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FLAVONOIDS ISOLATED FROM THE FLOWERS OF *CAMELLIA CHRYSANTHA*

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Abstract. *Camellia chrysantha* (Hu) Tuyama (the golden camellia, golden tea) is a species of evergreen shrub or small tree belonging to the family Theaceae. The flowers and the leaves of this plant are used as tea due to their health benefits. The aim of this study was to investigate the chemical constituents of the flowers of *Camellia chrysantha* (Hu) Tuyama. Five flavonoids were isolated from the flowers of *Camellia chrysantha* (Theaceae), including (+)-catechin (**1**), (-)-epicatechin (**2**), quercetin (**3**), quercetin-3-O-methyl ether (**4**) and kaempferol (**5**). Their chemical structures were elucidated by spectroscopic data analysis and by comparison with those reported in the literature. Among five compounds, compound **4** was isolated for the first time from this species.

Keywords: Camellia chrysantha (Hu) Tuyama, Theaceae, catechin, epicatechin, quercetin, quercetin-3-O-methyl ether, kaempferol.

Classification numbers: 1.1.3; 1.3.1.

1. INTRODUCTION

Camellia chrysantha (Hu) Tuyama (the golden camellia) is a species of evergreen shrub or small tree belonging to the family Theaceae and restricted to wet areas of forest below 500 m. It is found in China and Vietnam. In Vietnam, this species was found in Ba Che (Quang Ninh) [1]. It is noticed that *Camellia chrysantha* contains many trace elements such as germanium, selenium, manganese, molypdenum, vanadium, and zinc; the bioactive compounds found in this species have the ability to inhibit the growth of tumors up to 33.8 % and help reduce up to 35 % of cholesterol in the blood [2]. The plant is also able to relieve the symptoms of atherosclerosis caused by blood fats, control blood pressure and treat cardiovascular diseases and diabetes. *Camellia chrysantha* was included in The IUCN Red List of Threatened Species 1998: e.T32315A9695863 [3]. Although this is a precious medicinal plant and has many uses, the knowledge about its chemical constituents is still limited. There are some studies about this plant but almost all studies focused on its botanical characteristics and values for bonsai. Recently,

Luong Phu Hoang et al. have studied on preparation and characterization of chitosan/alginate film loading Golden Flower Tea (*Camellia chrysantha*) extract [4]. In the previous study [5], we have presented five flavonoid glycosides isolated from the flowers of *Camellia chrysantha* (Hu) Tuyama, collected at Ba Che (Quang Ninh province). In the current study, we continue to report the isolation and elucidation of chemical structures of five flavonoids from this plant.

2. MATERIALS AND METHODS

2.1. Materials

Camellia chrysantha (Hu) Tuyama was collected in December 2016 at Ba Che, Quang Ninh province. Plants were identified by Dr. Nguyen Quoc Binh of the Viet Nam National Museum of Nature, Vietnam Academy of Science and Technology (VAST). A voucher specimen (THV01/BC-QN) was deposited in the Institute of Natural Product Chemistry (INPC), VAST.

2.2. Chemicals and equipments

Chemicals: Thin layer chromatography (TLC) used a pre-coated silica-gel 60 F_{254} (0.25 mm, Merck) and RP-18 F_{254S} plates (0.25 mm, Merck). Column chromatography (CC) was performed using a silica gel 60 (70 - 230 mesh or 230 - 400 mesh, Merck, Germany) or YMC RP-18 resins (30 - 50 μm, Fujisilisa Chemical Ltd., Japan).

Equipments: The nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AM500 FT-NMR Spectrometer using tetramethylsilane as internal standard (ppm). The mass spectra (ESI-MS) were obtained from an AGILENT 1100 LC-MSD Trap spectrometer.

2.3. Extraction and isolation of compounds

The dried flowers of *Camellia chrysantha* (Hu) Tuyama (1.0 kg) were extracted with ethanol by the sonication three times at temperature 50^{o}C to yield 200.0 g of a dark solid extract, which was then suspended in water and successively partitioned with hexane, chloroform and ethyl acetate (EtOAc) (each, 2.0 L) to obtain the hexane (THV/A), the chloroform (THV/B) and the EtOAc (THV/C) layers after removal solvent *in vacuo*. The aqueous phase was chromatographed on a Diaion HP-20P column (7 × 20 cm) eluting with water and then methanol; the mathanolic fraction was evaporated *in vacuo* to yield THV/D.

THV/C (35 g) was chromatographed on a silica gel column and eluated with gradient elution of dichloromethane:methanol (100 % CH_2Cl_2, 9:1, 8:2, 7:3, 6:4, 5:5 and 100 % MeOH) to obtain seven sub-fractions, signed from THV/C-1 to THV/C-7. The fraction THV/C-2 (1.5 g) was chromatographed on a silica gel column eluting with hexane:ethyl acetate (4:1, v:v) to give five smaller fractions (signed from THV/C2/1 to THV/C2/5). The fraction THV/C2/3 was chromatographed on a silica gel column eluting with hexane:ethyl acetate (3:1, v:v) to yield **4**. The fraction THV/C2/5 was chromatographed on an YMC RP-18 column eluting with methanol:water (5:1, v:v) to yield **3** and **5.**

The fraction THV/C-5 (1.5 g) was chromatographed on a silica gel column eluting with dichloromethane:methanol (8:1, v:v) to give eight smaller fractions (signed from THV/C5/1 to THV/C5/8). The fraction THV/C5/3 was continuously chromatographed on a silica gel column eluting with hexane:acetone (2.5:1, v:v) to yield **1** and **2**.

Compound 1: (+)-catechin

Yellowish powder;

ESI-MS: *m/z* 289 $[M-H]^-$, $C_{15}H_{14}O_6$

^{1}H-NMR (CD_3OD, 500 MHz) δ (ppm): 4.58 (1H, d, *J* = 7.0 Hz, H-2), 3.99 (1H, m, H-3), 2.52 (dd, *J* = 8.0 Hz, 16.25 Hz, H_a-4), 2.87 (dd, *J* = 5.5Hz, 16.5Hz, H_b-4), 5.95 (d, *J* = 2.0 Hz, H-6), 5.88 (d, *J* = 2.0 Hz, H-8), 6.86 (d, *J* = 2.0 Hz, H-2'), 6.78 (d, *J* = 8.0 Hz, H-5'), 6.74 (dd, *J* = 2.0 Hz, 8.0 Hz, H-6').

^{13}C-NMR (CD_3OD, 100 MHz) δ (ppm): 82.87 (C-2), 68.82 (C-3), 28.52 (C-4), 157.58 (C-5), 96.32 (C-6), 157.85 (C-7), 95.53 (C-8), 156.93 (C-9), 100.84 (C-10), 132.25 (C-1'), 115.28 (C-2'), 146.24 (C-3'), 146.26 (C-4'), 116.10 (C-5'), 120.04 (C-6').

Compound 2: (-)-epicatechin

Yellow solid;

ESI-MS: *m/z* 289 $[M-H]^-$, $C_{15}H_{14}O_6$

^{1}H-NMR (CD_3OD, 500 MHz) δ (ppm): 4.83 (1H, s, H-2), 4.20 (1H, m, H-3), 2.75 (dd, *J* = 3.0 Hz, 17.0 Hz, H_a-4), 2.88 (dd, *J* = 4.5Hz, 16.5Hz, H_b-4), 5.96 (d, *J* = 2.5 Hz, H-6), 5.94 (d, *J* = 2.0 Hz, H-8), 6.99 (d, *J* = 1.5 Hz, H-2'), 6.78 (d, *J* = 8.0 Hz, H-5'), 6.84 (dd, *J* = 2.0 Hz, 8.1 Hz, H-6').

^{13}C-NMR (CD_3OD, 100 MHz) δ (ppm): 79.88 (C-2), 67.48 (C-3), 29.24 (C-4), 157.67 (C-5), 96.42 (C-6), 157.99 (C-7), 95.91 (C-8), 157.37 (C-9), 100.10 (C-10), 132.29 (C-1'), 115.33 (C-2'), 145.94 (C-3'), 145.78 (C-4'), 115.91 (C-5'), 119.41 (C-6').

Compound 3: quercetin

Yellow solid;

ESI-MS: *m/z* 303 $[M+H]^+$, $C_{15}H_{10}O_7$.

^{1}H-NMR (500 MHz, CD_3OD) δ (ppm): 6.20 (1H, d, *J* = 2.0 Hz, H-6), 6.41 (1H, d, *J* = 2.0 Hz, H-8), 7.75 (1H, d, *J* = 2.0 Hz, H-2'), 6.90 (1H, d, *J* = 8.5 Hz, H-5'), 7.65 (1H, dd, *J* = 8.5 Hz, 2.0 Hz, H-6').

^{13}C-NMR (125 MHz, CD_3OD) δ (ppm): 148.6 (C-2), 137.2 (C-3), 177.3 (C-4), 162.6 (C-5), 99.1 (C-6), 165.6 (C-7), 94.4 (C-8), 158.2 (C-9), 104.5 (C-10), 124.1 (C-1'), 116.0 (C-2'), 146.2 (C-3'), 148.0 (C-4'), 116.2 (C-5'), 121.7 (C-6').

Compound 4: quercetin-3-*O*-methyl ether

Yellow powder;

ESI-MS: *m/z* 315 $[M-H]^-$, $C_{16}H_{12}O_7$.

^{1}H-NMR (500MHz, DMSO) δ (ppm): 3.77 (3H, s, $-OCH_3$), 6.18 (1H, d, *J* = 2.0 Hz, H-6), 6.39 (1H, d, *J* = 2.0 Hz, H-8), 6.90 (1H, d, *J* = 8.0 Hz, H-5'), 7.44 (1H, dd, *J* = 8.0, 2.0 Hz, H-6'), 7.61 (1H, d, *J* = 2.0 Hz, H-2').

^{13}C-NMR (125 MHz, DMSO) δ (ppm): 59.6 (OCH_3), 93.5 (C-8), 98.5 (C-6), 104 (C-10), 115.4 (C-5'), 115.7 (C-2'), 120.5 (C-1'), 120.7 (C-6'), 137.6 (C-3), 145.2 (C-3'), 148.7 (C-4'), 155.5 (C-2), 156.3 (C-9), 161.2 (C-5), 164.2 (C-7), 177.9 (C-4).

Compound 5: kaempferol

Yellow solid;

ESI-MS: *m/z* 285 $[M-H]^-$, $C_{15}H_{10}O_6$.

^{1}H-NMR (500 MHz, CD_3OD), δ (ppm): 8.08 (2H, dd, *J* = 2.0 Hz, 9.0 Hz, H-2', 6'), 6.90 (2H, dd, *J* = 2.0 Hz, 9.0 Hz, H-3', 5') 6.39 (1H, d, *J* = 2.0 Hz, H-8), 6.19 (1H, d, *J* = 2.0 Hz, H-6).

^{13}C NMR (125 MHz, CD_3OD), δ (ppm) 177.32 (C-4), 165.53 (C-7), 160.51 (C-4'), 158.23 (C-5), 156.50 (C-9), 130.66 (C-2'), 130.66 (C-6'), 148.01 (C-2), 137.09 (C-3), 123.72 (C-1'), 116.29 (C-3'), 116.29 (C-5'), 104.5 (C-10), 99.26 (C-6), 94.47 (C-8).

3. RESULTS AND DISCUSSION

Compound **1** was isolated as yellowish powder. The 1D-NMR and 2D-NMR spectra of compound **1** suggested that this compound is a flavan-3-ol. The ^{1}H-NMR spectrum of **1** showed five aromatic proton signals, including two doublet signals with meta-coupling (*J* = 2.0 Hz) at δ_H 5.95 and 5.88 ppm characteristic for the presence of two protons at position 6 and 8 of A ring, three signals of an ABX type at δ_H 6.86 (1H, d, *J* = 2.0 Hz, H-2'), 6.78 (1H, d, *J* = 8.0 Hz, H-5') and 6.74 (1H, dd, *J* = 2.0 Hz and 8.0 Hz, H-6') characteristic for the presence of three protons of B ring substituted at three positions 1, 3 and 4. The basic structure of this compound was therefore deduced as 3,5,7,3',4'-pentahydroxyflavan of catechin. At the higher magnetic field, it also displayed two methylene proton signals at δ_H 2.52 (dd, *J* = 8.0 Hz, 16.25 Hz, H_a-4) and 2.87 (dd, *J* = 5.5 Hz, 16.5 Hz, H_b-4) typical of position 4 of catechin; another signals at δ_H 4.58 (1H, d, *J* = 7.0 Hz, H-2) of oximethin group and at δ_H 3.99 (1H, m, H-3) of carbinol proton characteristic of a flavan nucleus. The coupling constant ($J_{2,3}$ = 7.0 Hz, H-2) supports *trans*-2,3 stereochemistry for the structure of **1**.

The ^{13}C-NMR and DEPT spectra of **1** showed the presence of 15 carbon signals, including one methylene, seven methins and seven nonprotonated aromatic carbons. The HMBC spectrum of **1** showed the correlations between proton H-2 and carbons C-1', C-2', C-6' and C-9, between proton H-4 and C-2, C-3, C-5, C-9 and C-10, between proton H-8 and C-6 and C-10, and also between proton H-6 and C-8 and C-10.

Based on the above mentioned data, and based on the comparison with the data of literature [6], compound **1** was confirmed to be (+)-catechin. Catechin has been isolated from *Camellia sinensis* [7] and known to possess a variety of biological activities, such as anti-inflammatory [8], antimicrobial [9] and antioxidant [10].

Compound **2** was isolated as yellow solid. The 1D-NMR and 2D-NMR spectra of compound **2** are similar with those of compound **1**, suggesting that this compound is also a flavan-3-ol. The ^{1}H-NMR spectrum of compound **2** showed a multiplet signal at δ_H 4.20 ppm (1H, m, H-3) resulting from the splitting of H-3 by H-2 and H-4; two doublet doublet signals at δ_H 2.75 (1H, dd, *J* = 3.0 Hz, 17 Hz) and 2.88 (1H, dd, *J* = 4.5 Hz, 16.5 Hz) typical of methylene protons at position 4 which were split by H-3; three aromatic proton signals characteristic for the presence of an ABX type of B-ring at δ_H 6.99 (1H, d, *J* = 1.5 Hz), 6.78 (1H, d, *J* = 8.0 Hz) and 6.83 (1H, dd, *J* = 2.0 Hz and 8.5 Hz); two other aromatic proton signals characteristic for the presence of A-ring at δ_H 5.96 (d, *J* = 2.5 Hz, H-6) and δ_H 5.94 (d, *J* = 2.5 Hz, H-8). The difference between compound **2** and compound **1** is that: while the position of the proton H-2 chemical shift of compound **1** was observed as doublet signal at δ_H 4.58 (1H, d, *J* = 7.0 Hz, H-2), this position of compound **2** appeared as a broad singlet at δ_H 4.83, suggesting that the flavan structure of **2** possesses a *cis*-2,3 configuration and typically of epicatechin [11].

The ^{13}C-NMR and DEPT spectra of **2** showed fifteen carbon signals, which consisted of one methylene at δ_C 29.24 (C-4), seven methins at δ_C 79.88 (C-2), 67.48 (C-3), 96.42 (C-6),

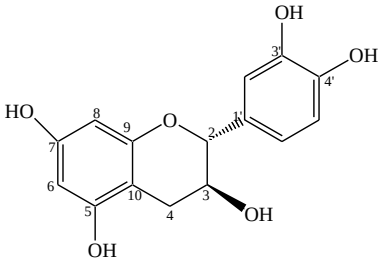
95.91 (C-8), 115.33 (C-2'), 115.91 (C-5'), 119.41 (C-6'), and seven quartenary carbons at δ_C 157.67 (C-5), 157.99 (C-7), 157.37 (C-9), 100.10 (C-10), 132.29 (C-1'), 145.94 (C-3'), 145.78 (C-4'). The HMBC spectrum of **2** showed the correlations between proton H-2 and C-1', C-2', C-6', between proton H-4 and C-2, C-3, C-5, C-9, C-10, between proton H-8 and C-6, C-10, also between proton H-6 and C-8, C-10.

The above data of compound **2** was compared with those reported in the literature [12], so compound **2** was identified as (-)-epicatechin. Epicatechin has been isolated from the *Camellia sinensis* [13], and reported to have antimicrobial [13], anti-inflammatory [14], and antioxidant activities [15].

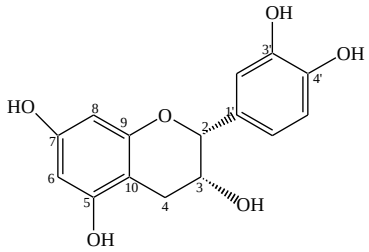
The compound **3** was isolated as yellow powder. The NMR spectra of compound **3** suggested that this compound is a flavonoid. The ^{1}H-NMR showed two doublet signals with meta-coupling (J = 2.0 Hz) at δ_H 6.20 and 6.41 that were assigned to H-6 and H-8 in the A-ring, three distinctive signals of aromatic protons of an ABX type ring B found at δ_H 7.75 (1H, d, J = 2.0 Hz, H-2′), 6.90 (1H, d, J = 8.5 Hz, H-5′) and 7.65 (1H, dd, J = 8.5 Hz, 2.0 Hz, H-6′). The ^{13}C-NMR and DEPT spectra of **3** showed fifteen carbon signals, which consisted of one carbonyl carbon (δ_C 177.3), five methines (δ_C 99.1, 94.4, 116.0, 116.2, 121.7), and nine other quartenary carbons (δ_C 148.6, 137.2, 162.6, 165.6, 158.2, 104.5, 124.1, 146.2, 148.0). The obtained results for compound **3** were in agreement with the spectral data of quercetin previously reported in the literature [16]. Thus, compound **3** was identified as quercetin.

The compound **4** was isolated as yellow powder. The 1D-NMR and 2D-NMR spectra of compound **4** are similar with those of compound **3**, suggesting that it is also a flavonoid: the ^{1}H-NMR showed meta coupled signals at δ_H 6.18 (1H, d, J = 2.0 Hz) and 6.39 (1H, d, J = 2.0 Hz) corresponding with two carbon signals at δ_C 98.5 and 93.5 ppm in HSQC spectrum characteristic for the presence of two protons at position 6 and 8 of A ring; three characteristic aromatic proton signals for one B ring substituted at three positions 1, 3 and 4 at δ_H 7.61 (1H, d, J_{meta} = 2.0 Hz, H-2′), 6.90 (1H, d, J_{ortho} = 8.0 Hz, H-5′) and 7.44 (1H, dd, $J_{ortho\ and\ meta}$ = 8.0 and 2.0 Hz, H-6′) corresponding with three carbon signals at δ_C 115.7, 115.4 and 120.7 ppm in HSQC spectrum. The presence of a methoxy group was indicated by the presence of a singlet signal at 3.77 (3H, s, -OCH_3) in the ^{1}H-NMR spectrum correspongding with a carbon signal at δ_C 59.6 ppm in the ^{13}C-NMR spectrum. The ^{13}C-NMR spectrum shown also the presence of one carbonyl group at δ_C 177.8 (C-4). The HMBC spectrum showed a clearly correlation between protons of methoxy group (δ_H 3.77 ppm) and carbon C-3 (δ_C 137.6 ppm). The molecular formula of **4** was established as $C_{16}H_{12}O_7$ based on spectral data obtained by ESI-MS (*m/z* 315 $[M-H]^+$) together ^{1}H and ^{13}C NMR analysis, including DEPT. The above data of **4** were consistent with the literature data of quercetin-3-*O*-methyl ether [17].

The compound **5** was isolated as yellow solid. The NMR spectra of compound **5** are similar with those of compound **3, 4**, suggesting that this compound is a flavonoid with the 3,3',4',5,7-pentahydroxy substitution pattern. The ^{1}H-NMR spectrum of **5** showed two doublet signals at δ_H 6.19 (1H, d, J = 2.0 Hz) and 6.39 (1H, d, J = 2.0 Hz), corresponding with two carbon signals at δ_C 99.26 and 94,47 ppm in HSQC spectrum, were attributed to aromatic protons H-6 and H-8 of the ring A; two doublet doublet signals at 8.08 (J = 2.0 Hz, 9.0 Hz) and 6.90 (J = 2.0 Hz, 9.0 Hz), with integration of 2 protons each, which confirm the presence of a A_2B_2 system in the ring B. The ^{13}C-NMR spectrum of **5** indicates the presence of fifteen carbon signals at δ_C 177.32 (C-4), 165.53 (C-7), 160,51 (C-4'), 158.23 (C-5), 156.50 (C-9), 130.66 (C-2'), 130.66 (C-6'), 148.01 (C-2), 137.09 (C-3), 123.72 (C-1'), 116.29 (C-3'), 116.29 (C-5'), 104.5 (C-10), 99.26 (C-6) and 94.47 (C-8). The ^{1}H-NMR and ^{13}C-NMR spectral data confirm that compound **5** is kaempferol. The results are identical to those obtained by the literature [16].

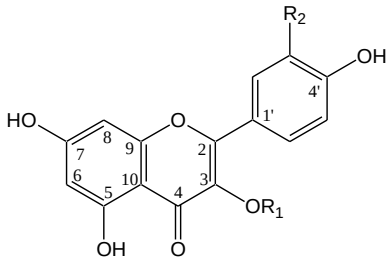


(+)-Catechin (**1**)



(-)-Epicatechin (**2**)

3 Quercetin: R_1=H, R_2=OH
4 Quercetin-3-*O*-methyl ether: R_1=CH_3, R_2=OH
5 Kaempferol: R_1=R_2=H



4. CONCLUSIONS

Five flavonoids were isolated from the flowers of *Camellia chrysantha* (Hu) Tuyama, including catechin, epicatechin, quercetin, quercetin-3-O-methyl ether and kaempferol. Their chemical structures were elucidated by spectroscopic data analysis and by comparison with those reported in the literatures. Among five compounds, quercetin-3-*O*-methyl ether **4** was isolated for the first time from this species.

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