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# A new flavanone glycoside with antimicrobial and cytotoxic activities from *Camellia hakodae* Ninh leaves

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**Abstract.** *Camellia hakodae* Ninh, belonging to the golden subgroup of *Camellia* (Theaceae), is a Camellia species native to Viet Nam and primarily found in Tam Dao, Vinh Phuc province. This study aimed to isolate and identify the flavonoids from the leaves of *Camellia hakodae* Ninh. As a result, a new flavanone glycoside, named camehakonin A (**1**), was discovered alongside six known flavonoids including isoxanthohumol (**2**), naringenin (**3**), (+)taxifolin (**4**), (-)epicatechin (**5**), macarangin (**6**), and 5,7,3',4'-tetrahydroxy-6-geranylflavonol (**7**). The chemical structures of these compounds were elucidated based on analysis of their spectroscopic data and by comparison with reported NMR spectroscopy data of known compounds. All isolated flavonoids were evaluated for their antimicrobial activity against various microorganisms, including *Enterococcus faecalis*, *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enterica* and *Candida albicans*. Compound **1** exhibited significant inhibition of the tested Gram-positive bacteria (*E. faecalis*, *S. aureus*, and *B. cereus*) with MIC values of 128, 64, and 256 µg/mL, respectively; as well as the human-pathogenic fungus *C. albicans* (MIC: 16 µg/mL). Furthermore, compound **1** displayed weak cytotoxicity *in vitro* against two cancer cell lines (KB and Hep-G2) with $IC_{50}$ values of 73.70 and 221.11 µM, respectively. Notably, compounds **2-7** were isolated from the leaves of *Camellia hakodae* Ninh for the first time.

*Keywords: Camellia hakodae* Ninh, flavanone glycoside, flavonoid, antimicrobial, cytotoxicity.

*Classification numbers*: 1.1.1, 1.1.6.

## 1. INTRODUCTION

Golden camellia species belong to a subgroup of the genus *Camellia* L., family Theraceae. To date, a total of sixty-nine species were identified in the regions of southern China and Viet Nam, of which fifty-six species were found in Viet Nam, mainly distributed at 100-1000 m high above sea level [1, 2]. Phytochemical studies demonstrated that golden camellias were natural sources enriched with flavonoids, polyphenols, triterpenoids, sterols, saponins, polysaccharides, and amino acids [1, 3, 4]. Recently, extracts and isolated compounds from these species have been proven to possess beneficial pharmacological properties, including antioxidant [5], anticancer [6 - 8], antibacterial [9], neuroprotective [10], hypoglycemic, hypolipidemic [11], anxiolytic [12], and antidepressant activities [12, 13]. *Camellia hakodae* Ninh, a golden camellia species native to Viet Nam, was first discovered in Tam Dao National Park, Tam Dao district, Vinh Phuc province, Viet Nam in 2007 [14]. As part of our phytochemical and bioactivity studies of *Camellia hakodae* Ninh [15, 16], we describe herein the isolation and structural elucidation of seven flavonoids including one undescribed flavanone glycoside from the leaves and their antimicrobial and *in vitro* cytotoxic effects.

## 2. MATERIALS AND METHODS

### 2.1. General experimental procedures

Silica gel 60 (40 - 63 µm and 63 - 200 µm, Merck), Sephadex LH-20 (GE Healthcare, Sweden) and reversed-phase RP-18 (Merck, Germany) were utilized for column chromatography (CC). Thin layer chromatography (TLC) was performed using silica gel 60 $F_{254}$ on aluminum plates (Merck, Germany). The fractions were monitored by TLC using precoated plates of silica gel 60 $F_{254}$ (Merck) and spots were visualized under UV light (254 and 365 nm), staining with 5 % vanillin-$H_2SO_4$ solution. Medium-pressure liquid chromatography (MPLC) was performed on a Biotage model Isolera instrument. ESI-MS spectra were obtained from an MS-Engine-5989-HP. HR-ESIMS data were obtained from an Agilent 6530 Accurate-Mass Q-TOF LC/MS (Agilent Technologies, Santa Clara, United States). NMR spectra were recorded on a Bruker AV500 or AM500 FT-NMR spectrometer (Bruker, Germany) operating at 500 MHz and 125 MHz for $^{1}H$ and $^{13}C$ NMR spectra, respectively. Optical rotations were measured on a Jasco P-2000 Series (Japan). Melting points were determined using a Büchi B-450 melting point apparatus (USA).

### 2.2. Plant material

The fresh leaves of *Camellia hakodae* Ninh were collected at Soc Son Medicinal Herbs Garden, Soc Son district, Ha Noi in February 2018. The voucher specimen (N5817) was identified by Pr. Tran Ninh, University of Natural Sciences, Vietnam National University, Hanoi and deposited at the Department of Botanical Chemistry, Faculty of Biology, University of Natural Sciences, Ha Noi and Soc Son cooperative for Conservation and Development of Medicinal Herbs, Soc Son district, Hanoi, Viet Nam.

### 2.3. Extraction and isolation

The fresh leaves of *Camellia hakodae* Ninh (10.0 kg) were dried at room temperature and ground to powder. The obtained powder (3.05 kg) was extracted three times with ethanol 96% under ultrasonic conditions (50 $^{o}$C, 1 h). The combined solution was filtered and the solvent was evaporated under reduced pressure to obtain a crude residue (120 g). The crude residue was separated by MPLC (silica gel) using a mobile phase of *n*-hexane (3L), dichloromethane (DCM, 3L), ethyl acetate (EtOAc, 3L), acetone (3L) and methanol (MeOH, 3L), then the solvents were removed under reduced pressure to obtain five main fractions: FH (10 g), FD (15 g), FE (20 g), FA (12 g) and FM (54 g).

Fraction FD was subjected to a silica gel CC eluted with a gradient of *n*-hexane/acetone (100:1→1:1) to yield seven fractions, FD1-FD7. Fraction FD6 was separated by silica gel CC eluted with DCM/acetone solvent systems (70:30) to obtain compound **2** (6.0 mg). Fraction FE was separated by silica gel MPLC using gradient DCM/MeOH to afford seven fractions, FE1-FE7. Fraction FE1 was separated by silica gel CC eluted with *n*-hexane/acetone (gradient) to yield compound **6** (3.5 mg) and **7** (3.1 mg). Fraction FA was separated by silica gel CC using DCM/MeOH (gradient) solvent system to give six fractions, FA1-FA6. Compound **3** (4.1 mg) and **4** (3.5 mg) were obtained by Sephadex LH-20 CC (MeOH) of fractions FA2 and FA3, respectively. Fraction FM was subjected to silica gel CC using a gradient of DCM/MeOH (70:1→1:1) to afford ten fractions, FM1-FM10. Fraction FM2 was purified by Sephadex LH-20 CC with MeOH giving compound **5** (15 mg). Fraction FM4 was loaded onto a RP-18 column with MeOH/$H_2O$ (4/1) to give compound **1** (18 mg).

**Camehakonin A** (dihydrokaempferol 3-*O*-[*β*-2,3,4-triacetyl-D-rhamnopyranosyl (1→2)-*β*-D-rhamnopyranosyl-(1→2)]-*α*-L-glucopyranoside, **1**): yellow amorphous, m.p. 217 - 219 $^{o}$C. $[\alpha]_D^{20}$ – 23.56 (c 0.08, MeOH). (+)HR-ESI-MS: *m/z* 891.2526 $[M+Na]^+$ (calcd. for $[C_{39}H_{48}O_{22}Na]^+$, 891.2523). IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3420, 1724, 1639, 1575, 1413, 1244, 1174, 835; $^1$H-NMR (500 MHz, $CD_3OD$) và $^{13}$C-NMR (125 MHz, $CD_3OD$): see Table 1.

**Isoxanthohumol (2)**: white needles, m.p. 204 - 206 $^{o}$C. ESI-MS: *m/z* 356.16 $[M+2H]^+$ (calcd. for $[C_{21}H_{24}O_5]^+$, 356.16). $[\alpha]_D^{20}$ - 29.6 (c 0.08, MeOH). $^1$H-NMR (500 MHz, $CDCl_3$) $\delta$ (ppm): 7.39 (2H, d, *J* = 8.5 Hz, H-2', H-6'), 6.95 (2H, d, *J* = 8.5 Hz, H-3', H-5'), 6.00 (1H, s, H-6), 5.34 (1H, dd, *J* = 3.0, 13.0 Hz, H-2), 5.17 (1H, m, H-2''), 3.82 (3H, s, 5-$OCH_3$), 3.20 (2H, br. d, *J* = 7.5 Hz, H-1''), 3.04 (1H, dd, *J* = 13.0, 17.0 Hz, H-3a), 2.75 (1H, dd, *J* = 3.0, 17.0 Hz, H-3b), 1.63 (3H, s, H-4''), 1.60 (3H, s, H-5''). $^{13}$C-NMR (125 MHz, $CDCl_3$) $\delta$ (ppm): 197.2 (C-4), 165.6 (C-7), 162.4 (C-5), 160.8 (C-4'), 160.5 (C-8a), 131.8 (C-3''), 131.5 (C-1'), 128.2 (C-2', C-6'), 123.3 (C-2''), 114.6 (C-3', C-5'), 108.8 (C-8), 103.0 (C-4a), 96.3 (C-6), 79.2 (C-2), 55.6 ($OCH_3$), 43.5 (C-3), 25.9 (C-5''), 22.1 (C-1''), 17.9 (C-4'').

**(-)Naringenin** (**3**): yellow amorphous. ESI-MS: *m/z* 273.07 $[M+H]^+$ (calcd. for $[C_{15}H_{13}O_5]^+$, 273.07). $[\alpha]^{25}{}_D$ -15.0 (*c* 0.14, EtOH). $^1$H-NMR (500 MHz, $CD_3OD$) $\delta$ (ppm): 7.34 (2H, d, *J* = 8.0 Hz, H-2', H-6'), 6.84 (2H, d, *J* = 8.0 Hz, H-3', H-5'), 5.91 (1H, d, *J* = 2.0 Hz, H-8), 5.90 (1H, d, *J* = 2.0 Hz, H-6), 5.36 (1H, dd, *J* = 3.0, 12.5 Hz, H-2), 3.13 (1H, dd, *J* =13.0, 17.0 Hz, H-3a ), 2.72 (1H, dd, *J* = 3.0, 17.5 Hz, H-3b ). $^{13}$C-NMR (125 MHz, $CD_3OD$) $\delta$ (ppm): 197.8 (C-4), 168.4 (C-7), 165.5 (C-5), 164.9 (C-9), 159.0 (C-4'), 131.8 (C-1'), 131.0 (C-2', C-6'), 116.4 (C-3', C-5'), 103.4 (C-10), 97.1 (C-6), 96.2 (C-8), 80.5 (C-2), 44.1 (C-3).

**(+)Taxifolin** (**4**): yellow amorphous. $[\alpha]^{25}{}_D$ + 20.0 (*c* 0.15, MeOH). ESI-MS m/z 305.06 $[M+H]^+$ (calcd. for $[C_{15}H_{13}O_7]^+$, 305.07). $^1$H-NMR (500 MHz, $CD_3OD$) $\delta$ (ppm): 6.98 (1H, d, *J* = 2.0 Hz, H-2'), 6.87 (1H, dd, *J* = 2.0, 8.5 Hz, H-6'), 6.82 (1H, d, *J* = 8.0 Hz, H-5'), 5.94 (1H, d, *J* = 2.0 Hz, H-8), 5.90 (1H, d, *J* = 2.0 Hz, H-6), 4.93 (1H, d, *J* = 11.5 Hz, H-2), 4.52 (1H, d, *J* = 11.5 Hz, H-3). $^{13}$C-NMR (125 MHz, $CD_3OD$) $\delta$ (ppm): 198.4 (C-4), 168.8 (C-7), 165.3 (C-5),

164.5 (C-9), 147.1 (C-4'), 146.3 (C-3'), 129.9 (C-1'), 120.9 (C-6'), 116.1 (C-2'), 115.9 (C-5'), 101.8 (C-10), 97.3 (C-6), 96.3 (C-8), 85.1 (C-2), 73.7 (C-3).

**(-) Epicatechin** (**5**): white amorphous. $[\alpha]^{25}{}_{D}$ -55.0 (*c* 0.21, EtOH). ESI-MS: *m/z* 291.08 $[M+H]^+$ (calcd. for $[C_{15}H_{15}O_6]^+$, 291.09). $^1$H-NMR (500 MHz, $CD_3OD$) $\delta$ (ppm): 6.99 (1H, d, *J* = 2.0 Hz, H-2'), 6.82 (1H, dd, *J* = 1.5, 8.5 Hz, H-6'), 6.78 (1H, d, *J* = 8.0 Hz, H-5'), 5.96 (1H, d, *J* = 2.5 Hz, H-6), 5.94 (1H, d, *J* = 2.0 Hz, H-8), 4.84 (1H, overlapped, H-2), 4.19 (1H, m, H-3), 2.88 (1H, dd, *J* = 4.5, 17.0 Hz, H-4a), 2.76 (1H, dd, *J* = 3.0, 16.5 Hz, H-4b). $^{13}$C-NMR (125 MHz, $CD_3OD$) $\delta$ (ppm): 158.0 (C-5), 157.6 (C-7), 157.4 (C-8a), 145.9 (C-3'), 145.8 (C-4'), 132.3 (C-1'), 119.4 (C-6'), 115.9 (C-5'), 115.3 (C-2'), 100.1 (C-4a), 96.4 (C-6), 95.9 (C-8), 79.9 (C-2), 67.5 (C-3), 29.2 (C-4).

**Macarangin** (**6**): yellow amorphous. ESI-MS: *m/z* 423.18 $[M+H]^+$ (calcd. for $[C_{25}H_{27}O_6]^+$, 423.18). $^1$H-NMR (500 MHz, $CD_3OD$) $\delta$ (ppm): 8.09 (2H, d, *J* = 7.5 Hz, H-2', H-6'), 6.93 (2H, d, *J* = 7.5 Hz, H-3', H-5'), 6.45 (1H, s, H-8), 5.27 (1H, dt, *J* = 1.0, 7.0 Hz, H-2"), 5.08 (1H, m, H-6"), 3.34 (2H, overlapped, H-1"), 2.07 (2H, dd, *J* = 7.0, 7.5 Hz, H-5"), 1.98 (2H, dd, *J* = 8.0, 7.0 Hz, H-4"), 1.80 (3H, s, H-10"), 1.62 (3H, s, H-8"), 1.57 (3H, s, H-9"). $^{13}$C-NMR (125 MHz, $CDCl_3$) $\delta$ (ppm):175.3 (C-4), 161.9 (C-7), 157.7 (C-4'), 157.3 (C-5), 155.1 (C-9), 145.4 (C-2), 140.1 (C-3), 135.5 (C-3"), 132.2 (C-7"), 129.7 (C-2', C-6'), 123.7 (C-6"), 123.6 (C-1'), 120.9 (C-2"), 115.6 (C-3', C-5'), 109.4 (C-6), 103.6 (C-10), 94.4 (C-8), 39.7 (C-4"), 26.3 (C-5"), 25.7 (C-10"), 21.4 (C-1"), 17.7 (C-9"), 16.3 (C-8").

**5,7,3′,4′-Tetrahydroxy-6-geranylflavonol** (**7**): yellow amorphous. ESI-MS: *m/z* 439.17 $[M+H]^+$ (calcd. for $[C_{25}H_{27}O_7]^+$, 439.17). $^1$H-NMR (500 MHz, $CD_3OD$) $\delta$ (ppm): 7.75 (1H, d, *J* = 2.0 Hz, H-2'), 7.63 (1H, dd, *J* = 2.0, 8.5 Hz, H-6'), 6.90 (1H, d, *J* = 8.5 Hz, H-5'), 6.45 (1H, s, H-8), 5.26 (1H, dt, *J* = 1.7, 7.5 Hz, 2"), 5.08 (1H, m, H-6"), 3.34 (2H, overlapped, H-1"), 2.08 (2H, dd, *J* = 7.5, 7.0 Hz, H-4"), 1.98 (2H, dd, *J* = 7.0, 8.0 Hz, H-5"), 1.80 (3H, s, H-10"), 1.62 (3H, s, H-8"), 1.57 (3H, s, H-9"). $^{13}$C-NMR (125 MHz, $CD_3OD$) $\delta$ (ppm):177.3 (C-4), 163.4 (C-7), 159.1 (C-5), 156.1 (C-10), 148.7 (C-4'), 147.7 (C-3'), 146.2 (C-2), 137.1 (C-3), 135.6 (C-3"), 132.0 (C-7"), 125.5 (C-6"), 124.3 (C-1'), 123.7 (C-2"), 121.6 (C-6'), 116.2 (C-5'), 116.0 (C-2'), 112.3 (C-6), 104.4 (C-10), 93.6 (C-8), 40.9 (C-4"), 27.7 (C-5"), 25.8 (C-10"), 22.1 (C-1"), 17.7 (C-9"), 16.3 (C-8").

### 2.4. Antimicrobial assay

***Microorganisms:*** The microorganisms used in this study consisted of three Gram-positive bacteria (*Enterococcus faecalis* ATCC29212, *Staphylococcus aureus* ATCC25923, *Bacillus cereus* ATCC13245), three Gram-negative bacteria (Escherichia coli ATCC25922, *Pseudomonas aeruginosa* ATCC27853, *Salmonella enterica* ATCC13076) and one yeast strain (*Candida albican*s ATCC10231).

***Determination of minimum inhibitory concentration (MIC)***: The MIC (minimal inhibitory concentration) values were determined using a modified method of broth microdilution [17]. Tested flavonoids were dissolved in DMSO at a decreasing concentration range: 256 µg/ml, 128 µg/ml, 64 µg/ml, 32 µg/ml, 16 µg/ml, 8 µg/ml, 4 µg/ml and 2 µg/ml. Streptomycin, nystatin and cyclohexamide (Merck) were used as positive control for bacteria and yeast, respectively.

### 2.5. Cytotoxic assay

KB (human epithelial carcinoma), Hep-G2 (human hepatoblastoma), SK-LU1 (human lung adenocarcinoma), and MCF7 (human breast adenocarcinoma) cells were purchased from the American Type Culture Collection (ATCC). The cells were grown in the HyClone Dulbecco's Modified Eagle Medium (Hyclone Co.) mixed with 10 % fetal bovine serum (Gibco Co.), 1 % penicillin/streptomycin, and 1 % L-glutamine using an incubator with 5 % $CO_2$ at 37 ºC. Cells were regularly subcultured using a mixture of trypsin/EDTA. The cytotoxicity of isolated compounds was determined using a modified MTT assay [18]. Cultured KB, Hep-G2, SK-Lu1, and MCF7 cells were added in 96-well plates at $3\times10^4$ cells/well density and grown for 24 h. Compounds **1**-**7** were dissolved and serially diluted with dimethyl sulfoxide (DMSO). Compounds with various concentrations (128, 32, 8, 2, and 0.5 µg/ml) or control (0.5 % of DMSO) were added to cells using a liquid handling station equipped with a pintool system. After incubating for 72 h, cells were treated with 10 µL of MTT solution (5 µg/ml) per well and incubated for 4 h until intracellular purple formazan crystals are visible. Absorbance was measured by a plate reader (Biotek) at 540 nm for the determination of cell viability. Ellipticine (Merck, purity > 98 %) was used as a positive control. The inhibition ratio was calculated based on the optical densities from the three replicate tests.

## 3. RESULTS AND DISCUSSION

Seven flavonoids (**1**-**7**) including camehakonin A (**1**), isoxanthohumol (**2**), naringenin (**3**), (+)taxifolin (**4**), (-)epicatechin (**5**), macarangin (**6**), and 5,7,3',4'-tetrahydroxy-6-geranylflavonol (**7**) were isolated from the ethanol extract of *Camellia hakodae* Ninh leaves by repeated column chromatography (Figure 1).

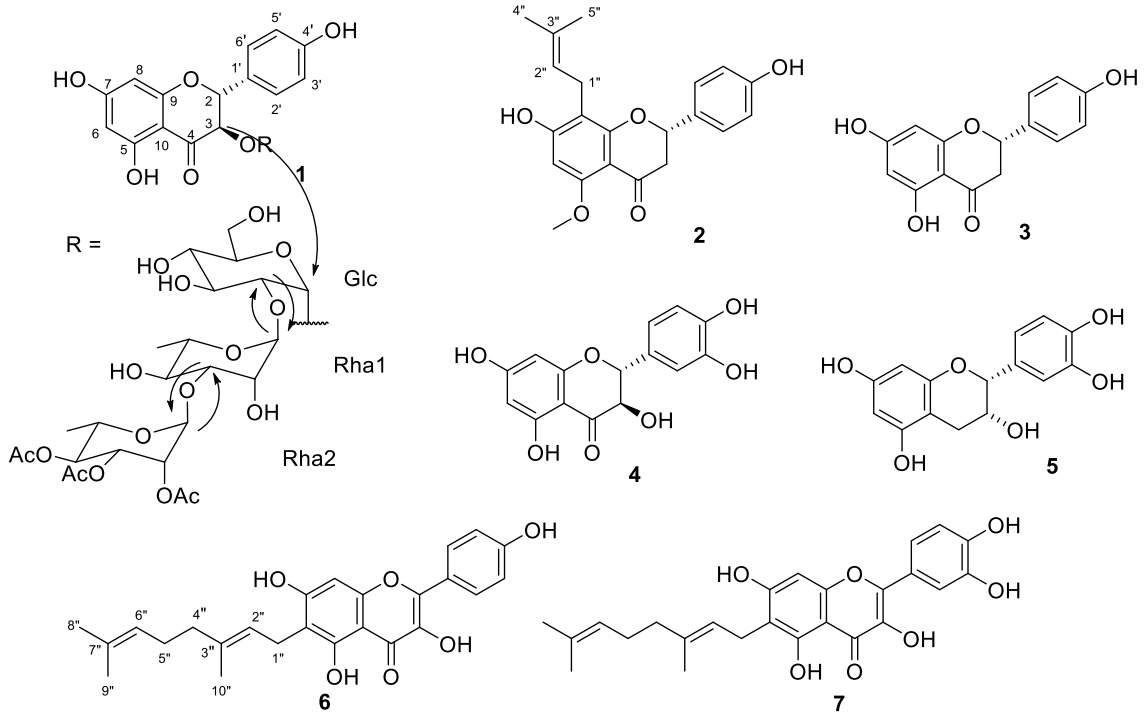


*Figure 1.* Chemical structures of isolated compounds (**1**-**7**) from *C. hakodae* Ninh leaves.

Compound **1** was isolated as a light yellow amorphous powder, mp. 217 - 219 ℃. Its molecular formula, $C_{39}H_{48}O_{22}$, was determined from the adduct molecular ion peak at *m/z* 891.2526 $[M+Na]^+$ (calcd. for $[C_{39}H_{48}O_{22}Na]^+$, 891.2523) in the positive HR-ESI-MS spectrum. The IR spectrum of **1** reveals the absorbance bands of hydroxyl (3420 $cm^{-1}$), carbonyl (1724 $cm^{-1}$), and alkenyl (1639 $cm^{-1}$) functional groups.

*Table 1.* $^1H$ and $^{13}C$ NMR data of compound **1** in $CD_3OD$ ($\delta$ in ppm, *J* in Hz).

| Position | ***Flavanone moeity*** | | ***Glc*** | | ***Rha1*** | | ***Rha2*** | |
|---|---|---|---|---|---|---|---|---|
| | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
| 1 | - | - | 4.63 (d, 1.5 ) | 101.9 | 4.27 (d, 8.0) | 104.1 | 5.37 (d, overlap.) | 98.8 |
| 2 | 5.23 (d, 10.0) | 83.4 | 3.67 (m, 1.5, 3.0) | 80.8 | 3.37 (dd, 8.0, 1.5) | 78.4 | 5.38-5.37 (overlap.) | 70.8 |
| 3 | 4.57 (d, 10.0) | 80.6 | 3.77 (dd, 3.0, 9.5) | 71.7 | 3.52 (dd, 9.0, 9.5) | 78.6 | 5.38-5.37 (overlap.) | 70.8 |
| 4 | - | 195.5 | 3.34 (overlap.) | 71.1 | 3.33 (overlap.) | 74.2 | 5.01(t, 10.0) | 72.4 |
| 5 | - | 165.5 | 3.17 (m) | 77.3 | 4.18 (dd, 9.5, 6.5) | 70.7 | 4.43 (dd, 9.5, 6.5) | 67.9 |
| 6 | 5.92 (overlap.) | 96.5 | 3.72-3.74 (m) | 62.2 | 1.20 (d, 6.0) | 17.7 | 1.17 (d, 6.0) | 18.0 |
| 7 | - | 169.2 | | | | | | |
| 8 | 5.92 (overlap.) | 96.5 | | | | | | |
| 4a | - | 102.4 | | | | | | |
| 8a | - | 164.0 | | | | | | |
| 1' | - | 128.7 | | | | | | |
| 2' | 7.38 (d, 7.0) | 130.1 | | | | | | |
| 3' | 6.89 (d, 7.0) | 116.6 | | | | | | |
| 4' | - | 159.2 | | | | | | |
| 5' | 6.89 (d, 7.0) | 116.6 | | | | | | |
| 6' | 7.38 (d, 7.0) | 130.1 | | | | | | |
| $\underline{CH_3}CO$ | | | | | | | 2.14 | 20.8 |
| $CH_3\underline{C}O$ | | | | | | | | 171.7 |
| $\underline{CH_3}CO$ | | | | | | | 2.08 | 20.7 |
| $CH_3\underline{C}O$ | | | | | | | | 172.0 |
| $\underline{CH_3}CO$ | | | | | | | 1.97 | 20.8 |
| $CH_3\underline{C}O$ | | | | | | | | 171.8 |

The $^1H$ and $^{13}C$ NMR spectra of **1** indicated characteristic signals of a flavonoid triacetylated triglycoside structure, including the signals of 15 carbons and 8 protons belonging to a flavanone moiety, three anomeric protons of three sugar units and three acetyl groups. The $^{13}C$ NMR and HSQC spectra revealed the presence of a flavanone unit with a carbonyl group ($\delta_C$

195.5, C-4), six aromatic methines [$\delta_H$ 5.92-7.38/ $\delta_C$ 96.5-130.1], six aromatic quaternary carbons and two oxygenated methines at [$\delta_H$ 5.23 (d, $J$ = 10.0 Hz, H-2)/$\delta_C$ 83.4 (C-2)] and [$\delta_H$ 4.57 (d, $J$ = 10.0 Hz, H-3)/ $\delta_C$ 80.6 (C-3)]. The $^1$H NMR spectrum of **1** also showed the presence of six aromatic protons belonging to two benzene rings including two *meta* protons [$\delta_H$ 5.3 (brs, H-5), 5.92 (brs, H-8)], and two pairs *ortho* protons in a AA'BB' system at [$\delta_H$ 7.38 (d, $J$ = 8.0 Hz, H-2', H-6'), 6.89 (d, $J$ = 8.0 Hz, H-3', H-5')]. The NMR data of the aglycon moiety (*Table 1*) were quite similar to those of dihydrokaempferol [19] and the aglycon moiety of engelitin (dihydrokaempferol-3-rhamnoside) [20]. The coupling constant value between H-2 and H-3 ($J$ = 10.0 Hz) suggested the (2*R*,3*R*) configuration of **1** while comparing with the reported value of (2*R*,3*R*)-engelitin ($J$ = 10.4 Hz). The (2*S*,3*R*), (2*R*,3*S*) and (2*S*,3*S*)-engelitin had a coupling constant value of 2.0, 2.4 and 11.2 Hz, respectively [20]. The (2*R*,3*R*) configuration of **1** was also confirmed by comparison of optical rotation value of aglycon moiety obtained by acidic hydrolysis [21] with that of 2*R*,3*R*-dihydrokaempferol [19]. Therefore, the structure of aglycon moiety of **1** was determined as 2*R*,3*R*-dihydrokaempferol.

The signals of a glucopyranose and two rhamnopyranose units were also observed in the $^1$H and $^{13}$C NMR spectra of **1**, including 3 anomeric protons [$\delta_H$ 4.63 (d, $J$ = 1.5 Hz, Glc H-1), 4.27 (d, $J$ = 8.0 Hz, Rha1 H-1), 5.37 (overlapped, Rha2 H-1)], twelves oxymethin groups, one oxymethylene group [$\delta_H$ 3.72-3.74 (2H, m, Glc H-6)/$\delta_C$ 62.2 (Glc C-6)] and two methyl groups [$\delta_H$ 1.20 (d, $J$ = 6.0 Hz, Rha1 H-6)/$\delta_C$ 17.7 (Rha1 C-6); $\delta_H$ 1.17 (d, $J$ = 6.0 Hz, Rha2 H-6)/$\delta_C$ 18.0 (Rha2 C-6)]. Based on 2D NMR experiments (COSY, NOESY, HSQC, HMBC) and $J_{1-2}$ values, the first and second sugar moieties were determined to be a $\alpha$-glucopyranosyl and a $\beta$-rhamnopyranosyl unit (*Table 1*). The acidic hydrolysis, followed by TLC analysis and comparison of the optical rotation values of sugar portions with those of authentic *L*-glucose and *D*-rhamnose [21] allowed to confirmed the presence of *L*-glucose (Glc) and *D*-rhamnose (Rha1, Rha2) in **1**. The relative configuration of the *D*-rhamnose 2 (Rha2) unit that was initially uncertain due to overlapped signals of H-1/H-2/H-3 at 5.37 ppm in $^1$H NMR spectra in **1,** were presumed to be $\beta$ from the obtained optical rotation of rhamnose fraction and natural occurrences of sugars. Furthermore, the sugar linkages were determined by the HMBC correlations between H-3 ($\delta_H$ 4.57) of the aglycon with Glc C-1 ($\delta_C$ 101.9) and Glc H-1 with C-3 ($\delta_C$ 80.6); Rha1 H-1 with Glc C-2 ($\delta_C$ 80.8) and Glc H-2 ($\delta_H$ 3.67) with Rha1 C-1 ($\delta_C$ 104.1); Rha2 H-1 with Rha1 C-2 ($\delta_C$ 78.4) and Rh1 H-2 ($\delta_H$ 3.37) with Rha2 C-1 ($\delta_C$ 98.8) (Figure 1). In addition, the $^1$H and $^{13}$C NMR spectra indicated the presence of three acetyl groups with three methyl groups [$\delta_H$ 2.14, 2.08, 1.97 (s, 3$CH_3$)/ $\delta_C$ 20.8, 20.7, 20.7] and three carbonyl groups ($\delta_C$ 172.0, 171.8, 171.7). These acetyl groups were found attached with Rha2 unit at C-2, C-3 and C-4 based on the downfield shift of Rha2 H-2, H-3, H-4 ($\delta_H$ 5.38-5.37, 5.01) [22] compare to the others sugars units. Moreover, the correlations between the methyl protons with carbonyl carbons and Rha2 H-2, H-3, and H-4 with these carbonyl groups were also observed (Figure 1).

Based on (+)HR-ESI-MS, NMR data, optical rotation of aglycon and sugars moieties, the structure of the new compound, camehakonin A (**1**), was concluded to be dihydrokaempferol 3-*O*-[$\beta$-2,3,4-triacetyl-D-rhamnopyranosyl(1→2)-β-D-rhamnopyranosyl-(1→2)]-$\alpha$-L-glucopyranoside.

The known isolated compounds were identified by comparison of physical and spectroscopic data (m.p, optical rotation, $^1$H and $^{13}$C NMR) with authentic samples or literature values, and this included isoxanthohumol (**2**) [23, 24], naringenin (**3**) [25], (+)taxifolin (**4**) [26], (-)epicatechin (**5**) [27], macarangin (**6**) [28], and 5,7,3',4'-tetrahydroxy-6-geranylflavonol (**7**)

[29]. All of these known compounds were reported for the first time from *Camellia hokodae* Ninh leaves.

The isolated flavonoids were evaluated in terms of their antimicrobial activities. Their MIC values against (+) Gram (*Enterococcus faecalis*, *Staphylococcus aureus*, *Bacillus cereus*), (-) Gram bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enterica*) and yeast (*Candida albican*s) are reported in Table 2. The results showed that only compound **1** exhibited inhibition effects against tested (+) Gram bacteria with MIC values in the range from 64 - 256 µg/mL. Interestingly, the antibacterial activities of **1** against *E. faecalis* and *S. aureus* (MIC 128, 64 µg/mL, respectively) were higher than that of streptomycin (MIC 256 µg/mL). Moreover, compound **1** inhibited significantly *C. albicans* with a MIC value of 16 µg/mL. Compounds **2-7** did not show any inhibition against tested microorganisms at the concentration range of 2 - 256 µg/mL.

*Table 2*. MIC (µg/mL) values of compound **1**.

| Sample | (+) Gram bacteria | | | (-) Gram bacteria | | | Yeast |
|---|---|---|---|---|---|---|---|
| | *E. faecalis* | *S. aureus* | *B. cereus* | *E. coli* | *P. aeruginosa* | *S. enterica* | *C. albicans* |
| **1** | 128 | 64 | 256 | - | - | - | 16 |
| Streptomycin | 256 | 256 | 128 | 32 | 256 | 128 | - |
| Nystatin | - | - | - | - | - | - | 8 |
| Cyclohexamide | - | - | - | - | - | - | 32 |

Furthermore, compound **1** was evaluated for its *in vitro* cytotoxicity against four human cancer cell lines including KB (human epithelial carcinoma), Hep-G2 (human hepatoblastoma), SK-LU1 (human lung adenocarcinoma), and MCF7 (human breast adenocarcinoma) using modified MTT method. The result showed that **1** displayed weak cytotoxicity against two cancer cell lines (KB and Hep-G2) with $IC_{50}$ values of 73.70 and 221.11 µM, respectively, and inactivity against SK-LU1 and MCF7 cell lines (*Table 3*).

*Table 3*. $IC_{50}$ (µM) values of compound **1**.

| Sample | KB | Hep-G2 | SK-LU1 | MCF7 |
|---|---|---|---|---|
| **1** | 73.70 | 221.11 | - | - |
| Ellipticine | 1.10 | 1.21 | 1.42 | 1.95 |

## 4. CONCLUSIONS

A new flavanone acetylated triglycoside (dihydrokaempferol 3-O-[*β*-2,3,4-triacetyl-D-rhamnopyranosyl (1→2)-*β*-D-rhamnopyranosyl-((1→2)]-*α*-L-glucopyranoside, camehakonin A, **1**) and six known flavonoids, including isoxanthohumol (**2**), naringenin (**3**), (+)taxifolin (**4**), (-) epicatechin (**5**), macarangin (**6**), and 5,7,3',4'-tetrahydroxy-6-geranylflavonol (**7**) were isolated from *Camellia hakodae* Ninh leaves for the first time. Compound **1** exhibited inhibition effects against tested (+) Gram bacteria (*E. faecalis*, *S. aureus*, *B. cereus*) with MIC values in the range from 64-256 µg/mL. Moreover, compound **1** inhibited significantly *C. albicans* with a MIC

value of 16 µg/mL. In addition, compound **1** displayed weak cytotoxicity against two cancer cell lines (KB and Hep-G2) with $IC_{50}$ values of 73.70 and 221.11 µM, respectively.

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