Flavonoids from the *Camellia hakodae* Ninh flowers

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Abstract

Nine flavonoids including quercetin-3-O-*β*-D-glucopyranoside (**1**), quercetin-7-O-*β*-D-glucopyranoside (**2**), quercetin (**3**), kaempferol (**4**), epicatechin (**5**), epigallocatechin (**6**), taxifolin (**7**), naringenin (**8**), epigallocatechin gallate (**9**) were isolated and characterized from the *Camellia hakodae* flowers. Their structures were determined by spectroscopic analyses including MS, 1D and 2D NMR, as well as by comparison with reported data in the literature.

Keywords. Flavonoids, *Camellia hakodae*, flower.

1. INTRODUCTION

Camellia is a genus of flowering plants in the family Theaceae. Several species of this genus have a long history of use as a social, medicinal and health drink, mainly to produce tea. This beverage has a multitude of used from lowering blood pressure to preventing cancer. The medicinal effects of tea are mainly attributed to flavonoids, polyphenol, theanine. These compounds have been shown to exhibit antibacterial, anticancer, and antioxidant properties.[1, 2] *Camellia hakodae,* belonging to the *Camellia* genus, was first identified by Assoc. Prof. Dr. Tran Ninh (Hanoi University of Science, Hanoi) in Tam Dao National Park, Vietnam, in 2007.[3] However, there are few reports about the chemical constituents and biological activities of this plant. In this paper, we report the isolation and structural elucidation of night flavonoids **1-9** from the flowers of *Camellia hakodae.*

2. EXPERIMENTAL

2.1. General experimental procedures

The ESI-MS were recorded on Agilent 1100 LC-MSD Trap spectrometer. The NMR spectra were recorded on a Bruker AM500 FT-NMR spectrometer using TMS as an internal standard. TLC silica gel (Merck 60 F_{254}) was used for Thin-layer chromatography. Column chromatography (CC) was carried out using silica gel 40-63 µm or Sephadex LH-20 (Sigma-Aldrich, USA).

2.2 Plant material

Camellia hakodae flowers were collected at Soc Son Cooperative of Conservation and Medicinal Development, Soc Son District, Hanoi, in September 2017 and identified by Assoc.

Prof. Dr. Tran Ninh (Vietnam National University, Hanoi). A voucher specimen (CH.01) has been deposited in Soc Son Cooperative of Conservation and Medicinal Development.

2.3 Extraction and isolation

The dried flowers of *Camellia hakodae* (5.0 kg) were powdered and extracted five times with EtOH under ultrasonic condition (1h, 45^{o}C). The extract was concentrated under reduced pressure to give 200 g of methanol extract. This extract was separated by silica gel (CC) using mobile phase of *n*-hexane (3 L), dichloromethane (3 L), ethyl acetate (3 L), acetone (3 L) and MeOH (3 L) to obtain 5 fractions: HVH (20 g), HVC (35 g), HVE (40 g), HVA (14 g), HVM (50 g). Fraction HVE (1.9 g) was separated into 6 subfractions (FE1–FE6) by silica gel CC using mobile phase of dichloromethane/methanol gradient. Purification of the subfraction FE1 (4 g) by silica gel CC, eluted with dichloromethane/ethanol gradient to give compound **8** (10 mg).

Fraction FE2 (10 g) was subjected to CC on silica gel, eluted with dichloromethane/ methanol gradient to obtain compound **4** (12 mg). Fraction FE3 (1.4 g) was purified by CC on silica gel using CH_2Cl_2/MeOH gradient to afford compound **3** (20 mg) and compound 7 (8 mg). Fraction HVM (50 g) was purified by CC on silica gel using CH_2Cl_2/MeOH gradient to afford 6 subfractions (FM1-FM6). Purification of the subfraction FM3 (8 g) by CC on silica gel using CH_2Cl_2/MeOH gradient was performed to obtain compound **5** (11 mg) and compound **6** (6.5 mg). Fraction FM4 (7 g) was purified by CC on silica gel, eluted with CH_2Cl_2/MeOH gradient to give compound **13** (7.0 mg). Purification of fraction FM5 (6 g) by Sephadex LH-20 using MeOH and CC on silica gel using CH_2Cl_2/MeOH gradient was performed to obtain compound **1** (10 mg). Fraction FM6 (5 g) was purified by CC on silica gel, eluted with CH_2Cl_2/MeOH gradient to give compound **2** (16 mg).

Quercetin-3-*O*-*β*-D-glucopyranoside (1): yellow powder, m.p. 230^{o}C, $[\alpha]^{25}$ - 66.8 (*c* 0.13, MeOH), ESI-MS *m/z* 465.3 $[M+H]^{+}$.^{1}H NMR (500 MHz, CD_3OD-d_4): 7.73 (1H, *d*, *J* = 2.0 Hz, H-2'), 7.60 (1H, *dd*, *J* = 2.0; 8.5 Hz, H-6'), 6.89 (1H, d, *J* = 8.5 Hz, H-5'), 6.41 (1H, *d*, *J* = 2.0 Hz, H-8), 6.22 (1H, *d, J* = 1.5 Hz, H-6), 5.26 (1H, *d*, *J* = 7.0 Hz, H-1"), 3.73 (1H, *dd*, *J* = 2.5; 12.0 Hz; H-6a"); 3.60 (1H, *dd*, *J* = 5.5; 12.0 Hz*, H-6b"*); 3.50 (1H, t, *J* = 9.0 Hz; H-2"), 3.44 (1H, t, *J* = 8.5 Hz; H-4"), 3.36 (1H, t, *J*= 9.5 Hz; H-3"), 3.24 (1H, m, H-5"). ^{13}C NMR (125 MHz, CD_3OD-d_4): 177.5 (C-4), 166.0 (C-7), 163.1 (C-5), 159.0 (C-9), 158.5 (C-2), 149.9 (C-4'), 145.9 (C-3'), 135.6 (C-3), 123.2 (C-6'), 123.1 (C-1'), 117.6 (C-5'), 116.0 (C-2'), 105.7 (C-10), 104.4 (C-1"), 99.9 (C-6), 94.7 (C-8), 78.4 (C-5"), 78.1 (C-3"), 75.7 (C-2"), 71.2 (C-4"), and 62.6 (C-6").

Quercetin-7-*O*-*β*-D-glucopyranoside (2): yellow powder, m.p. 248-250^{o}C. ESI-MS *m/z* 465.2 $[M+H]^{+}$. ^{1}H-NMR (500 MHz, CD_3OD-d_4); 7.75 (1H, *d*, *J* = 1.5 Hz, H-2'), 7.65 (1H, *dd*, *J* = 1.5; 8.5 Hz, H-6'), 6.89 (1H, d, *J* = 8.5 Hz, H-5'), 6.72 (1H, *d*, *J* = 1.5 Hz, H-8), 6.45 (1H, *d, J* = 2.0 Hz, H-6), 5.07 (1H, *d*, *J* = 7.0 Hz, H-1"), 3.95-3.32 (unresolved m, other sugar protons); ^{13}C NMR (125 MHz, CD_3OD-d_4): 177.4 (C-4), 164.4 (C-7), 162.1 (C-5), 157.7 (C-9), 148.9 (C-4'), 148.7 (C-2), 146.2 (C-3'), 137.6 (C-3), 123.9 (C-1'), 121.9 (C-6'), 116.3 (C-5'), 116.1 (C-2'), 106.2 (C-10), 101.7 (C-1"), 100.2 (C-6), 95.6 (C-8), *78.4 (C-5"),* 77.8 *(C-3"),* 74.7 (C-2"), 71.3 (C-4"), and 62.5 (C-6").

Quercetin (3): yellow needles (MeOH); m.p. 279-281°C; ESI-MS *m/z*: 303.1 $[M+H]^+$. ^{1}H NMR (CD_3OD) δ_H (ppm) 7.75 (1H, d, *J* = 2.0 Hz; H-2'); 7.65 (1H, dd, *J* = 2.5; 8.5 Hz, H-6'); 6.90 (1H, d, *J*= 8.5 Hz; H-5'); 6.40 (1H, d, *J* = 2.0 Hz; H-8); 6.20 (1H, d, *J* = 2.0 Hz; H-6).

Kaempferol (4): Yellow powder; m.p. 202-210^{o}C; ESI-MS *m/z*: 286 $[M+H]^+$. ^{1}H-NMR (500 MHz, CD_3OD): δ_H (ppm) 8.11 (2H, *d*, *J* = 8.5 Hz, H-2', 6'), 6.93 (2H, d, *J* = 8.5 Hz, H-3', 5'), 6.42 (1H, *d*, *J* = 1.5 Hz, H-8), 6.20 (1H, *d*, *J* = 1.5 Hz, H-6). ^{13}C-NMR: 175.2 (C-4); 163.7 (C-7); 160.4 (C-5); 158.7 (C-4'); 156.7 (C-9); 146.3 (C-2); 135.2 9 (C-3); 129.4;129.4 (C-2'); 122.1 (C-1'); 115.3 (C-3'); 115.3 (C-5'); 103.1 (C-10); 98.4 (C-6); 93.8 (C-6').

Epicatechin (5): yellow powder, m.p. 174-175^{o}C. ^{1}H-NMR (500 MHz, CD_3OD): δ_H (ppm) 2.76 (1H, dd, *J* = 3.0; 16.5 Hz; H-4a), 2.88 (1H, dd, *J* = 4.5; 17 Hz; H-4b), 4.19 (1H, m, H-3), 4.83 (1H, s, H-2), 5.94 (1H, d, *J* = 2.0 Hz, H-8), 5.96 (1H, d, *J* = 2.5 Hz, H-6), 6.78 (1H, d, *J* = 8.0 Hz, H-5'), 6.83 (1H, dd, *J* = 1.5; 8.5 Hz, H-6'), 6.99 (1H, d, *J* = 2.0 Hz, H-2'). ^{13}C-NMR (125 MHz, CD_3OD): δ_C (ppm) 29.24 (C-4), 67.47 (C-3), 79.85 (C-2); 95.89 (C-8); 96.39 (C-6); 100.07 (C-4a); 115.31 (C-2'); 115.89 (C-5'); 119.39 (C-6'); 132.27 (C-1'); 145.76 (C-4'); 145.92 (C-3'); 157.35 (C-8a); 157.64 (C-7); 157.98 (C-5);

Epigallocatechin (6): yellow powder, m.p. 208-210^{o}C. ESI-MS *m/z* 307.4 $[M+H]^+$. ^{1}H-NMR (500 MHz, CD_3OD): δ_H (ppm) 2.75 (1H, dd, *J* = 3.0; 16.5; H-4a), 2.87 (1H, dd, *J* = 4.5; 17.0; H-4b), 4.19 (1H, s, H-3), 4.77 (1H, s, H-2), 5.94 (1H, d, *J* = 2.0 Hz, H-8), 5.96 (1H, d, *J* = 2.0 Hz, H-6), 6.54 (2H, s, H-2', 6').

Taxifolin (7): yellow powder, m.p. 237-239^{o}C. ESI-MS *m/z* 305.6 $[M+H]^+$. ^{1}H-NMR (500 MHz, CD_3OD): δ_H (ppm) 4.52 (1H, d, *J* = 11.5 Hz, H-3), 4.93 (1H, d, *J* = 11.5 Hz, H-2), 5.90 (1H, d, *J* = 2.0 Hz, H-6), 5.94 (1H, d, *J* = 2.0 Hz, H-8), 6.82 (1H, d, *J* = 8.0 Hz, H-5'), 6.87 (1H, dd, *J* = 2.0; 8.5 Hz, H-6'), 6.98 (1H, d, *J* = 2.0, H-2').

Naringenin (8): yellow powder, m.p. 250-252^{o}C. ESI-MS *m/z* 373.0 $[M+H]^+$. ^{1}H-NMR (500 MHz, CD_3OD): δ_H (ppm) 2.72 (1H, dd , *J* = 3.0; 17.5 Hz, H-3a), 3.12 (1H, dd, *J* = 13.0; 17.0 Hz, H-3b), 5.36 (1H, dd, *J* = 3.0; 12.5Hz, H-2); 5.90 (1H, d, *J* = 2.0 Hz; H-6); 5.91(1H, d, *J* = 2.0 Hz; H-8); 6.84 (2H, d, *J* = 8.5 Hz, H-3', H-5'); 7.33 (2H, d, *J* = 8.5 Hz, H-2', H-6').

Epigallocatechin gallate (9): yellow solid, m.p. 218^{o}C. ESI-MS *m/z* 459.2 $[M+H]^+$. ^{1}H-NMR (500 MHz, CD_3OD) δ_H (ppm) 2.87 (1H,d, *J* = 17.0 Hz, H-4a), 3.00 (1H, dd, *J* = 5.0; 17.5 Hz, H-4b), 4.99 (1H, s, H-2), 5.55 (1H, s, H-3), 5.98 (1H, s, H-8), 5.99 (1H, s, H-6), 6.53 (2H, s, H-2'), 6.97 (2H, d, *J* = 1.5 Hz, H-2").

3 RESULTS AND DISCUSSION

Compound **1** was isolated as yellow powder; m.p. 230^{o}C and optically active $[\alpha]^{25}_D$ = 66.8 (*c* 0.13, MeOH). The ESI-MS spectrum indicated the pseudo-molecular ion peak at *m/z* 465.3 $[M+H]^+$. The ^{1}H-NMR showed three aromatic protons signals at δ_H 7.71 (d, *J* = 2 Hz, H-2'), 6.89 (d, *J* = 8.5 Hz, H-5') and 7.60 (dd, *J* = 2, 8.5 Hz) in the form of an ABX spin-system

suggesting a flavonol with 3`,4`- disubstituted B-ring and showed a pair of meta coupling proton signals at δ_H 6.22 (d, *J* = 2.0 Hz, H-6) and 6.41 (d, *J* = 2.0 Hz, H-8) for the A-ring. One anomeric proton at δ_H 5.26 (1H, *d*, *J*= 7.0 Hz, H-1"), assigned to a sugar moiety. The ^{13}C NMR spectra supported this hypothesis and showed 21 signals including carbonyl signal at δ_C 177.5 (C-4). It revealed chemical shifts at δ_C 135.6 (C-3), 163.1 (C-5), 166.0 (C-7), 145.9 (C-3`), 149.9 (C-4`) that suggested the 3, 5, 7, 3`, 4`- oxygenated flavone nucleus. This sugar was determined to be *β*-D-glucopyranoside by its NMR data δ_C 104.4 (C-1"), 78.4 (C-5"), 78.1 (C-3"), 75.7 (C-2"), 71.2 (C-4"), and 62.6 (C-6") and coupling constant between H-1" and H-2": *d*, *J* = 7.0 Hz. In addition, the position of this sugar at C-3 of flavonoid was by HMBC correlations between H-1" (δ_H 5.26) and C-3 (δ_C 135.6). On this basis, compound (1) was identified as quercetin-3-O-*β*-D-glucopyranoside. The identity of this compound was further substantiated by comparison of its spectral data with previously reported values.[4]

Compound **2** was isolated as yellow powder, m.p. 248-250^{o}C. The ESI-MS spectrum indicated the pseudo-molecular ion peak at *m/z* 465.2 $[M+H]^+$. The 1D NMR spectra (^{1}H and ^{13}C) of compound **2** were close to those of compound **1**, including a quercetin unit and glucopyranoside moiety. The ^{1}H-NMR showed five aromatic proton signals of quercetin skeleton such as ABX system at δ_H 7.75 (d, *J* = 1.5 Hz, H-2'), 6.89 (d, *J* = 8.5 Hz, H-5') and 7.65 (dd, *J* = 1.5, 8.5 Hz); a pair of meta coupling proton signals at δ_H 6.72 (d, *J* = 2.0 Hz, H-6) and 6.45 (d, *J* = 2.0 Hz, H-8). The glucopyranoside moiety was identified by its NMR data δ_C 101.7 (C-1"), *78.4 (C-5"),* 77.8 *(C-3"),* 74.7 (C-2"), 71.3 (C-4"), and 62.5 (C-6"). Configuration of the sugar moiety was determined to be *β* based on the coupling constant of the anomeric proton ($J_{1",2"}$ = 7.0 Hz). In addition, the anomeric proton showed a correlation in the HMBC spectrum with δ_C 163.7 (C-7). Thus, according to these findings and all the spectroscopic data, the structure of compound **2** was determined to be quercetin-7-O-*β*-D-glucopyranoside which exhibited antioxidant activitiy with ORAC value: 18÷4 μmol). [5]

Compound **3** was isolated was obtained as yellow solid, m.p. 279-281°C. The ^{1}H NMR spectra of 3 displayed the signals close to those of **2** in which ABX system at δ_H 7.75 (d, *J* = 2.0 Hz, H-2'), 6.89 (d, *J* = 8.5 Hz, H-5') and 7.65 (dd, *J* = 2.5, 8.5 Hz) and a pair of meta coupling proton signals at 6.20 (d, *J* = 2.0 Hz, H-6) and 6.40 (d, *J* = 2.0 Hz, H-8). However, in comparison with compound **2**, the sugar moiety was not noted in structure of **3**. Compared with the literature data and ESI-MS (*m/z* 303.1 $[M+H]^+$), compound **3** was identified as quercetin.[6]

Compound **4** was obtained as yellow solid, m.p. 202-210^{o}C. The ESI-MS spectrum indicated the pseudo-molecular ion peak at *m/z* 286 $[M+H]^+$. The 1D NMR spectra (^{1}H and ^{13}C) of compound **3** showed a signal of the flavonol skeleton. Difference **3**, the presence of an A_2B_2 system [δ_H 8.11 (2H, *d*, *J* = 8.5 Hz, H-2', 6'), 6.93 (2H, d, *J* = 8.5 Hz, H-3', 5')] was instead of ABX system in **3**. So, the structure of 4 was determined as kaempferol. Its NMR data were identical with those reported in the literature.[7]

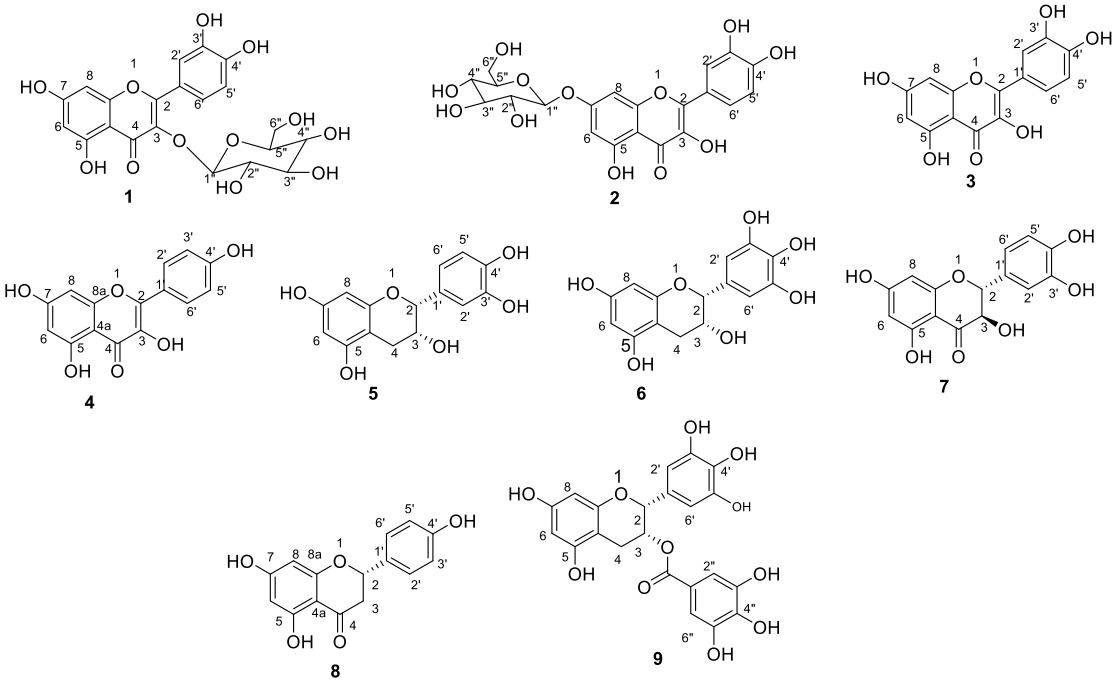
Compound **5** was obtained as yellow solid, m.p. 174-175^{o}C. Analysis of the ^{1}H NMR spectra was displayed signals of three aromatic proton of an ABX system at δ_H 6.78 (1H, d, *J* = 8.0 Hz, H-5'), 6.83 (1H, dd, *J* = 1.5; 8.5 Hz, H-6'), 6.99 (1H, d, *J* = 2.0 Hz, H-2'); a pair of meta coupling proton signals at δ_H 5.94 (1H, d, *J* = 2.0 Hz, H-8), 5.96 (1H, d, *J* = 2.5 Hz, H-6); protons of two oxymethine groups [δ_H 4.19 (1H, m, H-3), 4.83 (1H, s, H-2)]; and protons of methylene

at δ_H 2.75 (1H, dd, *J* = 3.0; 16.5; H-4a). This methylene was characterized by a flavanol skeleton. In the ^{13}C-NMR and DEPT spectra of 5, signals of 15 carbons was noted. It revealed chemical shifts at δ_C 157.98 (C-5), 157.64 (C-7), 145.92 (C-3`), 149.76 (C-4`) that suggested the 5, 7, 3`, 4`- oxygenated flavonoid nucleus. Carefully analysis of NMR, ESI-MS data and comparison with reported data showed that compound **5** to be epicatechin.[8]

Compound **6** was obtained as yellow powder, m.p. 208-210^{o}C. The ESI-MS spectrum indicated the pseudo-molecular ion peak at *m/z* 307.4 $[M+H]^+$. The ^{1}H NMR spectrum of 6 exhibited the presence of a flavanol skeleton as the structure of 5. The differences between **6** and **5** were signals of protons in B ring: a pair of meta coupling proton signals at δ_H 6.54 (2H, br s, H-2', 6') in compound **6** was instead ABX system in **5**. Based on the above NMR data and comparison with literature, the structure of **6** was identified as epigallocatechin.[9]

Compound **7** was isolated as yellow powder, m.p. 237-239^{o}C. The ESI-MS spectrum indicated the pseudo-molecular ion peak at *m/z* 305.6 $[M+H]^+$. The ^{1}H NMR spectrum showed signals of four aromatic protons at δ_H 5.90 (1H, d, *J* = 2.0 Hz, H-6), 5.94 (1H, d, *J* = 2.0 Hz, H-8), 6.82 (1H, d, *J* = 8.0 Hz, H-5'), 6.87 (1H, dd, *J* = 2.0; 8.5 Hz, H-6'), 6.98 (1H, d, *J* = 2.0, H-2').The ^{1}H NMR spectrum of **7** was similar to that of **5**, except for the methylene (C-4) was not noted in the structure of **7**. Comparing of the NMR data with reported data, compound **7** was elucidated as taxifolin.[10]

Figure 1: Isolated flavonoids **1-9** from the flowers of *Camellia hakodae*



Compound **8** was obtained as yellow powder, m.p. 250-252^{o}C. The ESI-MS spectrum indicated the pseudo-molecular ion peak at *m/z* 373.0 $[M+H]^+$. The ^{1}H NMR spectrum of **8** also displayed the signals of an A_2B_2 system [δ_H 6.84 (2H, d, *J* = 8.5 Hz, H-3', H-5'); 7.33 (2H, d, *J* = 8.5 Hz, H-2', H-6')]; two meta-coupled aromatic protons at δ_H 5.90 (1H, d, *J* = 2.0 Hz; H-6); 5.91(1H, d, *J* = 2.0 Hz; H-8); two methylene protons at δ_H 2.72 (1H, dd , *J* = 3.0; 17.5 Hz, H-3a), 3.12 (1H, dd, *J* = 13.0; 17.0 Hz, H-3b) and one oxymethine at δ_H 5.36 (1H, dd, *J* = 3.0; 12.5Hz, H-2). This observation suggested that compound **8** was a flavanone skeleton. Based on these data and compared with reported data, compound **8** was identified as naringenin.[11]

Compound **9** was isolated as yellow solid, m.p. 218-220^{o}C. The ^{1}H NMR spectrum of **9** also displayed the signals close to those of **6.** However, in comparison with **6**, the presence of a gallate moiety [δ_H 6.97 (2H, d, *J* = 1.5 Hz, H-2", 6")] linkage to C-3 of the flavanol skeleton. The combination of MS, NMR data and comparison with reported value confirmed the structure of **9** as epigallocatechin gallate.[10]

4 CONCLUSIONS

Nine flavonoids including quercetin-3-O-β-D-glucopyranoside (1), quercetin-7-O-β-D-glucopyranoside (2), quercetin (3), kaempferol (4), epicatechin (5), epigallocatechin (6), taxifolin (7), naringenin (8), epigallocatechin gallate (9) were isolated and characterized from the *Camellia hakodae* flowers. Their structures were determined by spectroscopic analyses including MS, 1D and 2D NMR, as well as by comparison with reported data in the literature.

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