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Constituents of *Vernonia gratiosa* Hance and their *α*-glucosidase and xanthine oxidase inhibitory activities

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Abstract

Eight compounds, including 11*β*,13-dihydrovernolide (**1**), apigenin (**2**), kaempferol (**3**), quercetin 3-*O*-methyl ether (**4**), quercetin (**5**), syringaresinol-*β*-D-glucoside (**6**), 3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-2-[4-(3-hydroxy-1-(*E*)-propenyl)-2,6-dimethoxyphenoxy]propyl-*β*-D-glucopyranoside (**7**), and 5-(methoxymethyl)-1*H*-pyrrole-2-carbaldehyde (**8**) were isolated from the aerial part of *Vernonia gratiosa*. Their chemical structures were identified by NMR experiments along with the comparison of their spectroscopic data with those reported in the literature. In addition, all compounds were evaluated for their inhibitory effects on *α*-glucosidase and xanthine oxidase. Among them, only compound **7** showed the significant inhibition of *α*-glucosidase with an IC_{50} value of 47.08±3.98 µg/mL and xanthine oxidase activity with an IC_{50} value of 26.92±1.04 µg/mL. Compounds **1-5** were firstly isolated from *V. gratiosa*, while compounds **6-8** were reported from the genus for the first time. This is the first study about this species growing in Vietnam.

Keywords: *Vernonia gratiosa*, *α*-glucosidase, xanthine oxidase

1. INTRODUCTION

The genus *Vernonia* is a larger and more diverse genus of family Asteraceae with 1000 species that grow throughout the world as well as mainly in South America, North America, Africa, and Southeast Asia.[1] Many species have been widely used for centuries in local and traditional medicine. They have been found to treat diseases such as dysentery, fever, malaria, hepatitis, stomachache, eczema, snakebites, burns, etc.[1] With the increasing interest paid to the pharmacologically active phytochemicals from the *Vernonia* genus, a lot of studies related to the phytochemical and pharmacological aspects of this genus have been carried out. In recent decades, phytochemical studies were carried out on *V. amygdalina*, *V. anthelmintica*, *V. cinerea*, *V. patula* and that demonstrated the presence of different classes of biologically active compounds, such as steroids,[2-4] sesquiterpenes, [5-7] flavonoids,[8,9] polyphenols[9] etc. Pharmacological studies revealed that the crude extracts and purified compounds possess a wide spectrum of biological activities, involving anti-diabetic, anti-oxidant, anti-microbial, anti-nociceptive, anti-pyretic, and insecticidal activities.[1] *V. gratiosa*, known in Vietnam as Bach dau thuon, and the phytochemical and biological research on this plant has been still rare by now [10], and there is still a large space to explore the chemicals especially the minor composition and their biological properties of this plant. In this paper, we report the isolation and structural elucidation of eight compounds, including a sesquiterpene, four flavonoids, two lignans, and a furan. All the isolates (**1-8**) were also examined the inhibitory effects on *α*-glucosidase and xanthine oxidase.

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2. MATERIALS AND METHODS

2.1. General experimental procedures

^{1}H-(500 MHz), ^{13}C-(125 MHz) NMR, and 2D-NMR spectra were acquired with a Bruker Avance Digital 500 MHz NMR spectrometer (Karlsruhe, Germany) in ppm relative to tetramethylsilane (TMS) as an internal standard, *J* in Hz at 294 K. Thin-layer chromatography was performed using glass plates pre-coated with silica gel (60F254 and RP-18 F254s; Merck, Germany). Chromatography column (CC) was carried out on a Merck silica gel (60-200 µm) and Merck Lichroprep RP-18 gel (40-63 µm).

2.2. Plant material

The dried aerial part of *V. gratiosa* was collected from Quang Tri Province, Vietnam during April 2019, and identified by Dr. Tran Thi Phuong Anh, Vietnam National Museum of Nature Vietnam. The voucher specimen (VG-2020) was deposited at the Center for Research and Technology Transfer, Vietnam Academy of Science and Technology.

2.3. Extraction and isolation

The dried aerial part of *V. gratiosa* (5.0 kg) was extracted three times with hot methanol (4 h × 20 L). Combined extracts were filtered and concentrated by a rotary evaporator, yielding a crude extract. Subsequently, the crude extract (400.0 g) was suspended in distilled water (1.2 L) and partitioned with *n*-hexane, CH_2Cl_2, and EtOAc, successively. After concentration in *vacuo*, the crude extracts of the *n*-hexane fraction (70.0 g), CH_2Cl_2 fraction (40.0 g), EtOAc fraction (42.0 g), and water layer were obtained. The water layer was subjected to HP-20 diaion using a solvent system of MeOH: H_2O (0:1-1:0, v/v) to give four fractions (VGW1-VGW4). Fraction VGW2 (27.0 g) was further isolated on a silica gel column eluting with a gradient of CH_2Cl_2:MeOH (20:1-1:2, v/v) to obtain four fractions (VGW2A-VGW2D). Sub-fraction VGW2B (3.0 g) was submitted to RP-18 silica gel column eluting with MeOH: H_2O (1:2, v/v) to yield compounds **3** (7.0 mg) and **4** (15.0 mg). Fraction VGW4 (31.0 g) was chromatographed over silica gel chromatography column (CC) eluting with gradient CH_2Cl_2: MeOH (20:1-1:2, v/v), and then purified by a RP-18 column with MeOH: H_2O (1:2, v/v) to afford compound **7** (4.0 mg). The CH_2Cl_2 fraction (40.0 g) was subjected to silica gel column eluting with a gradient of CH_2Cl_2: MeOH (50:1-1/1, v/v) to give five fractions (VGD1-VGD5). Fraction VGD2 (2.8 g) was further chromatographed on an RP-18 column eluting with MeOH: H_2O (2:1, v/v) to yield two sub-fractions (VGD2A-VGD2B). Fraction VGD2A (1.2 g) was purified on an RP-18 column with a gradient of MeOH: H_2O (2:1, v/v) to give compounds **5** (22.8 mg) and **6** (3.9 mg). The EA extract (42.0 g) was chromatographed on a silica gel CC eluting with a gradient of CH_2Cl_2: MeOH (20:1-1:2, v/v) to obtain eight fractions (VGE1-VGE8). Fraction VGE3 (3.6 g) was submitted to RP-18 silica gel and then purified by Sephadex LH-20 column with a solvent system of MeOH: H_2O (1:1, v/v) to give compounds **1** (10.0 mg). Fraction VGE5 (1.6 g) was subjected to a silica gel column eluting with an isocratic of CH_2Cl_2: MeOH (15:1, v/v) to afford four fractions (VGE5A-VGE5D). Compound **8** (5.0 mg) was obtained from fraction VGE5B by Sephadex LH-20 column eluting with MeOH: H_2O (1:1, v/v). In the same condition, compound **2** was also isolated from fraction VGE5D (800.3 mg).

11*β*,13-dihydrovernolide (1): white crystals, the ^{1}H (CD_3OD, 600 MHz) and ^{13}C NMR data (CD_3OD, 150 MHz), see table 1.[11]

Apigenin (2): Yellow crystals; ^{1}H-NMR (500 MHz, CD_3OD): δ_H 6.61 (1H, s, H-3), 6.48 (1H, d, *J* = 1.8 Hz, H-6), 6.23 (1H, d, *J* = 1.8 Hz, H-8), 7.87 (2H, d, *J* = 9.0 Hz, H-2', H-6'), and 6.95 (2H, d, *J* = 9.0 Hz, H-3', H-5'). ^{13}C-NMR (125 MHz, CD_3OD): δ_C 183.9 (C-4), 166.3 (C-7), 166.1 (C-2), 163.2 (C-5), 162.8 (C-4'), 159.5 (C-9), 129.5 (C-2', C-6'), 123.3 (C-1'), 117.0 (C-3', C-5'), 105.3 (C-10), 103.9 (C-3), 100.2 (C-8), and 95.1 (C-6).[12]

Kaempferol (3): Yellow crystals; ^{1}H-NMR (500 MHz, DMSO): δ_H 7.94 (2H, d, *J* = 8.8 Hz, H-2', H-6'), 6.82 (2H, d, *J* = 8.8 Hz, H-3', H-5'), 6.34 (1H, d, *J* = 1.8 Hz, H-8), and 6.09 (1H, d, *J* = 1.8 Hz, H-6). ^{13}C-NMR (125 MHz, DMSO): δ_C 176.0 (C-4), 164.0 (C-7), 159.2 (C-4'), 156.2 (C-9), 146.9 (C-2), 135.7 (C-3), 129.6 (C-2', C-6'), 121.7 (C-1') 115.6 (C-3', C-5'), 103.1 (C-10), 98.3 (C-6), and 93.5 (C-8). [13]

Quercetin 3-*O*-methyl ether (4): Yellow powder; ^{1}H-NMR (500 MHz, CD_3OD): δ_H 7.64 (1H, d, *J* = 2.0 Hz, H-2'), 7.54 (1H, dd, *J* = 2.0, 8.5 Hz, H-6'), 6.92 (1H, d, *J* = 8.5 Hz, H-5'), 6.41 (1H, d, *J* = 2.0 Hz, H-8), 6.21 (1H, d, *J* = 1.5 Hz, H-6), and 3.80 (3H, s, 3-OCH_3). ^{13}C-NMR (125 MHz, CD_3OD): δ_C 180.0 (C-4), 165.9 (C-7), 163.1 (C-5), 158.4 (C-9), 158.0 (C-2), 149.9 (C-4'), 146.4 (C-3'), 139.5 (C-3), 123.0 (C-1'), 122.3 (C-6'), 116.5 (C-5'), 116.4 (C-2'), 105.9 (C-10), 99.7 (C-6), 94.7 (C-8), and 60.5 (OCH_3).[14]

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Quercetin (5): Yellow powder; ^{1}H-NMR (500 MHz, DMSO): δ_H 7.69 (1H, s, H-2'), 7.55 (1H, d, *J* = 7.5 Hz, H-6'), 6.90 (1H, d, *J* = 8.5 Hz, H-5'), 6.40 (1H, s, H-8), and 6.20 (1H, s, H-6). ^{13}C-NMR (125 MHz, DMSO): δ_C 175.9 (C-4), 163.9 (C-7), 160.8 (C-5), 156.2 (C-9), 146.8 (C-2), 147.6 (C-4'), 145.1 (C-3'), 135.8 (C-3), 122.0 (C-1'), 120.1 (C-6'), 115.6 (C-5'), 115.1 (C-2'), 103.1 (C-10), 98.2 (C-6), and 93.4 (C-8).[15]

Syringaresinol-*β*-D-glucoside (6): Amorphous powder; ^{1}H-NMR (500 MHz, CD_3OD): δ_H 6.74 (2H, s, H-2, H-6), 6.68 (2H, s, H-2', H-6'), 4.79 (1H, d, *J* = 4.5 Hz, H-7), 4.74 (1H, d, *J* = 4.5 Hz, H-7'), 3.16 (2H, m, H-8, H-8'), 3.94 (1H, m, H-9a), 4.31 (1H, m, H-9b), 3.94 (1H, m, H-9'a), 4.31 (1H, m, H-9'b), 4.88 (1H, d, *J* = 7.5 Hz, H-1''), 3.50 (1H, m, H-2''), 3.44 (1H, m, H-3''), 3.43 (1H, m, H-4''), 3.22 (1H, m, H-5''), 3.68 (1H, dd, *J* = 5.5, 12.0 Hz, H-6''b), 3.79 (1H, m, H-6''a), 3.88 (6H, s, 3-, 5 -OCH_3), and 3.86 (6H, s, 3'-, 5'-OCH_3). ^{13}C-NMR (125 MHz, CD_3OD): δ_C 154.4 (C-3, C-5), 149.4 (C-3', C-5'), 139.6 (C-1), 136.3 (C-4'), 135.7 (C-4), 133.1 (C-1'), 104.9 (C-2, C-6), 104.6 (C-2', C-6'), 87.6 (C-7'), 87.2 (C-7), 72.9 (C-9, C-9'), 105.4 (C-1''), 78.3 (C-5''), 77.8 (C-3''), 75.7 (C-2''), 71.4 (C-4''), 75.7 (C-2''), 62.6 (C-6''), 57.1 (3'-, 5'-OCH_3), 56.9 (3-, 5-OCH_3).[16]

3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-2-[4-(3-hydroxy-1-(*E*)-propenyl)-2,6-dimethoxy phenoxy]propyl-*β*-D-glucopyranoside (7): Amorphous powder; ^{1}H-NMR (500 MHz, CD_3OD): δ_H 7.11 (1H, d, *J* = 2.0 Hz, H-2), 6.95 (1H, dd, *J* = 2.0, 9.0 Hz, H-6), 6.79 (2H, s, H-2', H-6'), 6.78 (1H, d, *J* = 9.0 Hz, H-5), 6.58 (1H, d, *J* = 19.0 Hz, H-7'), 6.36 (1H, dt, *J* = 19.0, 6.6 Hz, H-8'), 5.16 (1H, d, *J* = 6.5 Hz, H-7), 4.33 (1H, m, H-8), 4.25 (2H, dd, *J* = 2.0, 6.6 Hz, H-9'), 3.62 (1H, m, H-9), 3.23 (1H, m, H-9), 4.61(1H, d, *J* = 7.5, Hz, H-1''), 3.76 (1H, dd, *J* = 3.0, 11.4 Hz, H-6''), 3.62 (1H, m, H-6''), 3.21-3.41 (5H, m, H-2''- H-5''), 3.90 (6H, s, 3'-, 5'-OCH_3), and 3.88 (3H, s, 3-OCH_3). ^{13}C-NMR (125 MHz, CD_3OD): δ_C 154.6 (C-3', C-5'), 148.6 (C-3), 147.2 (C-4), 136.2 (C-4'), 135.1 (C-1'), 131.9 (C-7'), 131.3 (C-1), 130.8 (C-8'), 121.4 (C-6), 115.7 (C-5), 112.6 (C-2), 105.2 (C-1''), 87.0 (C-8), 82.3 (C-7), 78.0 (C-5''), 77.8 (C-3''), 75.6 (C-2''), 71.4 (C-4''), 62.5 (C-6''), 63.6 (C-9'), 61.2 (C-9'), 56.7 (3'-, 5'-OCH_3), and 56.5 (3-OCH_3).[17]

5-(methoxymethyl)-1H-pyrrole-2-carbaldehyde (8): Pale brown oil; ^{1}H-NMR (500 MHz, $CDCl_3$): δ_H 9.47 (1H, s, CHO), 6.90 (1H, dd, *J* = 2.4, 3.6 Hz, H-3), 6.21 (1H, dd, *J* = 2.4, 3.0 Hz, H-4), 4.49 (2H, s, OCH_2), 3.40 (3H, s, OCH_3). ^{13}C-NMR (150 MHz, $CDCl_3$): δ_C 178.9 (CHO), 137.4 (C-5), 132.7 (C-2), 121.3 (C-3), 109.6 (C-4), 67.1 (OCH_2), and 58.5 (OCH_3). [18]

2.4. α-Glucosidase inhibitory assay

The *α*-glucosidase inhibition was evaluated using previously published procedure.[19] Briefly, the reaction mixture (60 µL) containing 100 µM photphate buffer (pH 6.8, 20 µL), *p*-NPG (2.5 mM, 20 µL), and the test compounds in DMSO 10 % were added to 96-well plates. And then, 20 µl of α-glucosidase [0.2 U/mL in 10 mM phosphate buffer (pH 6.8)] was applied to each well. The plates were mixtured and incubated at 37 ºC for 15 min, and the reaction was stopped by the addition of sodium carbonate solution (0.2 M, 80 µl). The *α*-glucosidase activity was determined spectrophotometrically at 405 nm on spectrophotometer UV-Vis. Acarbose was used as a positive control. [19]

2.5. Xanthone oxidase inhibitory assay

The XO inhibitory activity was measured by spectrophotometer in 96-well plates as described previously by Abu-Gharbieh *et al*. Briefly, dissolved the isolates (**1-8**) in DMSO then diluted with buffer. The test solution consisting of 50 mL of the sample of compounds, 35 mL of the phosphate buffer (pH 7.5), 30 mL of XO solution (0.01 IU/mL xanthine oxidase in phosphate buffer pH 7.5) was incubated at 25 °C for 5 min, then 60 µL of substrate solution (0.15 mM of xanthine in phosphate buffer pH 7.5) was added. The reaction was terminated by the addition of 25mL of HCl 1 mol/L and then the absorbance was measured at 290 nm on a Bio Tek Epochmicroplate spectrophotometer. Allopurinol was used as a standard.[20]

3. RESULTS AND DISCUSSION

Compound **1** was isolated as colorless crystals. The ^{1}H-NMR spectrum of **1** showed signals of three olefinic protons at δ_H 5.56 (1H, d, *J* = 10.2 Hz, H-5), 5.71 (1H, brs, H-19), and 6.14 (1H, brs, H-19). This spectrum was also displayed signals of four oxymethines at δ_H 2.86 (1H, dd, *J* = 4.8, 11.4 Hz, H-1), 5.33 (1H, t, *J* = 10.2, 19.8 Hz, H-6), 5.98 (1H, t, *J* = 6.2, 13.6 Hz, H-8), and 4.60 (1H, s, H-14); two oxygenated methylene protons at δ_H 3.74 (1H, d, *J* = 13.2 Hz, H-15), 4.56 (1H, d, *J* = 13.2 Hz, H-15); and six methyl protons at δ_H 1.30 (3H, d, *J* = 7.2 Hz, H-13) and 1.99 (3H, s, H-18). The ^{13}C-NMR spectrum exhibited twenty carbon resonances as assignable to a sesquiterpene skeleton bearing a methylacryloyl moiety. The signals at δ_C 180.5 (C-12) and 167.8 (C-

16) were attributed to two carbonyl groups. The ^{13}C-NMR spectrum of **1** also exhibited signals of ethylenic carbons at δ_C 144.4 (C-4), 130.8 (C-5), 137.8 (C-17), 126.9 (C-19), and an oxygenated quaternary carbon at δ_C 60.7 (C-10). The signals at δ_C 67.0 (C-1), (C-6), (C-8), (C-14) were ascribable to oxymethine carbons, that of the oxymethylene carbon was observed at δ_C 64.9 (C-15). The COSY correlations of H-1/H-2/H-4, H-5/H-6/H-7/H-8/H-9, and H-7/H-11/H-13 indicated the presence of three main spin systems in **1**, a CH-CH_2-CH_2 unit, a CH-CH-CH-CH-CH_2, and a CH-CH-CH_3, respectively. The HMBC correlation between H-8 and C-16 suggested the position of methylacryloyl moiety at C-8 (figure 2). The NOESY spectrum of **1** showed the correlation between H-9α and H-1/H-7, between H-7 and H-13 suggesting these protons had the same orientation as being α-side while β-orientation assignments of H-11, H-8, H-6 were deduced by the NOESY correlation from H-8 to H-6 and H-11 (figure 2). The 1D- and 2D-NMR spectra allowed for the assignment of the NMR spectra for 11β,13-dihydrovernolide (figure 1).

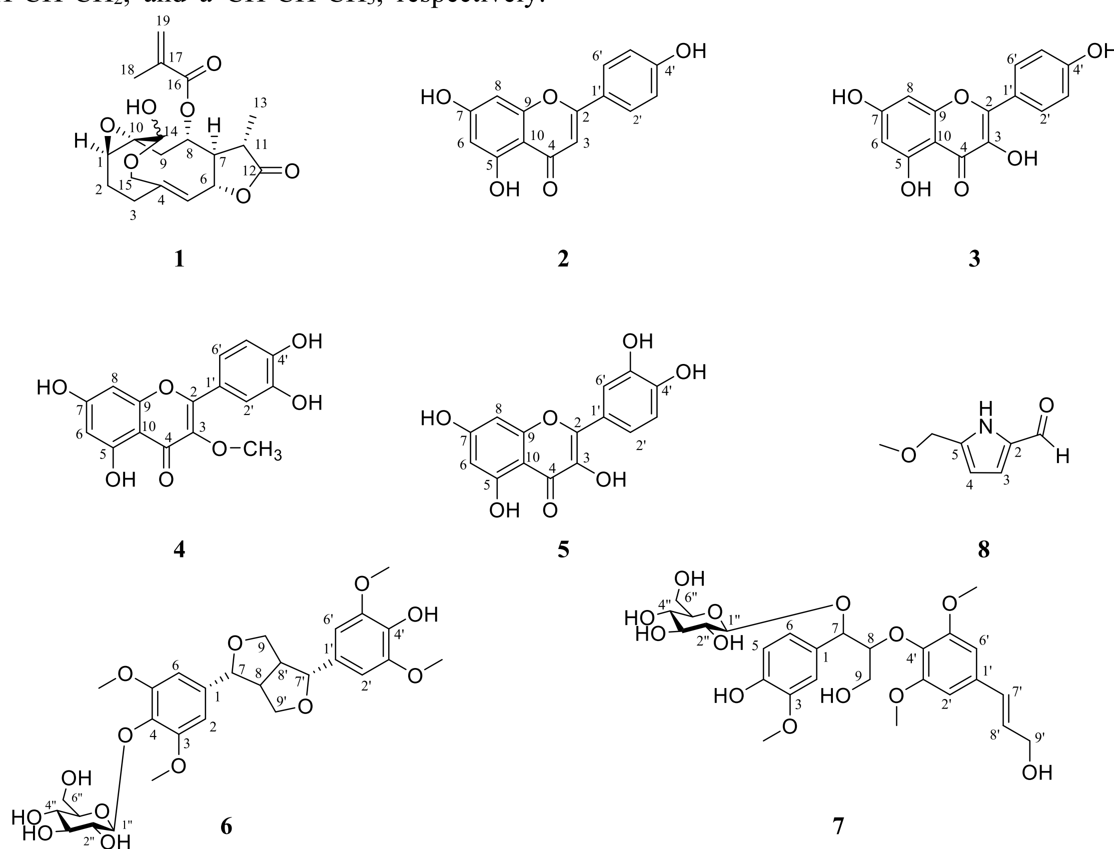

Figure 1: Chemical structures of compounds **1-8**

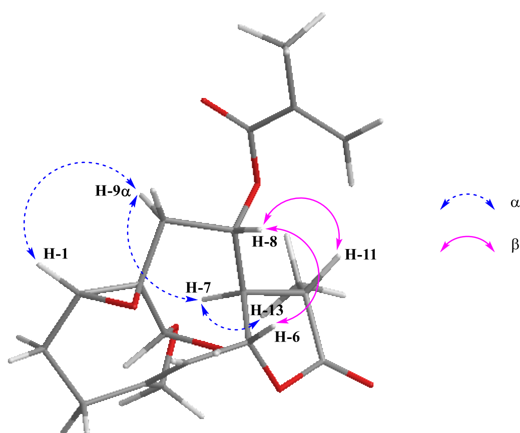

Figure 2: The key NOESY correlations of compound **1**

Compound **2** was isolated as a yellow crystal. The ^{1}H-NMR spectrum showed signals for six aromatic protons corresponding to the meta coupled protons at δ_H 6.48 (1H, d, J = 1.8 Hz, H-6) and 6.23 (1H, d, J = 1.8 Hz, H-8) on the A ring and an AABB coupling system at δ_H 7.87 (2H, d, J = 9.0 Hz, H-2', H-6'), 6.95 (2H, d, J = 9.0 Hz, H-3', H-5') on the B ring, and a singlet proton at δ_H 6.61 (1H, s, H-3) on ring C of the flavonoid skeleton. The ^{13}C-NMR spectrum of **2** displayed signals of 15 carbons, including a carbonyl group at δ_C 183.9 (C-4). These NMR spectroscopic data of **2** were in good agreement with those of apigenin. Thus, the chemical structure of **2** was elucidated as shown in figure 1.

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Table 1: NMR data of compound **1** in CD_3OD

Position	δ_H mult., (J in Hz)[a]	δ_C, type[b]	COSY	HMBC
1	2.86, dd (4.8, 11.4)	67.0, CH	H-2	C-2, C-10
2	1.75, m 2.23, m	24.0, CH_2	H-1, H-3	C-1, C-10, C-4, C-3
3	2.47, m 2.39, m	34.1, CH_2	H-2	C-1, C-2, C-4, C-5
4		144.4, C		
5	5.56, d (10.2)	130.8, CH	H-6	C-3, C-15,
6	5.33, t (10.2)	78.8, CH	H-5, H-7	C-7, C-8, C-5
7	2.35, m	58.0, CH	H-6, H-8, H-11	C-5, C-6, C-8, C-13
8	5.98, dd (1.8, 11.4)	72.9, CH	H-7, H-9	C-16, C-6
9	2.62, d (12.0) 1.34, d (12.0)	41.9, CH_2	H-8	C-7, C-8, C-10, C-1
10		60.7, C		
11	2.69, m	41.2, CH	H-13, H-7	C-13, C-12, C-7, C-8
12		180.5, C		
13	1.30, d (7.2)	16.9, CH_3	H-11	C-11, C-12, C-7
14	4.60, s	100.0, CH		C-1, C-10, C-9
15	3.74, d (13.2) 4.56, d (13.2)	64.9, CH_2		C-3, C-4, C-5, C-14
16		167.8, C		
17		137.8, C		
18	1.99, s	18.5, CH_3		C-16, C-17, C-19
19	6.14, s 5.71, s	126.9, CH_2		C-18, C-17, C-16

Recorded at [a)]600 MHz and [b)]150 MHz. [a,b)]Assigned by ^1H-^1H COSY (600 MHz), and HMBC (600/150 MHz).

Similarly, by analyzing the basis of the spectral and chemical data and comparing with those in the literature data, the structures of the compounds (**3-5**) were identified as kaempferol (**3**), quercetin 3-*O*-methyl ether (**4**), quercetin (**5**) (figure 1).

Compound **6** was obtained as a white powder. The ^{1}H-NMR spectrum of **6** showed the presence of four aromatic protons at δ_H 6.74 (2H, s, H-2, H-6), 6.68 (2H, s, H-2', H-6') together with two methine protons at δ_H 3.16 (2H, m, H-8, H-8'), two oxygenated methylene at δ_H 3.94 (1H, m, H-9a), 4.31 (1H, m, H-9b), 3.94 (1H, m, H-9'a), 4.31 (1H, m, H-9'b), and four methoxy groups at δ_H 3.88 (6H, s, 3-, 5-OCH_3), 3.86 (6H, s, 3'-, 5'-OCH_3), and two benzylic oxymethine at δ_H 4.79 (1H, d, J = 4.5 Hz, H-7), and 4.74 (1H, d, J = 4.5 Hz, H-7'). In addition, an anomeric proton at δ_H 4.88 (1H, d, J = 7.5 Hz, H-1'') with a large coupling constant (J = 7.5 Hz) was observed in the ^{1}H-NMR spectrum, suggesting the presence of β-glucopyranosyl moiety. The ^{13}C-NMR spectrum of **6** exhibited signals of twenty-one carbons, including 12 aromatic carbons, two oxygenated methylenes, four methines, and six carbons of the sugar moiety. Based on the above evidence, compound **6** was in good agreement with syringaresinol-β-D-glucoside.

Compound **7** was isolated as an amorphous powder. The ^{1}H- and ^{13}C-NMR data of compound **7** exhibited an 8-*O*-4' type neolignan glycoside comprised of two phenylpropanoid units. The ^{1}H-NMR spectrum of **7** displayed signals of a 1,3,4-trisubstituted benzene ring at δ_H 7.11 (1H, d, J = 2.0 Hz, H-2), 6.95 (1H, dd, J = 2.0, 9.0 Hz, H-6), 6.78 (1H, d, J = 9.0 Hz, H-5), a 1,3,4,5-tetrasubstituted benzene ring at δ_H 6.79 (2H, s, H-2', H-6'), a *trans* double bond at δ_H 6.36 (1H, dt, J = 5.5, 16.0 Hz, H-8'), and 6.58 (1H, brd, J = 5.5 Hz, H-7'), two oxymethines at δ_H 4.33 (1H, m, H-8), and 5.16 (1H, d, J = 6.5 Hz, H-7), two oxymethylenes at δ_H 3.76 (1H, dd, J = 3.0, 11.4 Hz, H-6''), 3.62 (1H, m, H-6''), and 4.25 (2H, dd, J = 2.0, 6.6 Hz, H-9'), and three methoxy groups at δ_H 3.89 (3H, s, 3-OCH_3), 3.90 (6H, s, 3'-, 5'-OCH_3). In addition, a glucopyranosyl moiety was assigned from the signal of anomeric proton at δ_H 4.61(1H, d, J = 7.5, Hz, H-1'') in the ^{1}H-NMR spectrum together with a set of characteristic signals

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at δ_C 105.2 (C-1″), 78.0 (C-5″), 77.8 (C-3″), 75.6 (C-2″), and 62.5 (C-6″) in the ^{13}C-NMR spectrum. Furthermore, the large coupling constant J = 7.5 Hz of an anomeric proton indicated the β-form of glucopyranosyl moiety. The location of this sugar moiety at C-7 was deduced by the HMBC correlation of H-1" and C-7 (figure 1). By comparison of the NMR data of compound **7** with those reported in the literature,[17] the structure of **7** was identified as 3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-2-[4-(3-hydroxy-1-(*E*)-propenyl)-2,6-dimethoxyphenoxy] propyl-β-D-glucopyranoside.

Compound **8** was obtained as pale brown oil. The ^{1}H-NMR spectrum exhibited one aldehyde proton at δ_H 9.47 (1H, s, CHO), two aromatic protons at δ_H 6.90 (1H, dd, J = 2.4, 3.6 Hz, H-3), and 6.21 (1H, dd, J = 2.4, 3.0 Hz, H-4), one oxygenated methylene at δ_H 4.49 (2H, s, OCH_2), a methoxy group at δ_H 3.40 (3H, s, OCH_3). The ^{13}C-NMR spectrum showed signals of seven carbons, including an aldehyde carbon at δ_C 178.9 (CHO), four aromatic carbons at δ_C 137.4 (C-5), 132.7 (C-2), 121.3 (C-3), and 109.6 (C-4), oxymethylene at δ_C 67.1 (OCH_2), a methoxy at δ_C 58.5 (OCH_3), which suggested that a 2,5-disubstituted pyrrole ring. The ^{1}H- and ^{13}C-NMR data were in good agreement with those of 5-(hydroxymethyl)-1H-pyrrole-2-carbaldehyde. Thus, the structure of **8** was determined as 5-(methoxymethyl)-1*H*-pyrrole-2-carbaldehyde.

Compound	IC_{50} (μg/mL)	
	α-	Xanthine
1	> 500	> 500
2	> 500	> 500
3	> 500	> 500
4	> 500	> 500
5	> 500	> 500
6	> 500	> 500
7	47.08±3.98	26.92±1.04
8	> 500	> 500
Allopurinol	-	1.12±0.15
Acarbose	146.64±8.85	-

α-Glucosidase inhibitors increase carbohydrates digestion time and thus decrease the rate of carbohydrate absorption by competitively blocking the activity of glucosidase. As a result, the peak concentration of postprandial blood glucose is reduced and the blood sugar level comes under control.[21] Whereas, xanthine oxidase is a critical enzyme that catalyzes hypoxanthine to xanthine then to uric acid in the purine metabolic pathway. Hyperuricemia caused by the high uric acid level in the blood leads to gout and cardiovascular diseases.[22] In this study, compounds (**1-8**) were evaluated for their inhibitory activities on α-glucosidase and xanthine oxidase. Compound **7** showed effective inhibition with IC_{50} of 47.08±3.98 µg/mL. The positive control, acarbose, showed enzyme inhibitory activity with IC_{50} of 146.64±8.85 µg/mL. In addition, compounds **7** also showed inhibition toward xanthine oxidase with IC_{50} of 26.92±1.04 µg/mL, whereas the positive control, allopurinol, inhibited xanthine oxidase activity with IC_{50} of 1.12±0.15 µg/mL.

4. CONCLUSION

This study detailed the chemical structure of eight compounds from *V. gratiosa*, intensive spectroscopy analyses, and comparison with those reported in the literature. Compounds **1-5** were firstly isolated from *V. gratiosa*, while compounds **6-8** were reported from the genus for the first time. Furthermore, only compound **7** showed the inhibitory effect on both α-glucosidase and xanthine oxidase with IC_{50} values 47.08±3.98 µg/mL, 26.92±1.04 µg/mL, respectively, and the other compounds did not show significant effects.

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