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CHEMICAL CONSTITUENTS FROM THE LEAVES OF *UVARIA BONIANA* IN VIET NAM

Nguyen Thanh Tam[1], Nguyen Ngoc Tuan[2], Hoang Van Trung[3], Le Thi My Chau[3], Dinh Thi Trung Anh[3], Hoang Van Luu[1,*]

[1] *School of Natural Sciences Education, Vinh University, 182 Le Duan, Vinh City, Nghe An*

[2]*Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City, 12 Nguyen Van Bao, Go Vap District, Ho Chi Minh City*

[3]*School of Chemistry, Biology and Environment, Vinh University, 182 Le Duan, Vinh, Nghe An*

[*]Email: *hoangluudhv@gmail.com*

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Abstract. A phytochemical study of *Uvaria boniana* Fin. & Gagnep collected at Pumat National Park, Nghe An province led to the isolation of five secondary metabolites, including uvaridacol G (**1**); 4-methyl-4-[(2*Z*)-3'-phenylprop-2'-en-1'-yl]cyclohex-2-en-1-one (**2**); 3,7- dimethoxy quercetin 4'-*O*-[*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranoside (**3**); *β*-sitosterol (**4**) and stigmasterol (**5**). Their structures were determined on the basis of one and two-dimensional NMR and spectrometric methods. This is the first report on the chemical constituents of *Uvaria boniana* in Viet Nam.

Keywords: Uvaria boniana, uvaridacol G, 3,7- dimethoxy quercetin 3'-*O*-[α-L-rhamnopyranosyl- (1→2)-β-D-glucopyranoside.

Classification numbers: 1.1.1, 1.1.6.

1. INTRODUCTION

Uvaria is a genus of flowering plants in Annonaceae family, which consists of approximately 150 species. Most plants of this genus are climbing shrubs or small trees. They are distributed in wet tropical regions such as Southeast Asia, tropical Africa, Northern Australia, Madagascar and Indochina [1, 2]. The phytochemical study on *Uvaria* species shows the presence of various chemical constituents, including flavonoids [3] and flavonoid glycosides [4], benzoylated derivatives [5], essential oils [6], oxygenated cyclohexanes [7] and polyoxygenated cyclohexenes [8]. *Uvaria boniana* Fin. & Gagnep is widely distributed in Viet Nam. All parts of this plant can be used in the traditional medicine. The squeezed leaves afford a cinnamon bark-like smell and the water decoction can be consumed directly, while the fruits are used to cure ulcers of the intestines diseases [9]. The water decoction of the roots is used to treat women with postpartum infection [10]. In this report, five compounds including uvaridacol G (**1**); 4-methyl-4-[(2Z)-3'-phenylprop-2'-en-1'-yl]cyclohex-2-en-1-one (**2**); 3,7- dimethoxy

quercetin 3'- O- [α-L- rhamnopyranosyl- (1 → 2) -β-D- glucopyranoside (**3**); β-sitosterol (**4**) and stigmasterol (**5**) have been isolated from *Uvaria boniana.*

2. MATERIAL AND METHODS

2.1. General

Melting points were determined using Yanagimoto MP-S3 apparatus without corrections. Optical rotations were measured using a JASCO DIP-370 polarimeter. The UV spectra were obtained on a Hitachi UV-3210 spectrophotometer, and IR spectra were recorded on a Shimadzu FTIR-8501 spectrophotometer. ^{1}H- and ^{13}C-NMR, COSY, NOESY, HMQC, and HMBC spectra were obtained on the Bruker AV-III 500 NMR spectrometer, with tetramethylsilane (TMS) as the internal standard and chemical shifts were reported in δ values (ppm). The electrospray ionization (ESI) and high resolution electrospray ionization (HR-ESI) mass spectra were determined using an Agilent 1200 LC-MSD Trap spectrometer. Column chromatography (CC) was performed on silica gel (Kieselgel 60, 70-230 mesh and 230-400 mesh, E. Merck). Thin layer chromatography (TLC) was conducted on precoated Kieselgel 60 F 254 plates (Merck) and the compounds were visualized by spraying with 10 % (v/v) H_2SO_4 followed by heating at 110 °C for 10 min.

2.2. Plant material

The leaves of *Uvaria boniana* Fin. & Gagnep were collected at the Pumat National Park of Nghe An province, Viet Nam, in August 2016 and identified by Prof. Dr. Tran Huy Thai, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology. A voucher specimen (Vinh-UHVL 20160821) was deposited at the herbarium of the Department of Chemistry, Vinh University.

2.3. Extraction and isolation

The dried leaves of *Uvaria boniana* (6.0 kg) were extracted with methanol at ambient temperature, and concentrated under reduced pressure to give the methanol extract (254 g). The crude extract was suspended in water and partitioned with ethyl acetate and butanol to afford ethyl acetate (172 g), butanol (33 g) and water soluble (40 g) fractions, respectively. The ethylacetate extract was applied to silica gel column chromatography with a mixture of hexane/acetone gradient (100:0, 50:1, 39:1, 30:1, 20:1, 15:1, 9:1, 4:1, 2:1, 1:1) to afford ten fractions (Frs. U1-U10). Fraction U1 (6.5 g) was subjected to the silica gel column chromatography (150 g, 80 × 2 cm) eluting with a mixture of hexane/acetone (15:1) to afford seven fractions (Frs. U1.1-U1.7). Fraction U1.1 (2.6 g) was subjected to the silica gel column chromatography (200 g, 60 × 3 cm), eluted with hexane/acetone mixture (15:1) to yield compound **4** (138 mg). Fraction U1.4 (2.5 g) was subjected to the silica gel column chromatography (300 g, 80 × 3 cm), eluted a mixture of hexane/acetone (9:1) to produce compound **1** (12 mg) and **2** (27 mg). Fraction U7 (1.9 g) was purified by silica gel column chromatography (350 g, 80 × 3 cm) eluting with $CHCl_3$: CH_3OH (7:1) to yield **5** (112 mg).

The butanol extract was applied to silica gel column chromatography with a mixture of chloroform and methanol (100:0, 40:1, 30: 1; 10:1, 4:1, 2:1) to afford minor fractions. Fraction UB5 was subjected to the silica gel column chromatography (150 g, 80 × 2 cm) and eluted with a mixture of chloroform and methanol (10:1; 8:1) to yield **3** (171 mg).

Compound **1:** colorless, amorphous solid, m.p.: 165-167 ^{0}C, $[\alpha]^{25}_D$ -12 (c 1.0, $CHCl_3$); HR ESI-MS *m/z* 385.1285 $[M + H]^+$ (calcd for $C_{21}H_{21}O_7$, 385.1287); ^{1}H-NMR (500 MHz, acetone-d_6, δ, ppm): 4.29 (1H, *t*, *J* = 13.5, 7.0 Hz, H-3), 4.43 (1H, *t*, *J* = 10.5, 4.5 Hz, H-4), 4.68 (1H, *d*, *J* = 11.0 Hz, H-7b), 4.75 (1H, *d*, *J* = 11.0 Hz, H-7a), 5.76 (1H, *d*, *J* = 8.0 Hz, H-2), 5.79 (1H, *d*, *J* = 11.0 Hz, H-6), 5.95 (1H, *dd*, *J* = 11.5, 2.5 Hz, H-5),7.49 (2H, *m*, H-3",5"), 7.49 (2H, *m*, H-3',5'), 7.63 (1H, *m*, H-4'), 7.63 (1H, *m*, H-4"), 8.03 (2H, *d*, *J* = 6.5 Hz, H-2',6'), 8.07 (2H, *d*, *J* = 6.5 Hz, H-2",6"); ^{13}C-NMR (125 MHz, acetone-d_6, δ, ppm): 68.0 (C-7), 69.7 (C-4), 70.9 (C-3), 75.5 (C-1), 76.3 (C-2), 127.3 (C-3', C-5'), 129.2 (C-3", C-5"), 129.2 (C-1'), 130.3 (C-6), 130.4 (C-2', C-6'), 131.1 (C-1"), 131.4 (C-2", C-6"), 131.5 (C-5), 133.7 (C-4'), 133.8 (C-4"), 166.8 (C-7'), 167.1 (C-7").

Compound **2**: colorless needles, m.p.: 176-178 ^{0}C; ^{1}H-NMR (500 MHz, $CDCl_3$, δ, ppm): 6.80 (1H, *d*, *J* = 10 Hz, H-2), 6.55 (1H, *d*, *J* = 16 Hz, H-3'), 6.23 (1H, *m*, H-2'), 5.97 (1H, *d*, *J* = 10 Hz, H-3), 2.64 (3H, s, 4-CH_3),7.24-7.39 (5H, H-2", 3", 4", 5" and 6") ; ^{13}C-NMR (125 MHz, $CDCl_3$, δ, ppm): 198.7 (C-1), 153.1 (C-2), 136.6 (C-3), 135.4 (C-3'), 129.0 (C-4"), 128.7 (C-3"), 128.7 (C-5"), 127.8 (C-1"), 126.3 (C-6"), 126.3 (C-2"), 122.9 (C-2'), 70.1 (C-4), 43.7 (4-CH_3), 35.0 (C-5), 35.0 (C-6), 34.4 (C-1').

Compound **3**: yellow powder, m.p. 241-242 ^{0}C; UVλ_{max}^{MeOH}nm (logε): 206, 269 and 355 nm; IRν_{max}^{KBr}cm^{-1}: 1662 (C=O) and 3443 (OH) cm^{-1}; HR-ESI-MS (negative) *m/z*: 637.1765 $[M-H]^-$; ^{1}H-NMR (DMSO-d_6, 500 MHz, δ, ppm): 12.66 (1H, *s*, OH-5), 9.84 (1H, *brs*, OH-3'), 7.79 (1H, *d*, *J* = 2.0 Hz, H-2'), 7.69 (1H, *dd*, *J* = 8.5, 2.0 Hz, H-6'), 7.01 (1H, *d*, *J* = 8.5 Hz, H-5'), 6.81 (1H, *d*, *J* = 2.0 Hz, H-8), 6.37 (1H, *d*, *J* = 2.0 Hz, H-6), 5.28 (1H, *d*, *J* = 6.0 Hz, OH), 5.20 (1H, br s, H-1'"), 5.10 (1H, *d*, *J* = 6.0 Hz, OH), 5.08 (1H, *m*, H-1"), 4.60 (2H, m, OH), 4.51 (1H, *m*, OH), 4.37 (1H, *d*, *J* = 6.0 Hz, OH), 3.88 (1H, *m*, H-5'"), 3.86 (3H, *s*, OCH_3-7), 3.82 (3H, *s*, OCH_3-3), 3.74 (1H, *m*, H-2'"), 3.70 (1H, *m*, H-6"), 3.59 (1H, *t*, *J* = 8.5 Hz, H-3"), 3.52-3.44 (3H, *m*, H-3"', -2", OH), 3.36 (1H, *m*, H-5"), 3.23 (1H, *m*, H-4"), 3.20 (1H, *m*, H-4"'), 1.09 (3H, *d*, *J* = 6.0 Hz, CH_3-6"'); ^{13}C-NMR (125 MHz, DMSO-d_6, δ, ppm): 178.0 (C-4), 165.1 (C-7), 160.8 (C-5), 156.2 (C-9), 155.6 (C-2), 144.9 (C-3'), 150.6 (C-4'), 137.9 (C-3), 116.5 (C-2'), 120.5 (C-1'), 123.6 (C-6'), 116.1 (C-5'), 105.1 (C-10), 100.4 (C-1'"), 99.2 (C-1"), 97.7 (C-6), 92.5 (C-8), 77.2 (C-5"), 77.0 (C-2"), 76.9 (C-3"), 72.0 (C-4"'), 70.5 (C-2"'), 70.4 (C-3"'), 69.8 (C-4"), 68.5 (C-5"'), 60.6 (C-6"), 59.7 (OCH_3-3), 56.0 (OCH_3-7), 17.9 (C-6"').

Compound **4**: white powder, m.p. 136 – 138 ^{o}C; IRν_{max}^{KBr}cm^{-1}: 3400, 3025, 1410, 1250; EI-MS m/z (%): 414 (M^+, $C_{29}H_{50}O$, 20), 413(41), 398 (28), 397(100), 395(32), 383 (11), 361 (11), 257 (3), 255 (6,3), 151 (5,6), 139 (11); ^{1}H-NMR (500 MHz, $CDCl_3$, δ, ppm): 5.31(1H, m, H-6), 3.51 (1H, m, H-3), 1.01 (3H, s, 19-CH3), 0.92 (3H, *d*, *J* = 6.2 Hz, 21-CH_3), 0.84 (3H, *d*, *J* = 7.0 Hz, 29-CH_3), 0.83 (3H, *d*, *J* = 6.5 Hz, H-26), 0.81 (3H, *d*, *J* = 6.5 Hz, 27-CH_3), 0.68 (3H, *s*, 18-CH_3); ^{13}C-NMR (125 MHz, $CDCl_3$, δ, ppm): 140.8 (C-5), 121.7 (C-6), 71.8 (C-3), 56.8 (C-14), 56.1 (C-17), 50.2 (C-9), 45.9 (C-24), 42.3 (C-4), 42.3 (C-13), 34.0 (C-22), 39.8 (C-12), 37.3 (C-1), 36.5 (C-10), 36.2 (C- 20), 34.0 (C-8), 32.0 (C-7), 31.7 (C-2), 29.2 (C-25), 28.3 (C-16), 26.1 (C-23), 24.3 (C-15), 23.1 (C-28), 21.1 (C-11), 19.8 (C-26), 19.4 (C-19), 19.1 (C-27), 18.8 (C-21), 12.0 (C-29), 11.9 (C-18).

Compound **5:** white powder; m.p. 155-157 ^{0}C; IRν_{max}^{KBr}cm^{-1}: 3400, 3025, 1410, 1250; EI-MS: *m/z* $[M]^+$: 412; ^{1}H-NMR (500 MHz, $CDCl_3$, δ, ppm): 5.35 (1H, *m*, H-6), 5.14 (1H, *dd*, *J* = 12.0, 3.0 Hz, H-22), 5.03 (1H, *dd*, *J* = 12.0, 3.0 Hz, H-23), 3.28 (1H, *m*, H-3), 0.90 (3H, *d*, *J* = 6.5 Hz, 21-CH_3), 0.82 (3H, *d*, *J* = 6.6 Hz, 26-CH_3), 0.83 (3H, *t*, *J* = 7.0 Hz, 29-CH_3), 0.80 (3H, *d*, *J*=6.5 Hz, 27-CH_3), 0.79 (3H, *s*, 19-CH_3), 0.64 (3H, *s*, 18-CH_3); ^{13}C-NMR (125 MHz, $CDCl_3$, δ, ppm): 140.8 (C-5), 138.3 (C-22), 129.3 (C-23), 121.7 (C-6), 71.8 (C-3), 56.9 (C-14), 56.0 (C-17), 51.3 (C-9), 50.2 (C-24), 42.3 (C-4, C-13), 40.5 (C-20), 39.7 (C-12), 36.5 (C-10), 37.3 (C-1),

31.9 (C-7, C-8), 31.7 (C-2, C-25), 28.9 (C-16), 25.4 (C-28), 24.4 (C-15), 21.2 (C-27), 21.1 (C-11), 19.4 (C-19), 19.0 (C-26), 12.2 (C-21), 12.0 (C-29), 11.9 (C-18).

3. RESULTS AND DISCUSSION

The dried leaves of *Uvaria boniana* was powdered and extracted with methanol, and the methanol extract was partitioned with ethyl acetate and butanol to afford ethyl acetate and butanol fractions successively. The ethyl acetate extract was purified by column chromatography to afford five compounds uvaridacol G (**1**); 4-methyl-4-[(2Z)-3'-phenylprop-2'-en-1'-yl]cyclohex-2-en-1-one (**2**); 3,7-dimethoxyquercetin-4'-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (**3**); *β*-sitosterol (**4**) and stigmasterol (**5**).

Compound **1** was isolated as a colorless, amorphous solid. Its molecular formula was deduced to be $C_{21}H_{20}O_7$ (*m/z* 385.1285 $[M+H]^+$) based on HR-ESIMS. The ^1H and ^{13}C-NMR spectra of **1** showed the signals of two benzoyl groups, three oxymethines, an oxymethylene, and two olefinic methines. However, they were characterized by the downfield shift of H-2 to δ_H 5.76 (1H, *d*, *J* = 8.0 Hz) and the upfield shift of H-3 to 4.29 (1H, t, J = 13.5, 7.0 Hz, H-3). The ^{13}C NMR spectrum showed 21 carbon signals including five oxygenated carbons, two olefinic carbons [δ_C131.5 (C-5), 130.3 (C-6)], and two benzoyl groups [δ_C 129.2 (C-1'), 130.4 (C-2', C-6'), 127.3 (C-3', C-5'), 131.1 (C-1''), 131.4 (C-2'', C-6''), 129.2 (C-3'', C-5''), 133.7 (C-4'), 133.8 (C-4'')]. In the HMBC spectrum of **1**, the long range correlations from the oxymethylene protons at δ_H 4.75,4.68 (H-7) to the oxymethine carbon at δ_C 76.3 (C-2), the olefinic methine carbon at δ_C 129.5 (C-6), and the nonprotonated oxygenated carbon at C-1 suggested the connectivity of C-2, C-6, and C-7 via the tertiary carbon C-1.The oxymethine proton at δ_H 5.76 (H-2) and the aromatic protons at δ_H 8.07 (H-2″, 6″), having HMBC correlation to the ester carbonyl carbon at δ_C 167.1, indicated the locations of the two benzoyl groups to be at C-2 and C-7, respectively. The above spectroscopic data were consistent with those reported for uvaridacol G in the literature [11]. Therefore, compound **1** was characterized as uvaridacol G.

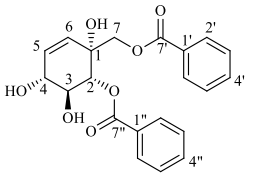
Compound **2** was obtained as a white powder. The ^1H-NMR spectrum displayed the presence of five aromatic protons at δ_H 7.24 - 7.39 ppm, four olefinic protons at δ_H 6.80 and 5.97 ppm, 6.55 and 6.23 ppm and the signal of methyl and methylene protons at δ_H 2.64, 2.46, 2,19 and 2,12. The ^{13}C-NMR of **2** showed signals of 16 carbons: a carbonyl carbon at δ_C 198.7 (C=O); 6 aromatic carbons at δ_C 127.8 (C-1"); 126.3 (C-2", 6"); 128.7 (C-3" , 5"); 129.0 (C-4"); two trans-olefinic carbons at δ_C 122.9 (C-2'); 135.4 (C-3'); two *cis*- olefinic carbons at δ_C 136.6 (C-3), 153.1(C-2) and sp^3carbons at δ_C 35.0 (C-5), 35.0 (C-6); 34.4 (C-1'); 43.7 (CH_3); 70.1 (C-4). In comparison with those reported in the literature, compound **2** was determined as known 4-methyl-4-[(2*Z*)-3'-phenylprop-2'-en-1'-yl]cyclohex-2-en- 1-one [12].

Compound **3** was obtained as yellow powder, m.p.241-242^{o}C. The HR-ESI-MS displayed the *pseudo*-molecular ion peak at *m/z* 637.1765 $[M-H]^+$, corresponding to a molecular formula of $C_{29}H_{34}O_{16}$ (cal. 637.5152). The UV absorption maxima at 355, 269 and 206 nm were the characteristic of a flavone skeleton. The IR absorption bands at 3443 and 1662 cm^{-1} displayed the presence of a hydroxyl and carbonyl group. In the ^1H-NMR spectrum, a typical set of ABX signals at δ 7.79 (1H, *d*, *J* = 2.0 Hz); 7.69 (1H, *dd*, *J* = 8.5, 2.0 Hz) and 7.01 (1H, *d*, *J* = 8.5 Hz) were attributed to the trisubstituted B-ring. Two doublets at δ 6.37 (1H, *d*, *J* = 2.0 Hz) and 6.81 (1H, *d*, *J* = 2.0 Hz) was assumed to be H-6 and H-8 due to the correlations with the carbon signals at 165.1 (C-7); 160.8 (C-5); 105.1 (C-10); 156.2 (C-9); and 97.7 (C-6), respectively. Two anomeric proton signals at 5.20 (1H, *br s*) and 5.08 (1H, *m*) suggested the presence of two sugar units. In addition, there are oxygenated methine and methylene protons at δ 3.88 (1H, *m*); 3.74

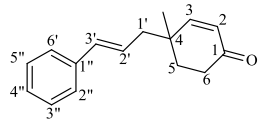
(1H, *m*); 3.59 (1H, t, *J* = 8.5 Hz); 3.52-3.44 (3H, *m*); 3.36 (1H, *m*); 3.23 (1H, *m*); 3.20 (1H, *m*) which were identified as the proton signals of the sugar moieties. Moreover, the upfield methyl doublet at δ_H 1.09 (3H, *d*, *J* = 6.0 Hz) was the characteristic absorption for the rhamnose unit. The ^{13}C NMR and DEPT spectra showed 29 carbons, including 17 carbons of flavone skeleton and 12 carbons of two sugar moieties. The sugar portion of **3** displayed a methyl (δ 17.9); an oxymethylene (δ 60.6); eight oxymethine (δ 77.2; 77.0; 76.9; 72.0; 70.5; 70.4; 69.8; 68.5) and two anomeric signals (δ 99.2 and δ 100.4). The structure of **3** was identified as 3,7-dimethoxyquercetin-3'-*O*-[*α*-L-rhamnopyranosyl- (1 → 2) -*β*-D- glucopyranosit] by comparison of its physical and spectroscopic data with those reported in the literature [13].

Compound **4** was obtained as optically active white powder, m.p. 135-136 ^{o}C. The EI-MS showed the molecular ion peak at *m/z* 414 $[M]^+$ corresponding to a molecular formula of $C_{29}H_{50}O$. The ^{1}H-NMR, ^{13}C-NMR and DEPT spectra of **4** showed signals of oxygenated proton at δ_H 3.51 ppm in the downfield region, which suggested the C-3 hydroxylation. The signal proton H-6 at 5.31 ppm suggested the characteristic of olefinic proton. Moreover, the signal of six methyl groups appeared at δ_H 0.68, 0.87, 0.91, 1.01, 1.10, 1.17. Compounds **4** was identified as *β*-sitosterol by comparison of its physical and spectroscopic data with those reported in the literature [14]. This compound exists very commonly in the plant [12].

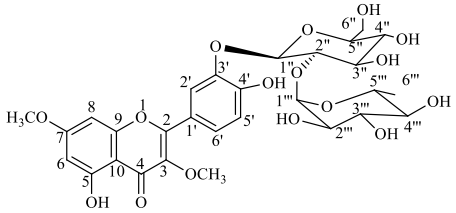
Compound **5** was obtained as a white powder, m.p. 155-157 ^{o}C. The IR, ^{1}H-NMR and ^{13}C-NMR spectra of **5** suggested the signal of oxygenated proton at C-3 corresponding to 3.28 (1H, *m*), 71.8 (C-3). Moreover, the ^{1}H-NMR spectrum of **5** showed signal at δ_H 5,35 (1H, *m*, H-6), and two *trans*-olefinic protons at δ_H 5.14 (1H, *m*, *J*=12.0, 3.0 Hz, H-22), and 5.03 (1H, *dd*, *J* =12.0, 3.0 Hz, H-23). The EI-MS of compound **5** showed the molecular ion peak at *m/z* 412 $[M]^+$ suggesting the molecular formula of $C_{29}H_{48}O$. The structure of **5** was identified as stigmasterol by comparison of its physical and spectroscopic data with those reported in the literature [14].



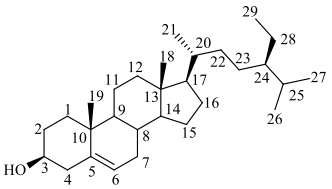
Uvaridacol G (**1**)



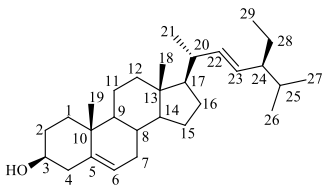
4-methyl-4-[(2*Z*)-3'-phenylprop-2'-en-1'-yl]cyclohex-2-en-1-one (**2**)



3,7- dimethoxy quercetin 3'-*O*- [*α*-L- rhamnopyranosyl- (1 → 2) -*β*-D- glucopyranoside (**3**)



β-sitosterol (**4**)



Stigmasterol (**5**)

Figure 1. The isolated compounds from *Uvaria boniana*.

4. CONCLUSION

In this study, five compounds, including uvaridacol G (**1**); 4-methyl-4-[(2*Z*)-3'-phenylprop-2'-en-1'-yl] cyclohex-2-en-1-one (**2**); 3,7- dimethoxy quercetin 3'-*O*-[α-L- rhamnopyranosyl-(1$\rightarrow$2) -β-D- glucopyranoside (**3**); β-sitosterol (**4**) and stigmasterol (**5**) have been isolated from the leaves of *Uvaria boniana* Fin. & Gagnep collected in Nghe An province, Viet Nam. These compounds were isolated from this plant for the first time. The chemical structures of the these compounds were determined on the basis of 1D and 2D NMR, UV, IR and MS analytical results.

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