

Research Article

Antiglycemic Activity of Vietnamese *Scutellaria barbata* Extract in Streptozotocin-Induced Diabetic Mice

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Scutellaria barbata D. Don (*S. barbata*), a medicinal herb commonly used in traditional Chinese and Korean medicine, consists of bioactive neoclerodanes and flavonoids. However, its anti-hyperglycemic activity has not been reported. In this work, we used bioassay-guided fractionation to find effective anti-hyperglycemic fractions of *S. barbata*. The chemical components of these fractions were also determined structurally; they are scutellarin (1), luteolin (2), naringenin (3), tricetin (4), baicalein (5), baicalin (6), apigenin (7), and wogonin (8). The most potent fraction **SB6** consisting of flavonoids 5, 6, 7, and 8 showed 89% inhibition of α -glucosidase in the *in vitro* experiment. In further *in vivo* experiments on streptozotocin (STZ)-induced diabetic mice, **SB6** exhibited significant anti-hyperglycemic activity comparable to metformin after 28 days of treatment. This fraction, however, did not show efficiency in reducing the weight gain of the treated mice.

1. Introduction

Glucose transport is the rate-determining step in carbohydrate metabolism. A reduction in the insulin-mediated glucose uptake is observed in diabetes patients, especially those with type 2 diabetes [1]. A number of diseases, such as heart disease, stroke, kidney disease, eye problems, dental disease, nerve damage, and foot problems, are developed as diabetes-related health problems in diabetes patients [2]. More than 400 million people live with diabetes worldwide, and the chronic metabolic disease was the direct cause of 1.5 million deaths in 2019 [2]. Diabetes is characterized by an elevated level of glucose in the blood; therefore, compounds able to inhibit α -glucosidase that catalyzes the degradation of disaccharides are considered antidiabetic and could potentially lead to the development of new antidiabetic drugs

[3]. Phytochemicals with different structural types, such as flavonoids, phenolic acids, triterpenoids, and triterpene saponins, have been exposed in recent studies as promising α -glucosidase inhibitors [2, 3]. Several authors studied the antidiabetic activity and disclosed the mechanism of action of bioactive compounds through structure-activity relationship (SAR) studies and *in vitro* functional evaluation [4]. Although there is high interest in the development of antidiabetic therapy from medicinal plants, few of them have been validated by scientific criteria [5]. Streptozotocin (STZ, 69%) and alloxan (31%) are the most frequently used drugs to induce diabetes in animals. *Scutellaria baicalensis* and *S. barbata* are common medicinal plants of the Lamiaceae family. Several antidiabetic studies have been conducted with *S. baicalensis* in animal models induced by STZ [2, 6]; however, the information on the *in vivo* antidiabetic activity

of *S. barbata* is very limited. *S. barbata* D. Don is a medicinal herb of the family Lamiaceae that is used in traditional Chinese and Korean medicine for its heat-clearing and detoxifying effects [7].

In Vietnam, *S. barbata* is found to grow in Northern (Thai Nguyen, Bac Giang, and Hoa Binh provinces) and Central Vietnam (Thanh Hoa province) with its medicinal uses in the treatment of hepatitis, liver cancer, gastrointestinal bleeding, pimples, and snake bites [7]. Neoclerodanes and flavonoids are the main compounds among more than 200 compounds isolated from *S. barbata*. Most of the previous biological activity and pharmacological studies focused on *in vitro* cytotoxic effects against human cancer cell lines as well as anti-inflammatory, antibacterial, antiviral, antioxidant, and anti-tumor activities [8, 9]. The literature search shows that this plant is rich in bioactive compounds and safe for the clinical applications. However, the literature has not reported the anti-hyperglycemic activity of extracts, fractions, and phytochemicals from any parts of *S. barbata*.

The objectives of the present study were to examine the *in vitro* α -glucosidase inhibitory activity of extracts and fractions of the whole plant of *S. barbata* D. Don in a subcellular assay. Bioassay-guided fractionation is known as one of the most effective methods in the isolation and identification of biologically active molecules from plant extracts. The method protocol consists of step-by-step bioassay and chromatographic fractionation of extracts and chromatographic fractions until the biologically active constituents are isolated [10]. The most bioactive fractions were submitted to chromatographic fractionation, aiming at the isolation and structural characterization of bioactive components of *S. barbata*. Addressing the potential of *S. barbata* in the preparation of herbal functional foods in the treatment of diabetes, the most bioactive fraction was evaluated in streptozotocin (STZ)-induced diabetic mice.

2. Materials and Methods

2.1. General Procedures. ESI-MS spectra were measured on the Agilent LC-MSD-Trap-SL system. ^1H NMR, ^{13}C NMR, and DEPT spectra were recorded in DMSO- d_6 using a Bruker Avance 500 NMR spectrometer. The chemical shifts are expressed in ppm relative to tetramethylsilane (TMS) as the internal standard. The ^1H NMR spectrum was recorded with a spectrometer frequency (SF) of 500.20 MHz. The ^{13}C NMR spectrum was recorded with SF 125.13 MHz.

Silica gel (Merck, Germany) and reversed-phase C_{18} silica gel (Merck, Germany) were used for column chromatography (CC). Precoated silica gel Merck 60 F_{254} aluminum plates were used for thin-layer chromatography (TLC).

2.2. Plant Material. The whole plant (including root) of *S. barbata* D. Don (Vietnamese name: Ban chi lien) was collected in January 2020 in Chi Lang, Lang Son province, Vietnam. The plant was botanically identified by Dr. Trieu Anh Trung of the Faculty of Biology, Hanoi University of Education, Hanoi, Vietnam.

The voucher specimen (no. BCL-LS-01-20) of the plant was deposited at the same institute.

2.3. Extraction and Isolation of Compounds 1–8. The powder of the dried whole plant of *Scutellaria barbata* (2 kg) was extracted with ethyl acetate at room temperature for 10 days, and the extraction was repeated one more time. The combined extracts were filtered and evaporated *in vacuo* to give an EtOAc extract (375 g). The extract (301 g) was subjected to silica gel column chromatography (CC) eluting with *n*-hexane-EtOAc gradient solution 20:1 \rightarrow 1:1 to give 7 fractions. The most active fractions in the inhibitory assay against enzyme α -glucosidase were fractions 3 (40 g), 4 (55 g), 5 (60 g), and 6 (58 g). They were further fractionated to isolate the active compounds. Fraction 3 (22 g) was purified by reversed-phase C_{18} silica gel CC eluting with $\text{CH}_3\text{CN-H}_2\text{O}$ 1:4 to give compound **1** (15 mg). Fraction 4 (28 g) was purified by reversed-phase C_{18} silica gel CC eluting with $\text{CH}_3\text{CN-H}_2\text{O}$ 1:4 to give compound **2** (10 mg). Fraction 5 (25 g) was purified by reversed-phase C_{18} silica gel CC eluting with $\text{CH}_3\text{CN-H}_2\text{O}$ 3:7 to give compounds **3** (7 mg) and **4** (8 mg). Fraction 6 (21 g) was purified by reversed-phase C_{18} silica gel CC eluting with $\text{CH}_3\text{CN-H}_2\text{O}$ 3:7 to give compounds **5** (12 mg), **6** (12 mg), **7** (10 mg), and **8** (10 mg).

2.4. Spectroscopic Data of the Isolated Compounds

2.4.1. Scutellarin (1): Yellowish Powder. ESI-MS (positive-ion): m/z 462.3 $[\text{M} + \text{H}]^+$ ($\text{C}_{21}\text{H}_{18}\text{O}_{12}$). ^1H NMR (DMSO- d_6 , 500 MHz): δ (ppm) 6.80 (1H, s, H-3), 6.10 (1H, s, H-8), 7.77 (d, 2H, $J = 8.3$ Hz, H-2', H-6'), 6.86 (d, 2H, $J = 8.3$ Hz, H-3', H-5'), 5.80 (1H, s, H-1''), 4.20 (1H, s, H-2''), 3.70 (1H, s, H-3''), 4.02 (1H, s, H-4''), 4.47 (1H, s, H-5''), 6.70 (2H, s, OH-6, OH-4'), 1.40 (1H, s, OH-3''), 13.9 (1H, s, OH-5), 11.0 (1H, s, OH-6''). ^{13}C NMR (DMSO- d_6 , 125 MHz): δ (ppm) 163.6 (C-2), 104.5 (C-3), 182.1 (C-4), 152.7 (C-5), 129.2 (C-6), 152.4 (C-7), 93.1 (C-8), 150.6 (C-9), 105.9 (C-10), 122.9 (C-1'), 129.2 (C-2', C-6'), 115.8 (C-3', C-5'), 157.7 (C-4'), 113.2 (C-1''), 73.1 (C-2''), 73.9 (C-3''), 72.7 (C-4''), 80.7 (C-5''), 173.2 (C-6'').

2.4.2. Luteolin (2): Yellow Powder. ESI-MS (positive-ion): m/z 286.2 $[\text{M} + \text{H}]^+$ ($\text{C}_{15}\text{H}_{10}\text{O}_6$). ^1H NMR (DMSO- d_6 , 500 MHz): δ (ppm) 6.71 (1H, s, H-3), 5.94 (1H, d, $J = 2.0$ Hz, H-6), 6.02 (1H, d, $J = 2.0$ Hz, H-8), 7.33 (1H, d, $J = 8.5$ Hz, H-2'), 7.03 (1H, d, $J = 8.5$ Hz, H-3'), 6.81 (1H, d, $J = 8.5$ Hz, H-6'), 11.0 (1H, s, OH-5), 6.0 (3H, s, OH-3', OH-4', OH-7). ^{13}C NMR (DMSO- d_6 , 125 MHz): δ (ppm) 165.1 (C-2), 107.1 (C-3), 186.1 (C-4), 161.8 (C-5), 98.3 (C-6), 166.4 (C-7), 94.0 (C-8), 158.8 (C-9), 108.4 (C-10), 123.0 (C-1'), 115.3 (C-2'), 145.9 (C-3'), 146.5 (C-4'), 117.2 (C-5'), 131.8 (C-6').

2.4.3. Naringenin (3): Colorless Powder. ESI-MS (positive-ion): m/z 272.2 $[\text{M} + \text{H}]^+$ ($\text{C}_{15}\text{H}_{12}\text{O}_5$). ^1H NMR (DMSO- d_6 , 500 MHz), δ (ppm): 5.51 (1H, s, H-2), 3.38 (1H, s, H-3), 5.80 (1H, d, $J = 2.0$ Hz, H-6), 6.20 (d, 1H, $J = 2.0$ Hz, H-8), 7.08

(2H, d, $J = 8.5$ Hz, H-2', H-6'), 6.78 (2H, d, $J = 8.5$ Hz, H-3', H-5'), 6.0 (2H, s, OH-7, OH-4'), 12.2 (1H, s, OH-5). ^{13}C NMR (DMSO- d_6 , 125 MHz): δ (ppm) 82.5 (C-2), 43.0 (C-3), 196.8 (C-4), 163.8 (C-5), 95.1 (C-6), 164.9 (C-7), 95.1 (C-8), 163.6 (C-9), 102.8 (C-10), 130.9 (C-1'), 127.4 (C-2', C-6'), 116.1 (C-3', C-5'), 150.4 (C-4').

2.4.4. Tricin (4): Yellow Powder. ESI-MS (positive-ion): 330.2 $[\text{M} + \text{H}]^+$ ($\text{C}_{17}\text{H}_{14}\text{O}_7$). ^1H NMR (DMSO- d_6 , 500 MHz): δ (ppm) 6.91 (1H, s, H-3), 6.10 (1H, d, $J = 2.0$ Hz, H-6), 6.62 (1H, d, $J = 2.0$ Hz, H-8), 7.81 (2H, s, H-2', H-6'), 6.5 (2H, s, OH-7, OH-4'), 12.0 (1H, s, OH-5), 3.83 (6H, s, OCH_3 -3', OCH_3 -5'). ^{13}C NMR (DMSO- d_6 , 500 MHz): δ (ppm) 170.1 (C-2), 115.4 (C-3), 190.0 (C-4), 179.8 (C-5), 100.1 (C-6), 172.4 (C-7), 98.8 (C-8), 160.8 (C-9), 111.2 (C-10), 126.8 (C-1'), 104.2 (C-2', C-6'), 148.0 (C-3', C-5'), 136.6 (C-4'), 56.1 (CH_3 -3', CH_3 -5').

2.4.5. Baicalein (5): Yellow Powder. ESI-MS (positive-ion): 270.2 $[\text{M} + \text{H}]^+$ ($\text{C}_{15}\text{H}_{10}\text{O}_5$). ^1H NMR (DMSO- d_6 , 500 MHz): δ (ppm) 6.80 (1H, s, H-3), 5.85 (1H, d, $J = 2.0$ Hz, H-8), 7.68 (2H, d, $J = 8.5$ Hz, H-3', H-5'), 7.71 (1H, d, $J = 8.5$ Hz, H-4'), 8.06 (2H, d, $J = 8.5$ Hz, H-2', H-6'), 6.90 (2H, s, OH-6, OH-7), 13.5 (1H, s, OH-5). ^{13}C NMR (DMSO- d_6 , 500 MHz): δ (ppm) 167.9 (C-2), 110.0 (C-3), 190.1 (C-4), 160.9 (C-5), 143.9 (C-6), 158.6 (C-7), 97.7 (C-8), 151.4 (C-9), 109.2 (C-10), 139.9 (C-1'), 137.0 (C-2', C-6'), 128.6 (C-3', C-5'), 127.9 (C-4').

2.4.6. Baicalin (6): Yellow Powder. ESI-MS (positive-ion): 446.4 $[\text{M} + \text{H}]^+$ ($\text{C}_{21}\text{H}_{18}\text{O}_{11}$). ^1H NMR (DMSO- d_6 , 500 MHz): δ (ppm) 6.71 (1H, s, H-3), 6.5 (1H, d, $J = 8.5$ Hz, H-8), 8.3 (2H, d, $J = 8.5$ Hz, H-2', H-6'), 8.0 (2H, d, $J = 8.0$ Hz, H-3', H-5'), 7.9 (1H, d, $J = 8.0$ Hz, H-4'), 3.70 (2H, s, H-1'', H-3''), 3.60 (1H, s, H-2''), 4.02 (1H, s, H-4''), 4.47 (1H, s, H-5''), 12.7 (1H, s, OH-5), 6.50 (1H, s, OH-6), 1.40 (2H, s, OH-2'', OH-3''), 1.15 (1H, s, OH-4''), 11.0 (1H, s, OH-6''). ^{13}C NMR (DMSO- d_6 , 125 MHz): δ (ppm) 159.9 (C-2), 115.0 (C-3), 176.3 (C-4), 157.0 (C-5), 143.9 (C-6), 167.8 (C-7), 90.9 (C-8), 155.4 (C-9), 100.9 (C-10), 130.3 (C-1'), 133.1 (C-2', C-6'), 120.9 (C-3', C-5'), 130.9 (C-4'), 77.3 (C-1''), 73.4 (C-2''), 70.6 (C-3''), 69.8 (C-4''), 79.4 (C-5''), 180.9 (C-6'').

2.4.7. Apigenin (7): Yellow Powder. ESI-MS (positive-ion): 270.2 $[\text{M} + \text{H}]^+$ ($\text{C}_{15}\text{H}_{10}\text{O}_5$). ^1H NMR (DMSO- d_6 , 500 MHz): δ (ppm) 6.71 (1H, s, H-3), 5.50 (d, 1H, $J = 2.0$ Hz, H-6), 6.30 (d, 1H, $J = 2.0$ Hz, H-8), 8.50 (d, 2H, $J = 8.5$ Hz, H-2', H-6'), 6.86 (d, 2H, $J = 8.5$ Hz, H-3', H-5'), 12.9 (1H, s, OH-5), 6.60 (2H, s, OH-7, OH-4'). ^{13}C NMR (DMSO- d_6 , 125 MHz): 177.8 (C-2), 107.9 (C-3), 191.5 (C-4), 161.7 (C-5), 99.0 (C-6), 167.7 (C-7), 95.7 (C-8), 155.8 (C-9), 104.4 (C-10), 122.0 (C-1'), 120.9 (C-2', C-6'), 122.8 (C-3', C-5'), 168.3 (C-4').

2.4.8. Wogonin (8): Yellow Powder. ESI-MS (positive-ion): 270.2 $[\text{M} + \text{H}]^+$ ($\text{C}_{16}\text{H}_{12}\text{O}_5$). ESI-MS: m/z 284.2 $[\text{M}]^+$ ($\text{C}_{17}\text{H}_{26}\text{O}_{10}$). ^1H NMR (DMSO- d_6 , 500 MHz): δ (ppm) 7.01 (1H, s, H-3), 6.02 (1H, d, $J = 2.0$ Hz, H-6), 7.99 (2H, d,

$J = 8.0$ Hz, H-2', H-6'), 8.30 (2H, d, $J = 8.0$ Hz, H-3', H-5'), 8.01 (1H, s, $J = 8.0$ Hz, H-4'), 13.0 (1H, s, OH-5), 5.90 (1H, s, OH-7), 3.71 (3H, s, OCH_3). ^{13}C NMR (DMSO- d_6 , 125 MHz): δ (ppm) 166.7 (C-2), 111.5 (C-3), 180.1 (C-4), 157.2 (C-5), 99.3 (C-6), 157.5 (C-7), 127.2 (C-8), 129.3 (C-1'), 120.1 (C-2', C-6'), 126.2 (C-3', C-5'), 130.0 (C-4'), 60.8 (OCH_3).

2.5. α -Glucosidase Inhibitory Activity Assay. The inhibition of the α -glucosidase activity was carried out following the method described by Hullatti and Telagari [11]. A 2.5 mM solution of *p*-NPG (*p*-nitrophenyl α -D-glucopyranoside) (Sigma-Aldrich) and 0.2 U/ml α -glucosidase from *Saccharomyces cerevisiae* (Sigma-Aldrich) were prepared in 100 mM potassium phosphate buffer at pH 6.8. Samples were prepared in dimethyl sulfoxide (DMSO) (Sigma-Aldrich) and serially diluted in concentrations of 1, 4, 16, 64, and 256 $\mu\text{g}/\text{mL}$. 10 μL of the sample was added to a reaction mixture consisting of 40 μL of 100 mM phosphate buffer at pH 6.8 and 25 μL of 0.2 U/mL α -glucosidase in a 96-well microplate, and the reaction mixture was incubated for 10 min at 37°C. Then, 25 μL of 2.5 mM *p*-NPG was added, and the reaction mixture was further incubated for 20 min at 37°C. After 30 min, 100 mM sodium carbonate solution (100 μL) was added to the stop reaction. The absorbance of the mixture was measured at λ 410 nm on a UV-vis spectrophotometer (BioTek Instruments, USA). To make a control reaction, the tested sample was replaced by 10 μL of 100 mM phosphate buffer (pH 6.8). Acarbose was used as the reference standard. The experiments were repeated three times.

The α -glucosidase inhibitory activity was calculated using the following equation: α -glucosidase inhibitory activity (%) = $(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}} \times 100$, where A_{control} is the absorbance of the control and A_{sample} is the absorbance of the sample. IC_{50} values (half maximal inhibitory concentration) were calculated using Table Curve software. All analyses were performed in triplicate, and data were reported as mean \pm SEM.

2.6. Design of the In Vivo Experiment

2.6.1. Animals and Diabetic Model. Male Swiss mice weighing 18–22 g were obtained from the National Institute of Drug Quality Control, Hanoi, Vietnam, and were humanely treated in accordance with the Ministry of Health Guidelines for the Care and Use of Laboratory Animals. The protocols were approved by the Ethics Committee of Vietnam National Institute of Drug Quality Control (Permit No. 53NC 05) and were employed in the experiment. Housing conditions were kept as follows: a temperature of 27–28°C with a dark-light cycle of 12–12 h. The mice were freely fed with standard chow and tap water (15 g of food and 15 ml of water/100 g BW/day) [12].

2.6.2. Investigation of Semichronic Toxicity of the Preparations. Based on the results on the inhibitory ability of the extract fractions to the activity of the α -glucosidase enzyme, the inhibitory ability of the SB6 fraction was the

most superior (89%). Therefore, the SB6 preparation was selected to conduct the experiment. Semichronicity toxicity was checked at different doses of 100, 200, and 300 mg/kg BW [13, 14].

Mice were divided into 4 groups of 6 mice, and the preparation was given at different concentrations once a day after lunch for two weeks as follows:

- + Lot 1: vehicle with normal saline.
- + Lot 2: extract with a dose of 100 mg/kg BW.
- + Lot 3: extract with a dose of 200 mg/kg BW.
- + Lot 4: extract with a dose of 300 mg/kg BW. Vital signs and BW were checked on a daily basis. The dose of 200 mg/kg BW was found to induce the highest changes in BW with no adverse effects. Therefore, the extract was injected through an oral catheter into mice with that dose.

2.6.3. Induction of Diabetic Model. All mice were checked for tail blood glucose with a glucose oxidase method (OneTouch Ultra, LifeScan, USA) before diabetic induction to ensure all mice had similar blood glucose levels. The mice were then divided into two groups: the diabetic group (intraperitoneally injected with streptozocin at a dose of 120 mg/kg body weight (BW)) and the vehicle group (intraperitoneally injected with normal saline). All mice were checked for caudal blood glucose at 48 h, 72 h, 5 days, 7 days, and 10 days after injection. If caudal blood glucose was greater than 18 mmol/L in the diabetic group, the mice were designated as successfully diabetic-induced.

2.6.4. Investigation of Extract Effectiveness. After injection, all mice were then subdivided into four groups, namely, VG, the vehicle group treated with tapping water; DN, the diabetic group treated without any further interventions ($n=6$); DM, the diabetic group treated with metformin with a dose of 120 mg/kg BW ($n=6$); and DSB6, the diabetic group treated with SB6 with a dose of 200 mg/kg BW ($n=6$). All mice were treated for 28 days with a 7-day cycle of blood glucose checks.

2.6.5. Statistics. Data were presented as mean and standard deviation, or SEM and percentages where available. The differences among groups in blood glucose and body weight were checked with two-way ANOVA followed by Bonferroni corrections. A $p < 0.05$ was set as significant. All data were processed with SPSS version 20.0 and Microsoft Excel.

3. Results and Discussion

The whole plant of *S. barbata* was extracted twice with EtOAc at room temperature, and the extract was subjected to bioassay-guided fractionation targeting the inhibition of α -glucosidase (Table 1). In the inhibitory assay four fractions, Frs. 3–6 were determined as the most active. They showed more potent inhibitory activity than the original EtOAc extract.

Eight compounds were isolated by using column chromatography from the fractions (Figure 1), and their

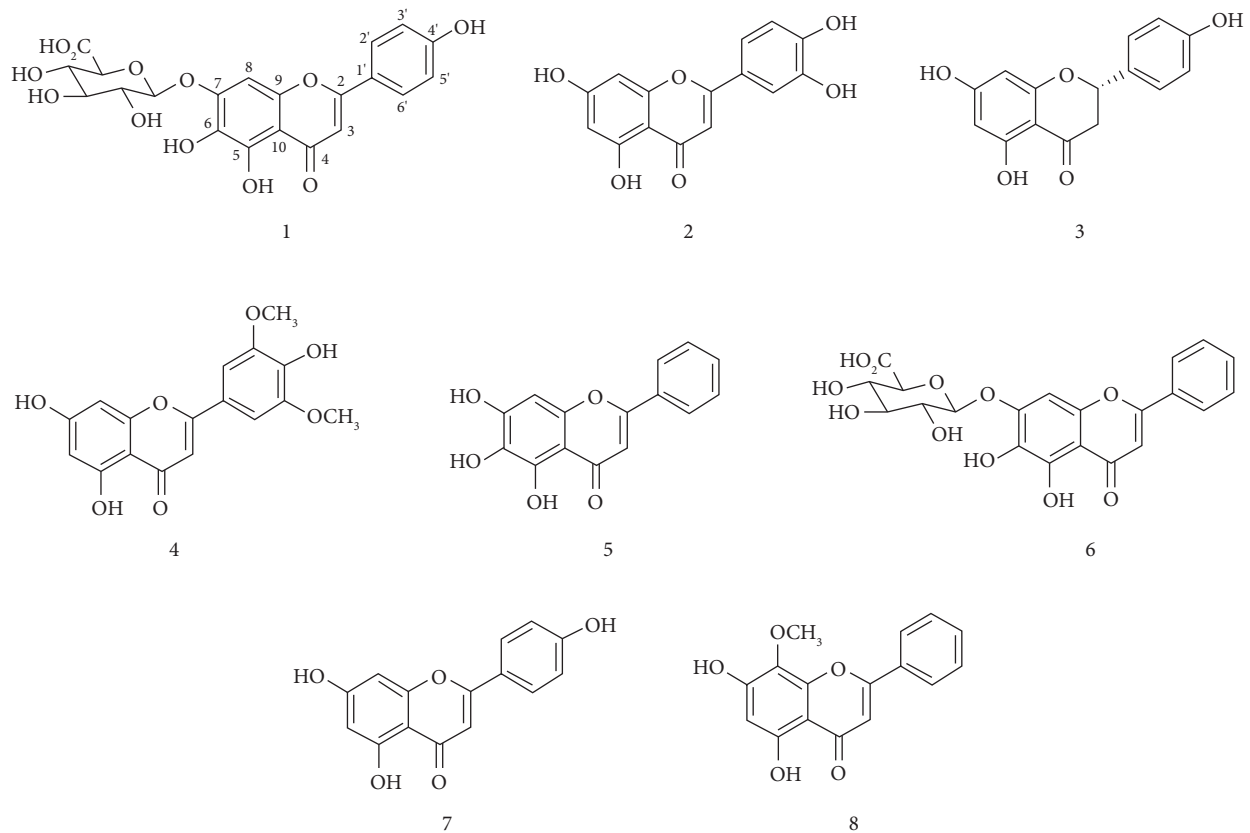
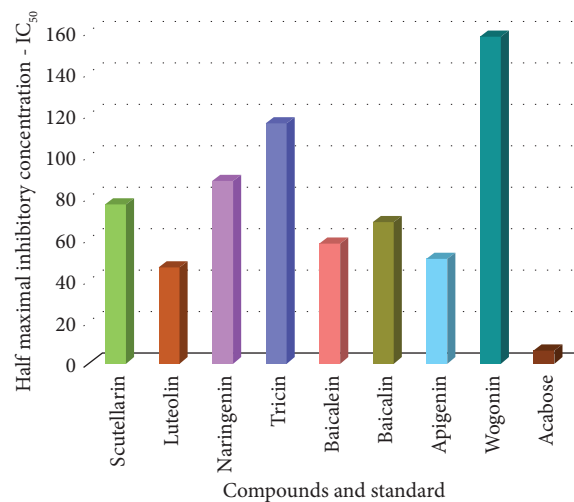
structures were determined on the basis of MS and NMR analysis, which were in agreement with the reference values, as scutellarin (1) [15], luteolin (2) [16], naringenin (3) [17], tricin (4) [18], baicalein (5) [19], baicalin (6) [20], apigenin (7) [21], and wogonin (8) [20].

The ability of the eight isolated flavonoids to inhibit α -glucosidases activities *in vitro* was also assayed (Figure 2). The results reported that compounds luteolin (2), baicalein (5), baicalin (6), and apigenin (7) have higher inhibition compared to other compounds (less than 7.4–11.01-fold compared to the standard acarbose), and fraction 6 contains a high amounts of active chemical components baicalein, baicalin, and apigenin, similar to the results of fraction 6 survey. The -OCH₃ group present in the structure of compounds tricin (4) and wogonin (8) may be the cause of hindering the compound inhibition; -OH groups at C5, C6, and C7 on A and B rings play an important role in the enzyme activity inhibition; this has been proven in structure correlation studies, and by the enzyme inhibitory ability of plant flavonoids [22, 23].

In the treatment recommendations for type 2 diabetes, metformin is always the preferred drug of the first choice for patients without cardiovascular or renal complications [24]. Therefore, in this study, we used metformin as a controlled drug in evaluating the blood glucose-lowering effect of SB6. Following the injection of streptozocin, vehicle-treated DN mice rapidly developed diabetes during the experiment, with blood glucose consistently above 20 mmol/L and reaching about 35 mmol/L on day 28 (Figure 3). In the remaining two groups of mice treated with metformin or SB6, the DM group had a marked decrease in blood glucose after 7 days of treatment. The rate of reduction was maintained steadily until the end of the experiment, and blood glucose reached a near-stable level on day 21 after treatment. The DSB6 group also exhibited significant glycemic reduction. Although the blood glucose levels of the DSB6 group at days 7, 14, and 21 after treatment were still higher than those of the DM group, at day 28 after treatment, the difference between these two groups was not significant. However, on day 28 after treatment, blood glucose in both groups was still significantly higher than that of the VG group. This could be explained by our use of a diabetes model with streptozocin, leading to the irreversible damage of a part of pancreatic tissue [25] and to a decrease in insulin levels in response to the need to maintain basal blood glucose. The remaining pancreatic parenchyma must work compensatory to secrete insulin even with a higher workload but cannot maintain normal blood glucose levels and rapidly degrades, leading to a complete loss of function later on. Under the glucose-lowering effect of treatment, this reserved part of the pancreatic parenchyma has a rest period and can prolong the time to maintain function but still cannot be like a normal pancreas. We, therefore, assumed that this extract has a hypoglycemic effect based on its ability to partially restore the beta cell function, which is also recognized in some other extracts [26, 27]. However, further studies must be conducted to shed light on the mechanism of SB6's effect on glucose lowering.

TABLE 1: α -Glucosidase inhibitory activity of EtOAc extract and column fractions.

Conc. ($\mu\text{g/ml}$)	% inhibitory activity								
	EtOAc extract	Fr. 1	Fr. 2	Fr. 3	Fr. 4	Fr. 5	Fr. 6	Fr. 7	Acarbose
2	22	6	72	70	65	61	65	66	22
4	37	9	75	74	67	65	71	68	45
16	42	17	76	75	70	73	74	70	63
64	49	39	84	80	79	79	76	73	74
256	57	42	85	86	88	87	89	74	86

FIGURE 1: Compounds 1–8 isolated from *S. barbata*.FIGURE 2: α -Glucosidase inhibitory activity of isolated compounds.

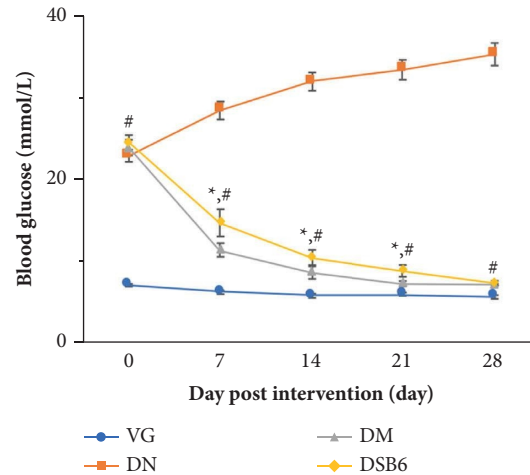


FIGURE 3: Blood glucose changes in mmol/L during the experimental period. * and # are significantly different between the DSB6 and DM groups and between the BSD6 and DM with VG groups, respectively.

Hyperglycemia is often accompanied by changes in body weight due to increased water loss due to increased osmotic diuresis and decreased ability to use glucose as an energy source for the body's demands. So, the improvement in the body weight is also an indicator of a good response to anti-hyperglycemic treatment. In our study, the weight gain of the DN group was significantly lower than that of the DM and DSB6 groups (Figure 4). Due to the short follow-up time (28 days after the intervention), the weight loss expression in the DN group was not clear. Besides, in this study, we selected mice that are in the immature stage, leading to a strong weight gain during the developmental stage. This may overshadow the effect of hyperglycemia on weight. However, the DM and DSB6 groups still maintained their weight gains, although not as much as the VG group. This phenomenon might be the result of the anti-hyperglycemic effect of SB6. The hemoconcentration after streptozocin induction of diabetes was easily recognized by the increases in the red blood cell and white blood cell counts in all 3 groups, namely, the DN, DM, and DSB6 groups, at day 0 (Table 2). These increases in the concentration lasted until day 28 in the DN group. However, in the DM and DSB6 groups, this phenomenon gradually decreased, and the red blood cell and white blood cell counts in these two groups at day 28 were not statistically different from those of the VG group. The increase in blood cells might be due to hypovolemia due to hyperosmolarity in diabetes. After treatment with our extract, plasma glucose decreased in the treated group, leading to a decline in the number of blood cells. Hemodynamic changes are also dependent on the causative agent of the diabetic model. In a study by Helal et al., alloxan-induced diabetes rats exhibited low red blood cell and white blood cell counts compared with control ones [28]. The authors explained these differences between the two groups due to the combined effects of alloxan and diabetes. Alloxan induces diabetes due to many mechanisms, in which dialuric acid establishes the formation of superoxide radicals, leading to the toxication of hematological

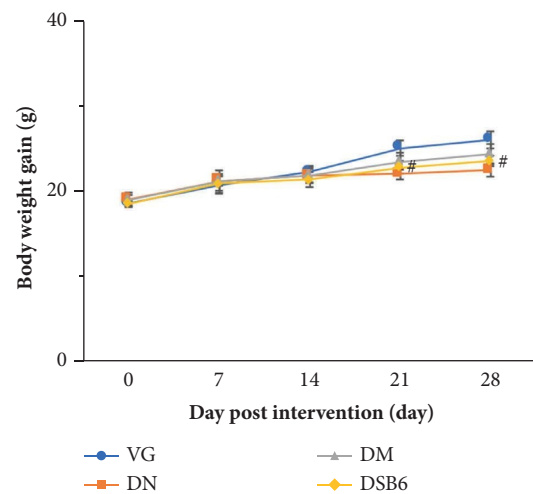


FIGURE 4: Body weight changes in g during the experimental period. # and # are significantly different between the DSB6 and DM with VG groups.

systems [29]. As a result, low white blood cell and red blood cell counts were observed. In contrast to alloxan, streptozocin does not lead to this formation. In our study, we used streptozocin to induce diabetes. Thus, the increase in blood cells could be explained by the increased concentration in a hyperosmolar state caused by hyperglycemia.

Regarding vital signs and locomotion, mice in the VG group did not show any abnormal expressions (Table 3). All mice in the DN group showed signs of slowing down, drinking more water, eating less, excreting more, and eyes turning red. In contrast, mice in the DM and DSB6 groups in the first week showed a reduction in locomotion and poor appetite, possibly due to high blood glucose; however, from day 7 onwards, the mice moved more, ate more, drank a lot of water, and excreted a lot, but not as much as the mice in the DN group. No mice died during the experiment.

TABLE 2: Red blood cell count (RBC) and white blood cell count (WBC) in control, diabetes, drug-treated, and *S. barbata* extract-treated groups during the experiment.

		Day 0	Day 7	Day 14	Day 21	Day 28
RBC (T/L)	VG	7.12 ± 0.14	6.98 ± 0.27	7.33 ± 0.24	7.56 ± 0.22	7.41 ± 0.29
	DN	11.01 ± 0.37	10.78 ± 0.15	12.06 ± 0.24	13.14 ± 0.18	14.51 ± 0.15
	DM	10.77 ± 0.29	9.37 ± 0.15	8.16 ± 0.25	7.38 ± 0.29	7.61 ± 0.23
	DSB6	11.14 ± 0.25	11.93 ± 0.21	8.99 ± 0.24	9.01 ± 0.35	8.20 ± 0.28
WBC (G/L)	VG	9.42 ± 1.11	9.50 ± 0.56	9.75 ± 0.48	9.56 ± 0.22	9.36 ± 0.41
	DN	8.75 ± 0.19	7.61 ± 0.23	8.16 ± 0.20	7.71 ± 0.26	8.19 ± 0.21
	DM	8.56 ± 0.28	8.02 ± 0.22	7.14 ± 0.25	6.63 ± 0.31	6.42 ± 0.15
	DSB6	9.01 ± 0.21	7.92 ± 0.31	7.24 ± 0.20	7.04 ± 0.11	7.01 ± 0.31

TABLE 3: Physiological status of mice during the experiment.

	Hair	Eyes	Excretion	Locomotion	Death
VG	–	–	–	–	0/6
DN	–	+ –	+ –	+ –	0/6
DM	–	–	+ –	+ –	0/6
DSB6	–	–	+ –	+ –	0/6
(–)					

4. Conclusion

From the bioactive fractions of Vietnamese *Scutellaria barbata*, eight compounds including a flavanone, five flavones, and two flavone glucosides were isolated and structurally identified by using bioassay-guided fractionation. They may be the active components of *S. barbata* and are useful as chemical markers to standardize the pharmacologically active SB6 fraction by using the NMR or high-performance liquid chromatography analytical methods. *S. barbata* D. Don has been reported for its anticancer, antitumor proliferation, antitumor activities, antioxidant effects, pharmacodynamics influenced on the brain, and antimicrobial activities. . . [30]. Several studies using crude extract of *S. barbata* at a dose of 400 mg/kg–600 mg/kg showed the antidiabetic effects in STZ-induced diabetic rats based on the exerted synergistic [31, 32], or compound isolated from the plant such as Baicalin exhibited anti-hyperglycemic activities through the mechanism of hindering glucose production in the liver [33]. In our study, similar treatment effects were also obtained but using selected fractions from crude extracts that concentrate flavonoids as a survey sample but with a lower dose. Research has shown the α -glucosidase inhibitory activity of *S. barbata* fraction and isolated compounds; however, the mechanism of action evaluation for extract and compounds has not been studied. The data of the screening experiments of biological assay are exploited to provide more specific guidance for antidiabetic testing on animals. The blood glucose indicators were used to evaluate the anti-hyperglycemic effects of the drug; however, this is the first study to evaluate the effect of the extract on blood glucose. The research is at a basic experiment; HbA1c and insulin test and the necessary experiments for clinical research are the authors' wish in the future.

Data Availability

Data were presented as mean and standard deviation, or SEM and percentages where available.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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