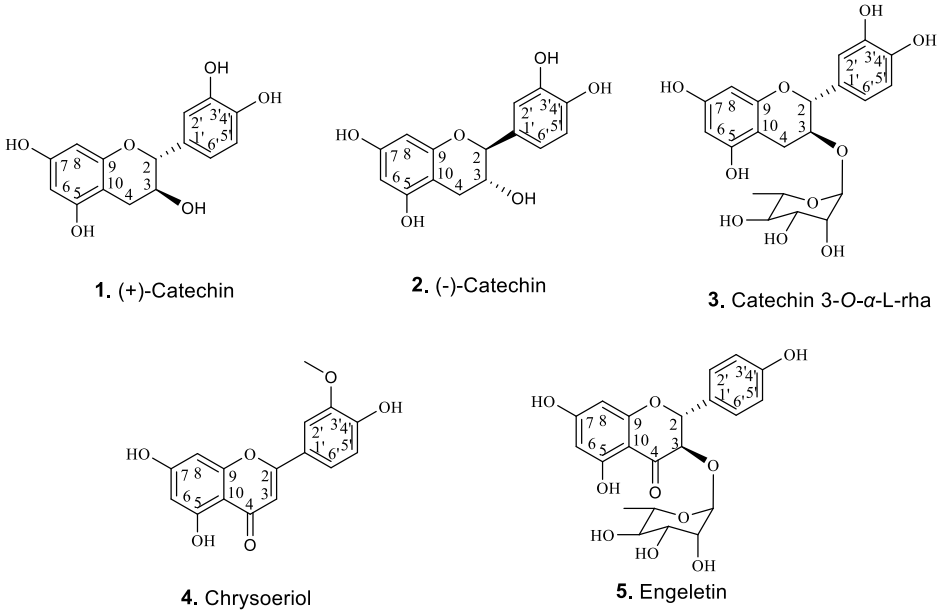


*Figure 1. Chemical structures of compounds **1**–**5***

Compound **2** was isolated as yellow solid. Similar compound **1**, the ^{13}C-NMR spectra of 2 (Table 1) gave signals of a flavanol with 15 carbons of 3 rings A, B and C including seven non-protonated carbons, five methines, two oxygenated aliphatic methines and one aliphatic methylene. The ^{1}H -NMR spectra of **2** (Table 1) also showed two meta coupling aromatic protons of A ring, three ABX system aromatic protons of B ring. Two protons of two oxymethine groups at δ_H 4.59 (d, *J* = 7.5 Hz, H-2) and 4.00 (ddd, 5.5, 7.5, 8.0 Hz, H-3), and two protons of a methylene group at δ_H 2.87 (dd, *J* = 5.4, 16.2 Hz, H-4eq) and 2.53 (dd, *J* = 8.4, 16.2 Hz, H-4ax), of C ring. The 1D, 2D NMR spectra analysis of **2** and comparision with literature [10] (Table 1 and Figure 2) also established compound **2** to be a catechin. The large coupling constant (*J* = 7.5 Hz) of H-2 with H-3 and the resonance position of C-2 at δ_C 82.9 confirmed 2,3-*trans* configuration. The optical rotation of **2** was determined to be $[\alpha]_D^{25}$ − 47 (c 0.1, MeOH), confirmed **2** to be (−)-catechin.

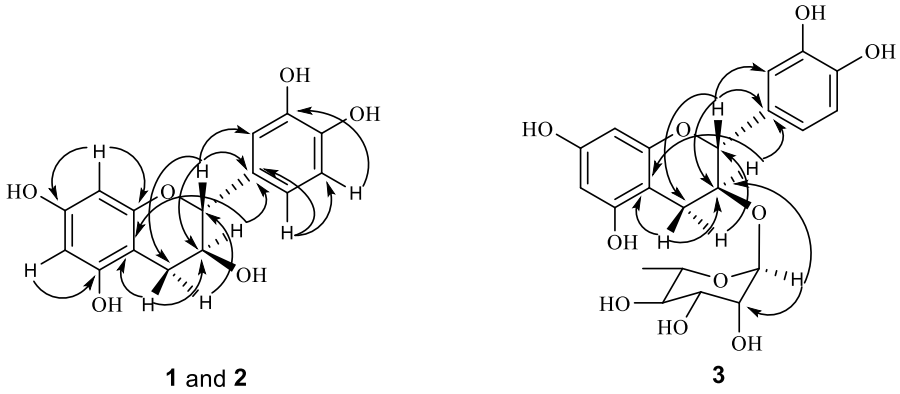
Compound **3** was isolated as yellow solid. The ESI-MS spectrum gave a negative quasi-molecular ion peak at m/z 435 $[M-H]^-$ ($C_{21}H_{23}O_{10}$) and ^{13}C-NMR spectrum of **3** indicated a molecular formula of $C_{21}H_{24}O_{10}$ (M = 436). The 1D (Table 1) and 2D NMR spectroscopic analysis of **3** revealed compound **3** to a flavanol glycosis. Moiety aglycone flavanol was a catechin similar compound **1** and **2** with signals of three rings system A, B and C. The proton signals of C ring were shown including two oxymethine groups at δ_H 4.64 (d, *J* = 7.8 Hz, H-2) and 3.96 (ddd, 5.4, 7.8, 8.4 Hz, H-3), and two protons of a methylene group at δ_H 2.90 (dd, *J* = 5.4, 16.2 Hz, H-4eq) and 2.66 (dd, *J* = 8.4, 16.2 Hz, H-4ax). The large coupling constant (*J* = 7.8 Hz) of H-2 with H-3 together the resonance position of C-2 at δ_C 81.1 confirmed 2, 3-*trans* configuration. The glycol moiety were identified to be a rhamnose sugar by recognisable signals shown including one anomeric carbon at δ_C 102.1 (C-1″) with anomeric proton at δ_H 4.32 (d, *J* = 1.2 Hz, H-1″); four oxygenated methines at δ_C 72.0 (C-2″)/δ_H 3.54 (dd, *J* = 1.2, 3.0 Hz, H-2″), δ_C 72.3 (C-3″)/δ_H 3.60 (dd, *J* = 3.0, 9.0 Hz, H-3″), δ_C 74.0 (C-4″)/ δ_H 3.33 (m, H-4″) and δ_C 70.3 (C-5″)/ δ_H 3.70 (m, H-5″); and one methyl at δ_C 17.9 (C-6″)/1.27 (d, *J* = 6.0 Hz, H-6″). The HMBC correlation (Figure 2) between H-1″ and C-3 showed sugar moiety linked to aglycone at C-3. The small coupling constant between H-1″ and H-2″ (*J* = 1.2 Hz) confirmed *α*-orientation of rhamnose sugar moiety. Above 1D, 2D NMR spectra analysis compound **3**, and comparison with literature [11] established compound **3** to be catechin 3-*O*-α-L-rhamnopyranoside.

*Table 1. The ^1H, ^{13}C-NMR spectroscopic data of compounds **1-3***

No.	**1**			**2**			**3**		
	δ_C ppm[a, b] [10]	δ_C ppm[c, d]	δ_H ppm[c, e] (mult., *J*= Hz)	δ_C ppm[a, b] [10]	δ_C ppm[c, d]	δ_H ppm[c, e] (mult., *J*= Hz)	δ_C ppm[a, f] [11]	δ_C ppm[a, g]	δ_H ppm[a, h] (mult., *J*= Hz)
2	82.8	82.9	4.55 (d, 7.5)	82.8	82.9	4.59 (d, 7.5)	80.2	81.1	4.64 (d, 7.8)
3	68.4	68.8	3.96 (m)	68.3	68.8	4.00 (ddd, 5.5, 7.5, 8.0)	74.5	75.9	3.96 (ddd, 5.4, 7.8, 8.4)
4	28.8	28.6	2.49 (dd, 8.0, 16.0) 2.84 (dd, 5.6, 16.0)	28.8	28.5	2.53 (dd 8.4, 16.2) 2.87 dd (5.4, 16.2)	27.6	27.9	2.66 (dd, 8.4, 16.2) 2.90 (dd, 5.4, 16.2)
5	157.1	157.6	-	157.2	157.5	-	157.0	157.5	-
6	96.2	96.2	5.93 (d, 2.2)	96.1	96.3	5.88 d (2.4)	95.4	96.4	5.96 (d, 2.4)
7	157.6	157.8	-	157.7	157.8	-	157.6	157.9	-
8	95.5	95.4	5.84 (d, 2.2)	95.3	95.5	5.95 d (2.4)	96.4	95.5	5.88 (d, 2.4)
9	156.7	156.9	-	156.9	156.9	-	156.5	156.9	-
10	100.7	100.8	-	100.6	100.8	-	100.2	100.7	-
1'	132.3	132.2	-	131.8	132.2	-	131.6	132.0	-
2'	115.3	115.2	6.82 (d, 1.6)	115.2	115.3	6.86 d (1.8)	114.9	115.1	6.86 (d, 1.8)
3'	145.6	146.2	-	146.1	146.2	-	145.6	146.4	-
4'	145.7	146.2	-	146.0	146.3	-	145.7	146.2	-
5'	115.7	116.0	6.75 (d, 8.0)	115.7	116.1	6.78 d (8.4)	115.8	116.1	6.79 (d, 7.8)
6'	120.1	120.0	6.70 (dd, 1.6, 8.0)	118.8	119.8	6.74 dd (1.8, 8.4, 1.8)	119.6	119.8	6.74 (dd, 1.8, 7.8)

1"	-	-	-	-	-	-	101.2	102.1	4.32 (d, 1.2)
2"	-	-	-	-	-	-	71.5	72.0	3.54 (dd,1.2, 3.0)
3"	-	-	-	-	-	-	72.2	72.3	3.60 (dd,3.0, 9.0)
4"	-	-	-	-	-	-	73.6	74.0	3.33 (m)
5"	-	-	-	-	-	-	69.6	70.3	3.70 (m)
6"	-	-	-	-	-	-	18.0	17.9	1.27 (d, 6.0)

Recorded in [a]acetone-*d6* at [b]125 MHz, [c]CD_3OD, [d]100 MHz, [e]500 MHz, [f]25.05 MHz, [g]150 MHz, [h]600 MH



1 and **2** **3**

*Figure 2. Key HMBC correlations of catechin (**1** and **2**) and **3***

Compound **4** was isolated as a yellow solid. ^{1}H-NMR spectrum of compound **4** (Table 2) exhibited signals of a flavone including two meta coupling aromatic protons of A ring at δ_H 6.55 (d, *J* = 1.9 Hz, H-8) and δ_H 6.26 (d, *J* = 1.9 Hz, H-6); three ABX coupling aromatic protons of B ring at δ_H 7.61 (d, *J* = 2.0 Hz, H-2′), 7.57 (dd, *J* = 2.0, 8.2 Hz, H-6′) and 7.00 (d, *J* = 8.2 Hz, H-5′); and one methine aromatic proton of C ring at δ_H 6.69 (s, H-3). The ^{13}C-NMR (Table 2) and DEPT spectra indicated signals of fifteen carbons including one carbonyl at δ_C 183.2 (C-4); eight non-protonated carbons at 163.4 (C-5), 165.1 (C-7), 158.8 (C-9), 148.9 (C-3′), 151.4 (C-4′), 165.0 (C-2), 105.2 (C-10) and 123.5 (C-1′); and six methines at δ_H 99.7 (C-6), 94.8 (C-8), 110.5 (C-2′), 116.5 (C-5′), 121.4 (C-6′) and 104.5 (C-3). Furthermore, the HMBC correlations between H-3 and C-1′/C-2/C-4/C-10 and between H-8 and C-6/C-7/C-9/C-10 determined two protons at C-3 and C-8, respectively; between H-6′ and C-2′/C-4′/C-2 and between protons of OCH_3 and C-3′(δ_C 148.9) identified the methoxy group located on position C-3′of B ring. On the ESI-MS of **4**, a positive quasi-molecular ion peak at m/z 301[M+H]+ ($C_{16}H_{13}O_6$+) was observed, together 13C-NMR spectrum of **4** determined a molecular formula of **4** to be $C_{16}H_{12}O_6$ (M = 300). The analysis of ESI-MS and NMR spectra of **3** and comparison with those reported in literature [12] identified compound **4** to be chrysoeriol (3′-methoxy-4′, 5, 7-trihydroxyflavone).

Compound **5** was isolated as a yellow solid. The 1D and 2D NMR spectroscopic analysis (Table 2) of **5** shown compound **5** to a flavanonol glycosis. Similar compounds **1-3,** moiety aglycone flavanonol of is three rings system A, B and C with a methylene at C-4 oxidated to a cetone group (4-C=O). The ^{1}H-NMR spectrum of compound **5** showed signals of a flavanonol with two meta coupling aromatic protons of A ring at δ_H 5.94 (d, *J* = 2.4 Hz, H-8) and δ_H 5.91 (d, *J* = 2.4 Hz, H-6); four protons of the 1,4-substituted aromatic B ring at δ_H 7.37 (d, *J* = 9.0 Hz, H-2′, H-6′), 6.86 (d, *J* = 9.0 Hz, H-3′, H-5′); and two protons of oxymethine groups of C ring at δ_H 5.15 (d, *J* = 10.8 Hz, H-2) and δ_H 4.63 (d, *J* = 10.8 Hz, H-3). The large coupling constant of H-2 with H-3 determined 2,3-*trans* configuration. The ^{13}C-NMR and DEPT spectra exhibited signals of a

flavanonol with fifteen carbons including one carbonyl at δ_C 195.9 (C-4); six non-protonated aromatic carbons at 165.3 (C-5), 168.8 (C-7), 164.1 (C-9), 102.5 (C-10), 128.6 (C-1'), 159.4 (C-4'); six aromatic methine carbone at 96.4 (C-6), 97.2 (C-8), 130.0 (C-2' C-3'), 116.4 (C-5', C-6'); two oxymethine groups at δ_C 83.8 (C-2), 78.7 (C-3). The glycol moiety was identified similarly as compound **5** to be a rhamnose sugar by recognisable signals shown including one anomeric carbon at δ_C102.2 (C-1″) and an anomeric proton at δ_H 4.04 (d, *J* = 1.2 Hz, H-1″); four oxygenated methines, and one methyl. The HMBC correlation between H-1″ and C-3 revealed sugar moiety linked to aglycone at C-3. The small coupling constant between H-1″ and H-2″ (*J* = 1.2) determined *α*-orientation of rhamnoside sugar moiety. Above 1D, 2D NMR spectra analysis compound **5** and in comparison with literature [13], determined compound **5** to be engeletin.

*Table 2. The ^{1}H, ^{13}C-NMR spectroscopic data of compounds **4** and **5***

No.	**4**			**5**		
	δ_C ppm[a, b] [12]	δ_C ppm[c, d]	δ_H ppm[c, e] (mult., *J*= Hz)	δ_C ppm[c,f] [13]	δ_C ppm[c,b]	δ_H ppm[c, h] (mult., *J*= Hz)
2	164.9	165.0	-	83.8	83.8	5.15 (d, 10.8)
3	104.5	104.5	6.69 (s)	78.7	78.7	4.63 (d, 10.8)
4	182.9	183.2	-	196.1	195.9	-
5	163.3	163.4	-	165.5	165.3	-
6	99.7	99.7	6.26 (d, 1.9)	97.4	96.4	5.94 (d, 2.4)
7	165.0	165.1	-	168.5	168.8	-
8	94.7	94.8	6.55 (d, 1.9)	96.3	97.2	5.91 (d, 2.4)
9	158.7	158.8	-	164.1	164.1	-
10	105.2	105.2	-	102.2	102.5	-
1'	123.6	123.5	-	128.6	128.6	-
2'	110.7	110.5	7.61 (d, 2.0)	130.1	130.0	7.37 (d, 9.0)
3'	148.8	148.9	-	116.4	116.4	6.86 (d, 9.0)
4'	151.3	151.4	-	159.4	159.4	-
5'	116.4	116.5	7.0 (d, *J* = 8.2 Hz)	116.4	116.4	6.86 (d, 9.0)
6'	121.4	121.4	7.57 (dd, *J* = 2.0; 8.2 Hz)	159.4	159.4	7.37 (d, 9.0)
1"	-	-	-	102.5	102.2	4.04 (d, 1.2)
2"	-	-	-	71.7	71.8	3.52 (dd, 3.0, 1.2)
3"	-	-	-	72.1	72.2	3.68 (dd, 3.0, 9.0)
4"	-	-	-	73.8	73.8	3.33 (m)
5"	-	-	-	70.5	70.5	4.27 (m)
6"	-	-	-	17.9	17.8	1.21 (d, 6.0)
3' -OCH_3	56.6	56.6	3.97 (s)	-	-	-
5-OH	-	-	12.97 (s)	-	-	-

Recorded in [a,] acetone-d_6 , at [b,]150 MHz, [c,] CD_3OD, [d,] 125 MHz; [e,] 500 MHz, [f,] 100 MHz, [h,] 600 MHz.

CONCLUSION

From leaves of *Carallia brachiata*, five known flavonoids including three flavan-3-ol compounds, (+)-catechin (**1**), (-)- catechin (**2**), and catechin 3-*O*-α-L-rhamnopyranoside (**3**), one flavone, chrysoeriol (**4**), and one flavanonol, engeletin (**5**), were isolated and chemical structural identified. It was the first time these compounds (**2**-**5**) had been

isolated from *Carallia* genus as well as *C. brachiata*.

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