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Research article

Chemical constituents and the inhibition of α-glucosidase of *Gynura procumbens* (Lour.) Merr.

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ABSTRACT

Introduction: *Gynura procumbens* (Lour.) Merr. (Family: Asteraceae) is mainly popular in South-East Asian countries for its traditional medicinal properties. It is usually used as a traditional medicine for the treatment of eruptive fevers, rash, kidney disease, migraines, constipation, hypertension, diabetes mellitus, and cancer. It is commonly used as a traditional medicine in Vietnam for the treatment of many diseases. **Methods:** The leaves and trunks of *G. procumbens* were collected, macerated with methanol. The extracts from MeOH-soluble extract were processed by the column chromatographic technique to give pure compounds, and the nuclear magnetic resonance methods were applied to determine their chemical structures. The inhibitory activities of these extracts against α-glucosidase were conducted and compared with acarbose. **Results:** Seven organic compounds were isolated and determined the structures, including syringic acid **(1)**, quercetin **(2)**, *N,N*-dimethylanthranilic acid **(3)**, dehydrovomifoliol **(4)**, β-sitosterol 3-*O*-β-D-glucopyranoside **(5)**, schottenol **(6)**, montanic acid **(7)**. The inhibition of α-glucosidase test results in the IC_{50} values of the four extracts, which were lower than those of acarbose. **Conclusion:** Seven pure compounds were identified from the leaves and trunks of *G. procumbens,* including two compounds being isolated from *G. procumbens* for the first time. The test results showed that the parts of *G. procumbens* were active as α-glucosidase inhibitor, which would be useful to support the treatment for diabetes.

Key words: Gynura procumbens, syringic acid, quercetin, α-glucosidase

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INTRODUCTION

Gynura is the genus of the Asteraceae family, includes 20 species spread all over the world, particularly in Vietnam, China, Malaysia, Thailand, Indonesia, Korea, and the Philippines. *G. procumbens* (Lour.) Merr. (**Figure 1**) is a herbal material widely used in tropical countries for the treatment of various health ailments such as cancers, lymphatic pain, hypertension, skin diseases, diabetes mellitus…)[1,2]. Nowadays, people in various tropical regions consume an increasing amount of *G. procumbens* leaves in diet and tea. Research shows that the leaves do not have any toxicity[2]. Pharmacologic studies have reported that *G. procumbens* has antioxidant, anti-Herpes simplex, anti-hyperglycemic, anti-hyperlipidemic, anti-inflammatory, analgesic, and reducing blood hypertension properties. The health benefits of *G. procumbens* are related to some of its bioactive compounds, such as flavonoids, saponins, and alkaloids[3]. However, there were not many studies about the chemicals constituent of this plant, especially in Vietnam. This study aimed to investigate the chemical constituents from the leaves and trunks of *G. procumbens*, growing in Gia Lai Province, Vietnam. By column chromatographic and spectroscopic methods seven compounds (**1**-**7**) (**Figure 2**) were identified. Besides, we also tested the inhibitory activity of α-glucosidase on four extracts of this plant.

METHODS

Chemicals and equipment

Column chromatography was performed on silica gel (HiMedia) (230-400 Mesh). Thin-layer chromatography (TLC) and preparative TLC were performed on silica gel GF_{254} (Merck), visualized by hot 10 % solution of H_2SO_4. NMR spectra were acquired on Bruker 500 Avance III at 500 MHz for ^1H-NMR and 125 MHz for ^{13}C-NMR spectra.

The pure solvents methanol, ethyl acetate, *n*-butanol, petroleum ether, chloroform were from Chemsol Vina, Vietnam.

Acarbose, an α-glucosidase inhibitor, was from Chem Cruz, Santa Cruz Biotechnology, Inc., USA.

Plant material

The leaves and trunks of *G. procumbens* were collected at Gia Lai province, Vietnam, in July 2016 and authenticated by Dr. Dang Van Son, Department of Biolog-

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Figure 1: ***Gynura procumbens*** **(Lour.) Merr.**

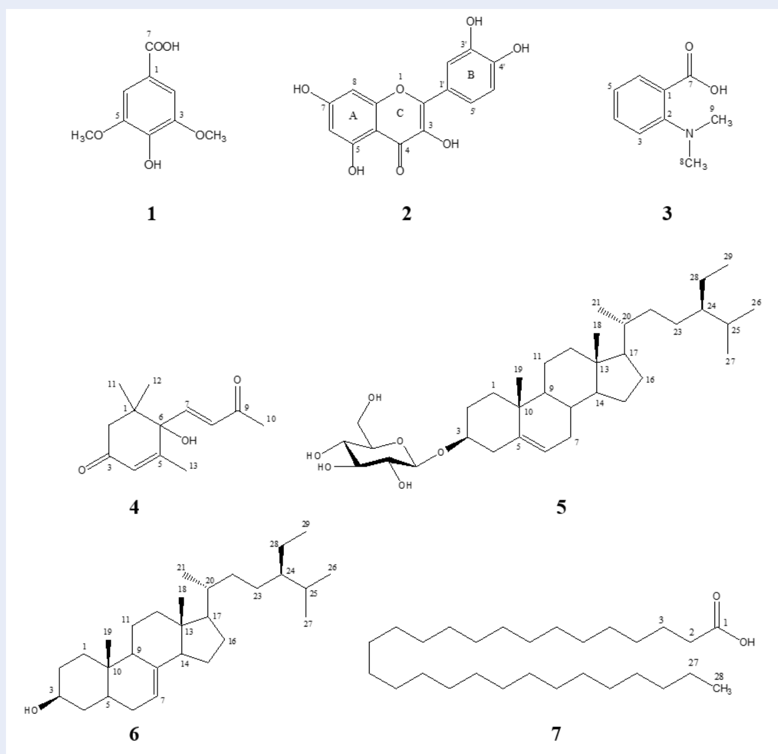

Figure 2: **Chemical structures for 1-7**.

ical Resources, Institute of Tropical Biology – Ho Chi Minh City, Vietnam.

Extraction and isolation

Dried leaves and trunks were ground into powder (2.9 kg) and extracted with hot methanol (4 × 7 L) for four hours each time. The methanolic filtrate was then evaporated to dryness under reduced pressure to obtain a methanolic residue (375.0 g). The methanolic residue was then dissolved in aqueous methanol (10 % methanol) and extracted with petroleum ether (10 x 500 mL), ethyl acetate (10 x 500 mL), *n*-butanol (10 x 500 mL), consecutively, to afford petroleum ether (PE, 75.0 g), ethyl acetate extract (EA, 7.2 g), *n*-butanol (Bu, 9.9 g) and crystal compound (102.9 g). The ethyl acetate extract (EA, 7.2 g) was subjected to a silica gel column chromatography and eluted with petroleum ether–ethyl acetate (stepwise, 9:1 → 0:10) followed by ethyl acetate–methanol (stepwise, 8:2 → 6:4) to afford five main fractions EA1 (26.1 mg), EA2 (498.1 mg), EA3 (696.2 mg), EA4 (1200.6 mg), EA5 (627.9 mg). Fraction EA1 (26.1 mg) was washed and cleaned with methanol (MeOH) to give compound **1** (9.0 mg). Fraction EA5 (1200.6 mg) was subjected to a silica gel column chromatography, eluted with chloroform–methanol ($CHCl_3$–MeOH) (stepwise, 99:1 → 9:1) to give compound **3** (11.0 mg). Fraction EA3 (627.9 mg) was subjected to column chromatographic separation over silica gel and eluted with $CHCl_3$–MeOH (stepwise, 99:1 → 9:1) to give compound **2** (4.4 mg). The same manner was applied on the EA2 (498.1 mg), eluted with $CHCl_3$–MeOH (95:5) to give compound **4** (4.5 mg). Fraction EA4 (696.2 mg) was fractionated by a silica gel column chromatography using $CHCl_3$–MeOH (stepwise, 95:5 → 8:2) to give compound **5** (4.5 mg). The petroleum ether extract (PE, 75.0 g) was subjected to a silica gel column chromatography and eluted with petroleum ether–ethyl acetate (PE–EA) (stepwise, 9:1 → 0:10) to afford fractions, in these, there were two fractions which were coded as PE1 (1294.7 mg), PE2 (2851.0 mg). By subjecting to a silica gel column chromatographic and eluting with appropriate solvents, fraction PE1 gave compound **6**, PE2 gave compound **7**.

Compound **1** (syringic acid): white needle-shaped crystals, ^{1}H- and ^{13}C-NMR (**Table 1**).

Compound **2** (quercetin): yellow powder, ^{1}H- and ^{13}C-NMR (**Table 1**).

Compound **3** (*N,N*-dimethylanthranilic acid): white powder, ^{1}H- and ^{13}C-NMR (**Table 1**), ESI/MS *m/z* 188.0723 $[M+Na]^+$.

Compound **4** (dehydrovomifoliol): white crystals, ^{1}H- and ^{13}C-NMR (**Table 1**).

Compound **5** (β-sitosterol 3-*O*-β-D-glucopyranoside): white powder, ^{1}H-NMR (pyridine-d_5) δ_H(ppm): 3.93 (1H, *m*, H-3),5.34 (1H, *m*, H-6), 0.65 (3H, *s*, H-18), 0.92 (3H, *s*, H-19), 0.98 (3H, *d*, *J* = 6.5, H-21), 0.85 (3H, *d*, *J* = 7.0, H-26), 0.87 (3H, *d*, *J* = 7.0, H-27), 0.88 (3H, *t*, *J* = 7.0, H-29) ^{13}C-NMR (pyridine-d_5) δ_C (ppm): 37.7 (C-1), 30.4 (C-2), 78.7 (C-3), 40.1 (C-4), 141.1 (C-5), 122.1 (C-6), 32.4 (C-7), 32.3 (C-8), 50.5 (C-9), 37.1 (C-10), 21.5 (C-11), 39.5 (C-12), 42.7 (C-13), 57.0 (C-14), 24.7 (C-15), 28.7 (C-16), 56.4 (C-17), 12.2 (C-18), 19.6 (C-19), 36.6 (C-20), 19.2 (C-21), 34.4 (C-22), 26.6 (C-23), 46.2 (C-24), 29.7 (C-25), 19.4 (C-26), 20.2 (C-27), 23.6 (C-28), 12.4 (C29), 102.7 (C-1'), 75.5 (C-2'), 78.6 (C-3'), 71.9 (C-4'), 78.4 (C-5'), 63.0 (C-6').

Compound **6** (schottenol): white crystals, ^{1}H-NMR ($CDCl_3$) δ_H(ppm): 3.60 (1H, *m*, H-3), 5.18 (1H, *m*, *J* = 4.6 Hz, H-7), 0.55 (3H, *s*, C-18), 0.80 (3H, *s*, H-19), 0.83 (3H, *d*, H-26), 0.85 (3H, *d*, H-27), 0.98 (3H, *d*, *J*=7.0 Hz, H-21), 0.86 (3H, *d*, H-29); ^{13}C-NMR ($CDCl_3$) δ_C (ppm): 37.16 (C-1), 31.48 (C-2), 71.09 (C-3), 37.99 (C-4), 40.28 (C-5), 29.66 (C-6), 117.42 (C-7), 139.69 (C-8), 49.48 (C-9), 22.97 (C-11), 39.59 (C-12), 55.06 (C-14), 23.10 (C-15), 27.95 (C-16), 56.12 (C-17), 11.84 (C-18), 13.03 (C-19), 36.59 (C-20), 21.56 (C-21), 34.22 (C-22), 26.25 (C-23), 45.88 (C-24), 29.21 (C-25), 18.91 (C-26), 19.05 (C-27), 19.81 (C-28), 11.97 (C-29).

Compound **7** (montanic acid): white crystals, HR-ESI-MS *m/z* 423.4234 $[M-H]^-$, ^{1}H-NMR ($CDCl_3$) δ_H (ppm): 2.35 (2H, *t*, *J*=7.5 Hz, H-2), 1.63 (2H, *quint*, H-3), 1.25 (2nH, *s*), 0.88 (3H, *t*, *J* =6.9 Hz); ^{13}C-NMR ($CDCl_3$) δ_C (ppm): 178.85 (C-1), 33.84 (C-2), 31.92 (C-3), 29.06-29.69 (C-4 to C-26), 24.70 (C-27), 22.68 (C-28), 14.10 (C-29).

Test of inhibition of α-glucosidase

Test of inhibition of α-glucosidase was performed at Research Center Of Ginseng & Materia Medica, Ho Chi Minh City on four extracts methanol (GP - Me), ethyl acetate (GP - EA), *n*-butanol (GP - Bu) and petroleum ether (GP - PE). The inhibitory activity of α-glucosidase was determined by the previous method[4] with some adjustments. Samples were dissolved in the DMSO solvent. A mixture of 60 μL of sample and 50 μL of phosphate buffer 0.1 M (pH 6.8) containing α-glucosidase solution (0.2 U.mL^{-1}) was incubated in the wells of 96-well plates at 37 °C for 10 minutes. After incubating, added 50 μL of *p*-nitrophenyl-α-D-glucopyranoside (*p*-NPG) prepared in phosphate buffer 0.1 M (pH 6.8) into each

well and the wells were continuously incubated for 20 minutes. OD was measured on the spectrophotometer at 405 nm with a microdisk reader (Bio Tek, USA) and compared it with a control sample containing a 60 μL buffer solution in place of the test sample. The test result data was expressed by the average of triplicated experiments.

The IC_{50} value is the concentration of the extract required to inhibit 50 % of α-glucosidase activity under the assay conditions. Acarbose was used as a positive control.

RESULTS

The powdered leaves and trunks of *G. procumbens* were extracted with hot methanol. The MeOH-soluble extract was successively partitioned to yield petroleum ether, ethyl acetate, and n-butanol-soluble fractions. By using column chromatographic technique and the nuclear magnetic resonance methods, seven organic compounds were isolated and determined to be syringic acid (**1**), quercetin (**2**), N,N-dimethylanthranilic acid (**3**), dehydrovomifoliol (**4**), β-sitosterol 3-O-β-D-glucopyranoside (**5**), schottenol (**6**), montanic acid (**7**). In these, two compounds (**3**), (**4**) were isolated from G. procumbens for the first time.

Compound 1 (**Figure 2**) was obtained as white needle-shaped crystals, completely soluble in MeOH, acetone, $CHCl_3$. The ^{1}H-NMR spectrum of compound 1 showed the resonance signal of eight protons, including six protons of the two methoxyl groups at δ_H3.88 (6H, *s*) and two cumulative protons at δ_H 7.33 (2H, *s*, H-6). It showed that compound 1 contains 1, 3, 4, 5 four-substituted aromatic nucleus. The ^{13}C-NMR spectrum of compound **1** has six carbon signals. There is a carbonyl carbon signal of the carboxyl group at δ_C 167.5 (C-7), carbon signals of the two methoxyl groups at δ_C 56.7 (3-OCH_3, 5-OCH_3) and the six carbons of the benzene ring, composed of tertiary carbons at δ_C 148.4 (C-3, C-5); 141.6 (C-4); 121.5 (C-1) and methine carbons at δ_C 108.2 (C-2, C-6). The HMBC spectra of compound **1** showed that the proton signal of the methoxyl group δ_H 3.88 (6H, s) correlated to the signal at δ_C 148.4 (C-3, C-5) of a oxygen-carrying carbon. Therefore, two methoxyl groups bind to the C-3 and C-5 positions of the benzene ring. In addition, HMBC spectrum of **1** also showed a correlation of the proton signal at δ_H 7.33 (2H, *s*, H-2, H-6) to the signals at δ_C 148.4 (C-3, C-5), 141.6 (C-4); 121.5 (C-1); 108.2 (C-2, C-6); 167.5 (C-7). Comparing the spectral data of compound 1 with syringic acid[5] gave the similarities. These above facts showed that compound **1** was syringic acid.

Compound 2 (**Figure 2**) was obtained as a yellow powder, completely soluble in DMSO. The ^{1}H-NMR spectrum displayed five aromatic protons at δ_H 6.16 (1H, *d*, *J* = 1.5 Hz, H-6), 6.39 (1H, *d*, *J* = 1.5 Hz, H-8), 7.86 (1H, *dd*, *J*1 = 8.5 Hz, *J*2= 2.5 Hz, H-6'), 6.86 (1H, *d*, *J* = 8.5, H-5'), 7.64 (1H, *d*, *J* = 2.0 Hz, H-2'), of which H-6 grafted meta with H-8, H-6' grafted ortho with H-5' and grafted meta with H-2'. Therefore, compound 2 contains two benzene rings, in that, H-6 and H-8 were in the first ring, H-2' and H-6' were in the second ring. One signal at δ_H 12.44 (1H, *s*, 5-OH) indicated a proton which made intramolecular hydrogen bonding with a carbonyl group at δ_C 147.7 (C-4). In 9.0 to 13.0 ppm region, there were signals characterized hydroxyl protons at δ_H 10.75, 9.49, 9.28. The ^{13}C-NMR spectrum showed fifteen carbon signals. The signal at δ_C 175.7 (C-4) displayed a carbonyl carbon. In the low-field magnetic resonance, there were seven signals of aromatic carbons which linked to oxygen at δ_C146.8 (C-2), 135.5 (C-3), 160.6 (C-5), 163.8 (C-7), 155.9 (C-9), 144.9 (C-3'), 147.7 (C-4). The carbon signals were attributed to the first ring at δ_C102.87 (C-10), 98.2 (C-6), 93.3 (C-8) and to the second ring at δ_C 121.9 (C-1'), 114.9 (C-2'), 115.6 (C-5'), 119.9 (C-6'). Comparing the spectral data of compound **2** with quercetin[6] gave the similarities. These above facts showed that compound **2** was quercetin.

Compound 3 (**Figure 2**) was obtained as a white powder, completely soluble in acetone. HR-ESI-MS of compound **3** exhibited an ion peak at *m/z* 188.0723 $[M+Na]^+$, consistent with a molecular formula of C9H11NO2. The ^{1}H-NMR spectrum showed four aromatic protons at δ_H7.72 (1H, *dd*, *J*1= 8.0 Hz, *J*2 = 0.8 Hz, H-3),7.41 (1H, *td*, *J*1= 7.9 Hz, *J*2 = 1.2 Hz, H-4), 7.66 (1H, *td*, *J*1 = 7.3 Hz, *J*2 = 1.6 Hz, H-5), 8.12 (1H, *dd*, *J*1 = 7.5 Hz, *J*2 = 1.5 Hz, H-6). The signal at δ_H 2.85 (6H, *s*) showed protons of two methyl groups linked with nitrogen. The ^{13}C-NMR spectrum exhibited eight carbon signals, of which six signals at δ_C 126.3 (C-1), 153.4 (C-2), 123.5 (C-3), 134.8 (C-4), 128.1 (C-5), 132.2 (C-6) were attributed to the aromatic ring, whereas a signal at δ_C 45.7 (C-8, C-9) characterized as two methyl groups linked with nitrogen and a signal at δC 167.2 (C-7) displayed a carbonyl carbon. The HMBC spectrum of compound 3 showed that the proton at δ_H 7.72 (1H, *dd*, H-3) correlated with signals at δ_C 128.1 (C-5); the proton at δ_H 7.41 (1H, *td*, H-4) correlated with signals at δ_C 126.3 (C-1), 123.5 (C-3); the proton at δH 7.66 (1H, *td*, H-5) correlated with signals at δC 153.4 (C-2), 132.2 (C-6); the signal at δH 8.12 (1H, dd, H-6) correlated with signals at δ_C 134.8 (C-4), 153.4 (C-2), 167.2

(C-7); the signal of protons at δ_H 2.85 (6H, *s*) correlated with the signal at δ_C 153.4 (C-2) and 45.7 (C-8, C-9). By analyzing the ^{1}H-NMR, ^{13}C-NMR, MS, HMBC spectral data and comparing the spectral data of compound 3 with reference[7], the structure of compound 3 was given as *N,N*-dimethylanthranilic acid.

Compound 4 (**Figure 2**) was obtained as white crystals, completely soluble in methanol, acetone. The ^{1}H-NMR spectrum gave nine proton signals, which included two olefin protons grafted trans at δ_H 7.04 (1H, d, 16 Hz, H-7) and 6.49 (1H, *d*, 16 Hz, H-8); one olefin proton at 5.98 (1H, *s*, H-4); two methylene protons at δ_H 2.29 (1H, *d*, 17 Hz, H-2), 2.58 (1H, *d*, 17 Hz, H-2); four proton signals of methyl group at δ_H 2.35 (3H, *s*, H-10), 1.12 (3H, *s*, H-11), 1.07 (3H, *s*, H-12), 1.95 (3H, *s*, H-13). The ^{13}C-NMR spectrum showed thirteen carbon signals. Two signals at δ_C 200.3 (C-3), 203.6 (C-9) characterized two carbonyl carbons; one quaternary olefin carbon at δ_C164.6 (C-5); three tertiary olefin carbons at δ_C 128.0 (C-4), 131.7 (C-8), 148.3 (C-7); two quaternary carbons at δ_C 80.0 (C-6), 42.6 (C-1); one methylene carbon at 50.6 (C-2) and four methyl carbons at δ_C 27.6 (C-10), 23.5 (C-11), 24.7 (C-12), 19.1 (C-13). By analyzing the ^{1}H-NMR and ^{13}C-NMR spectral data and comparing the spectral data of compound 4 with reference[8], the structure of compound **4** was given as dehydrovomifoliol.

Compound 5 (**Figure 2**) was obtained as a white powder, completely soluble in DMSO. The ^{1}H-NMR spectral data of **5** showed the present of six methyl groups at δH 0.65 (3H, *s*, H-18), 0.92 (3H, *s*, H-19), 0.98 (3H, *d*, *J* = 6.5, H-21), 0.85 (3H, *d*, *J* = 7.0, H-26), 0.87 (3H, *d*, *J* = 7.0, H-27), 0.88 (3H, *t*, *J* = 7.0, H-29). The signal at δ_H 3.93 (1H, *m*, H-3) appeared as multilet displayed proton H-3. A signal at δ_H 5.34 (1H, *m*, H-6) was the characteristics of double bond between quaternary carbon and methine carbon in the ring B. The ^{13}C-NMR spectrum showed compound 5 has 35 carbon signals. The signals at δ_C 12.2 (C-18), 19.6 (C-19), 19.2 (C-21), 19.4 (C-26), 20.2 (C-27), 12.4 (C29) were methyl carbons. Methylene carbons appeared at δ_C 37.7 (C-1), 30.4 (C-2), 40.1 (C-4), 32.4 (C-7), 21.5 (C-11), 39.5 (C-12), 24.7 (C-15), 28.7 (C-16), 34.4 (C-22), 26.6 (C-23), 23.6 (C-28). Methine carbons were at δ_C78.7 (C-3), 122.1 (C-6), 32.3 (C-8), 50.5 (C-9), 57.0 (C-14), 56.4 (C-17), 36.6 (C-20), 46.2 (C-24), 29.7 (C-25). Quaternary carbons appeared at δ_C 141.1 (C-5), 37.1 (C-10), 42.7 (C-13). Furthermore, the ^{1}H-NMR and ^{13}C-NMR spectral date of compound 5 displayed the present of a glucose unit. A signal among them appeared at δC 102.7 (C-1') presented anomeric carbon. Besides, the signal of methylene carbon C-6' appeared at δ_C 63.0 and the other four methine carbons, which linked to oxygen, appeared at δ_C75.5 (C-2'), 78.6 (C-3'), 71.9 (C-4'), 78.4 (C-5'). Comparing the spectral data of compound 5 with β-sitosterol 3-O-β-D-glucopyranoside[9] gave the similarities. These above facts indicated that compound 5 was β-sitosterol 3-O-β-D-glucopyranoside.

Compound 6 (**Figure 2**) was obtained as white crystals, completely soluble in chloroform. The ^{1}H-NMR spectrum gave an olefin proton at δ_H 5.18 (1H, *m*, *J* = 4.6 Hz, H-7) and one methyl proton at δ_H 3.60 (1H, *m*, H-3). In the high-field magnetic resonance, there were six signals characterized methyl protons including one methyl group grafted with secondary carbon at δ_H 0.86 (3H, *d*, H-29), three methyl groups grafted with tertiary carbons at δ_H 0.83 (3H, *d*, H-26), 0.85 (3H, *d*, H-27), 0.98 (3H, *d*, *J*=7.0 Hz, H-21), and two methyl groups grafted with quaternary carbons at δ_H 0.55 (3H, *s*, C-18), 0.80 (3H, *s*, H-19). The ^{13}C-NMR spectrum showed compound 6 has 29 carbon signals. In the low-field magnetic resonance, there were two signals of olefin carbons at δ_C 139.69 (C-8), δ_C 117.42 (C-7). Methyl carbon appeared at δ_C 71.09 (C-3). Two signals at δ_C 33.92, 43.41 characterized quaternary carbons C-10 and C-13. Seven methine carbons appeared at δ_C 40.28 (C-5), 49.48 (C-9), 55.06 (C-14), 56.12 (C-17), 36.59 (C-20), 45.88 (C-24), 29.21 (C-25). Eleven methylene carbons were at δ_C 37.16 (C-1), 31.48 (C-2), 37.99 (C-4), 29.66 (C-6), 22.97 (C-11), 39.59 (C-12), 23.10 (C-15), 27.95 (C-16), 34.22 (C-22), 26.25 (C-23), 19.81 (C-28). Six methyl carbons appeared at δ_C 11.84 (C-18), 13.03 (C-19), 21.56 (C-21), 18.91 (C-26), 19.05 (C-27), 11.97 (C-29). By analyzing the ^{1}H-NMR and ^{13}C-NMR spectral data and comparing the spectral data of compound 6 with reference[10], the structure of compound 6 was given as schottenol.

Compound 7 (**Figure 2**) was obtained as white crystals, completely soluble in chloroform. HR-ESI-MS of compound **7** exhibited an ion peak at *m/z* 423.4234 $[M-H]^-$, consistent with a molecular formula of $C_{28}H_{56}O_2$. The ^{1}H-NMR spectrum showed a signal of two methylene protons grafted with a carbonyl group at δ_H 2.35 (2H, *t*, *J*=7.5 Hz, H-2), a signal of two methylene protons defined H-3 at δ_H 1.63 (2H, *quint*, H-3). Furthermore, at δ_H 1.25 (2nH, *s*) there was a signal of accumulable protons of methylene groups in the saturated carbon chain. A signal at δ_H 0.88 (3H, *t*, *J* =6.9 Hz) characterized methyl protons. The ^{13}C-NMR and DEPT-NMR spectrum showed a carbonyl carbon signal at δ_C178.85, a carbon grafted with a carbonyl group δ_C 33.84, a methylene carbon separated carbonyl group by a carbon

at δ_C 31.92, a methyl carbon at δ_C 14.10, a methylene carbon grafted with methyl carbon at δ_C 22.68, a methylene carbon separated methyl group by a carbon at δ_C 24.70. Moreover, the other carbon signals at δ_C 29.06-29.69 described methylene groups in the saturated carbon chain. By analyzing the ^{1}H-NMR, ^{13}C-NMR, DEPT, MS spectral data, the structure of compound **7** was supposed to be montanic acid.

The inhibition of the α-glucosidase test was performed in optimal conditions for the enzyme that has been optimized. The data of the spectrophotometer (OD) was recorded and the inhibition (%) was expressed by the average of triplicated experiments and standard deviation (**Table 2**). The IC50 values were determined based on the logarithmic equations (**Figure 3**) drawn from the data in **Table 2**. The result showed that acarbose had the highest IC_{50} value of 0.722 μg.mL^{-1}. IC_{50} value of methanol, ethyl acetate, n-butanol, petroleum ether extracts were 0.244, 0.099, 0.209, 0.064 μg.mL^{-1}, respectively. The IC_{50} values of the four extracts were lower than those of acarbose. This indicates the extracts of *G. procumbens* could perform well in inhibiting α-glucosidase and petroleum ether extract showed the most potent effect.

DISCUSSION

Previous studies have shown that *G. procumbens* contains many compounds such as steroids, flavonoids, saponins, tannins, terpenoids, *etc*[2]. Among the seven compounds isolated, five compounds were known in *G. procumbens* syringic acid **(1)** (hydroxybenzoic acid structure), quercetin **(2)** (flavonoid glycoside structure), β-sitosterol 3-*O*-β-D-glucopyranoside **(5)**, schottenol **(6)** (steroid structure), montanic acid **(7)** (acid carboxylic), the two compounds *N,N*-dimethylanthranilic acid **(3)** and dehydrovomifoliol **(4)** were isolated in *G. procumbens* for the first time.

Previous studies have been conducted to investigate the anti-diabetic activities of *G. procumbens* leaves aqueous and ethanolic extracts and its possible underlying antihyperglycemic mechanisms of action involving liver carbohydrate metabolism in streptozotocin-induced diabetes in rats[3]. There was no previous study has ever conducted on anti-diabetes by inhibiting the enzyme α-glucosidase. From the results of the test on inhibiting α-glucosidase enzyme, which we have been doing in this study and the streptozotocin-induced diabetes treatment reported in previous studies, we can strongly believe that *G. proumbens* would be useful in the treatment of diabetes.

CONCLUSION

In the investigation of the chemical constituents of *G. procumbens* collected at Gia Lai province, seven compounds were isolated syringic acid **(1)**, quercetin **(2)**, *N,N*-dimethylanthranilic acid **(3)**, dehydrovomifoliol **(4)**, β-sitosterol 3-*O*-β-D-glucopyranoside **(5),** schottenol **(6),** montanic acid **(7).**

All four extracts (methanol, ethyl acetate, *n*-butanol, petroleum ether) showed inhibiting activity on α-glucosidase. The IC_{50} values of these four extracts were all smaller than those of the positive control acarbose. Petroleum ether extract gave the best ability to inhibit α-glucosidase with the lowest value of IC_{50} 0.064 μg.mL^{-1}. The results of this study showed that *G. procumbens* has great potential in treating diabetes.

LIST OF ABBREVIATIONS

IC_{50}: 50% Inhibitory Concentration
TLC: Thin-Layer Chromatography
NMR: Nuclear Magnetic Resonance
^{1} **H-NMR**: Proton Nuclear Magnetic Resonance
^{13} **C-NMR**: Carbon Nuclear Magnetic Resonance
DEPT: Distortionless Enhancement by Polarization Transfer
HR-ESI-MS: High-Resolution ElectroSpray Ionization Mass Spectrum
MeOH: Methanol
PE: Petroleum Ether
EA: Ethyl Acetate
n-Bu: *n*-Butanol
OD: Optical Density

AUTHOR CONTRIBUTIONS

The contributions of all authors are equal in selecting data, calculating descriptors, analyzing results, and writing a manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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Table 1: The ^{1}H-NMR and ^{13}C-NMR data of compounds (1 – 4)

No.	^{1}H-NMR				^{13}C-NMR			
	1[a]	2[b]	3[a]	4[c]	1[a]	2[b]	3[a]	4[c]
1	_	_	_	_	121.5		126.3	42.6
2	7.33 (2H, *s*)	_	_	2.29 (2H, *d*, 17.0) 2.58 (2H, *d*, 17.0)	108.2	146.8	153.4	50.6
3	_	_	7.72 (1H, *dd*, 8.0, 0.8)	_	148.4	135.5	123.5	200.3
4	_	_	7.41 (1H, *td*, 7.9, 1.2)	5.98 (1H, *s*)	141.6	175.7	134.8	128.0
5	_	_	7.66 (1H, *td*, 7.3, 1.6)	_	148.4	160.6	128.1	164.6
6	7.33 (2H, *s*)	6.16 (1H, *d*, 1.5)	8.12 (1H, *dd*, 7.5, 1.5)	_	108.2	98.2	132.2	80.0
7	_	_	-	7.04 (1H, *d*, 16.0)	167.5	163.7	167.1	148.3
8	3.88 (3H, *s*)	6.39 (1H, *d*, 1.5)	2.85 (3H, *s*)	6.49 (1H, *d*, 16.0)	56.7	93.3	45.7	131.7
9	3.88 (3H, *s*)	_	2.85 (3H, *s*)	_	56.7	156.0	45.7	203.6
10		_		2.35 (3H, *s*)		102.9		27.6
11				1.12 (3H, *s*)				23.5
12				1.07 (3H, *s*)				24.7
13				1.95 (3H, *s*)				19.1
1’		_				121.9		
2’		7.64 (1H, *d*, 2.0)				114.9		
3’		_				144.9		
4’		_				147.7		
5’		6.86 (1H, *d*, 8.5)				115.6		
6’		7.86 (1H, *dd*, 8.5, 2.5)				119.9		

a: Acetone; b: DMSO; c: MeOH

Table 2: The α-glucosidase inhibitory activity and their IC_{50} values

Extract	Concentration (μg.mL^{-1})	Triplicated experiment (%)			Average ± SD	IC_{50} (μg.mL^{-1})
GP - Me	0.075	-7.910	0.167	-4.330	-4.024 ± 3.304	0.244
	0.15	33.555	25.583	24.480	27.873 ± 4.043	
	0.3	58.285	59.583	59.450	59.106 ± 0.583	
	0.45	82.515	81.917	66.861	77.097 ± 7.242	
	0.6	94.005	91.750	90.258	92.004 ± 1.540	
	0.75	101.499	102.583	102.914	102.332 ±0.605	
GP - EA	0.0375	-16.403	-7.417	-1.249	-8.356 ± 6.222	0.099
	0.075	36.053	33.167	26.395	31.872 ± 4.048	
	0.1125	54.788	57.417	62.115	58.106 ± 3.031	
	0.15	81.932	77.750	69.359	76.347 ± 5.228	
	0.1875	88.260	89.250	89.509	89.006 ± 0.538	
GP - Bu	0.075	-10.241	0.167	6.495	-2.193 ± 6.847	0.209
	0.15	32.057	25.583	36.053	34.870 ± 1.998	
	0.3	75.937	59.583	63.281	69.684 ± 5.168	
	0.45	89.259	81.917	88.593	87.673 ± 1.793	
	0.6	100.416	91.750	100.416	100.583 ±0.236	
GP - PE	0.0375	14.821	25.167	25.396	21.794 ± 4.932	0.064
	0.075	59.867	58.167	67.027	61.687 ± 3.840	
	0.1125	74.022	82.250	72.773	76.348 ± 4.204	
	0.15	90.924	90.833	91.757	91.171 ± 0.416	
	0.1875	104.829	103.083	104.330	104.081 ±0.734	
Acarbose	0.038	-0.999	-4.500	11.657	2.053 ± 6.940	0.722
	0.188	34.305	32.333	24.480	30.373 ± 4.244	
	0.375	43.797	40.750	33.306	39.284 ± 4.407	
	0.563	49.958	41.250	38.385	43.198 ± 4.922	
	0.750	57.369	53.167	40.550	50.362 ± 7.147	
	1.125	56.536	56.750	52.040	55.109 ± 2.172	
	1.500	65.862	65.583	63.863	65.103 ± 0.884	

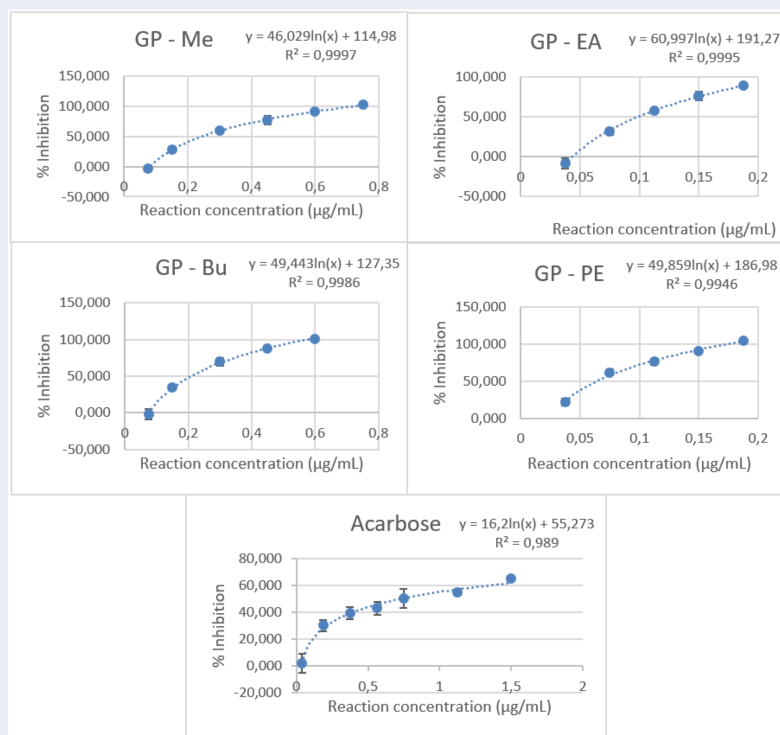

Figure 3: The graphs illustrating the inhibition of α-glucosidase of GP –Me, GP – EA, GP- Bu, GP – PE and acarbose.

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