α-GLUCOSIDASE INHIBITORS FROM THE LEAVES AND STEMS OF *ERYTHRINA VARIEGATA* L. COLLECTED IN HANOI

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Abstract - Four secondary metabolites, including daidzein (**1**), genistein (**2**), glycitein (**3**), and daidzin (**4**) have been isolated and structural elucidated from the methanolic extract of the stems and leaves of *Erythrina variegata* L. collected in Hanoi city, Vietnam. These compounds showed good in vitro inhibition on *α*-glucosidase enzyme with IC_{50} values of 97.6 ± 0.6, 230.4 ± 0.4, 34.3 ± 0.9, and 217.2 ± 0.2 µM, respectively as compared with that of acarbose (IC_{50} value of 125.8 ± 1.2 µM). This finding suggests the possible application of product containing high content of these compounds from the leaves and stems of *E. variegata* L. for research and development of anti-obese agent.

Key words - *Erythrina variegata*; *α*-glucosidase inhibitor; anti-diabetic; anti-obesity; Vong nem

1. Introduction

Erythrina variegata L. is a medicinal plant, grows widely in Asia, Africa, and South America. It has been used as sedative, antihypertensive, anti-inflammatory, antibacterial, anti-diabetic and anti-osteoporotic [1, 2]. This plant grows up to about 10-20 m tall. Its leaves have three ovate leaflets about 10-15 cm long and the flowers are bright red. The fruits are about 10-30 cm long and contain 5-6 red kidney-shaped [2]. The plant is a rich source of flavonoids, phenylpropanoids, and alkaloid such as erythrine, hypaphorine… [2 - 5]. Many published articles have indicated interestingly biological activities for this plant, such as anti-diabetic, anti-cancer, antimicrobial, and antifungal activities [6 - 7]. Recently, Tang et al. reported about 30 new dimeric alkaloids isolated from the stem bark of *E. variegate*, all of these were first detected in *E. variegate* and reported in the genus *Erythrina* for the first time [8-10]. In addition to natural alkaloids, a number of artificial Erythrina alkaloids have also been reported from *E. variegate*, *E. cristagalli* as well as *E. arborescens* [11].

In total, more than 60 alkaloids and more than 40 flavonoid compounds have been isolated and structurally identified from *E. variegata*. In addition, about 17 pterocarpinoids, 06 benzofurans, 12 steroids, 08 terpenoid compounds and about 20 other structural compounds have been isolated and identified from this species [12]. Most of these compounds were isolated and identified from the bark and roots, and a few compounds were from leaves. The studies of bioactivity on this plant species have been published the anti-anxiolytic and depressant activity [13], experimental anti-osteoporotic effects in rats [14], antibacterial effects, antioxidant, and antimalarial [15]. Some studies have showed that the leaf extract had anti-hair loss and larvicidal activity against mosquito [16], analgesic, anti-inflammatory [17], bio-sorption and removal of some metal elements [18], and anticonvulsant effects [19]. In addition to the activities studied above, the leaves, bark, and roots of *E. variegata* have antihypertensive, lipid-lowering and hypoglycemic activities in streptozotocin-induced diabetes rats [20-22].

In this report, the isolation, structural elucidation, and *α*-glucosidase inhibitory activity evaluation of the compounds from the medicinal plant are described.

2. Materials and Methods

2.1. Materials

Figure 1. *Flower and leaves of Erythrina variegata* L.

The stems and leaves of *Erythrina variegata* L. were collected on June 23rd, 2021 in Hanoi city. The plant sample was identified by Dr. Nguyen Quoc Binh, Vietnam National Museum of Nature, VAST. Its voucher specimen was deposited at Institute of Natural Product Chemistry, VAST.

^{1}H-NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were measured on a Bruker AVANCE 500 spectrometer at Institute of Chemistry, VAST. Mass spectra were determined by Agilent 1260 Series Single Quadrupole LC/MS system at Institute of Marine Biochemistry, VAST. Column chromatography (CC) was carried out on silica gel (Si 60 F_{254}, 230-400 mesh, Merck). Thin layer

chromatography was used for analytical purposes using silica gel and RP 60 F_{254} plates. HPLC was carried out using an Agilent 1260 HPLC system with a DAD detector and an Optima Pak C18 column (10 × 250 mm, 5 μm particle size, Korea) and HPLC solvents were from Fisher Scientific Korea Ltd.

2.2. Extraction and isolation of compounds

The stems and leaves of *E. variegata* (1.17 kg) were dried and cut into small pieces and then extracted with methanol (5 L for three times) using an ultrasonicator for 2 hours. The extracted solution was then filtered and combined before being evaporated by a rotary evaporator system to give the crude extract (125 g). The crude extract was partitioned between EtOAc and water. The EtOAc extract (55.1 g) was then chromatographed on a silica gel column (5.0 × 80 cm) using *n*-hexane-acetone as a mobile phase with a gradient system from 15:1 to 100% acetone to afford fifteen fractions. Sub-fraction 7 (780 mg) was purified by a reversed phase column C-18, using methanol-water gradient (from 1:5 to 1:0) to give compounds **1** (26 mg) and **3** (23 mg). Compounds **2** (19 mg) and **4** (27 mg) were isolated from sub-fraction 8 (1.2 g) by reversed phase column C-18, eluting with an acetone-water gradient (1:2 to 2:1).

*Compounds **1**–**3***: ^{1}H and ^{13}C NMR spectral data are listed in Tables 1 and 2.

*Compounds **4***: Light yellowish powder; MS $[M]^+$ *m/z* 416.8: $C_{21}H_{20}O_9$; ^{1}H NMR (methanol-d_4, 600 MHz) δ_H ppm: 8.22 (1H, s, H-2), 8.17 (1H, d, *J* = 9.0 Hz, H-5), 7.24 (1H, br d, *J* = 9.0 Hz, H-6), 7.27 (1H, br s, H-8), 7.40 (2H, d, *J* = 8.4 Hz, H-2′/6′), 6.87 (2H, d, *J* = 8.4 Hz, H-H-3′/5′), 5.12 (1H, d, *J* = 6.6 Hz, H-1″), 3.54 (2H, m, H-2″/4″), 3.44 (1H, m, H-3″), 3.57 (1H, m, H-5″), 3.95 (1H, br d, *J* = 12.0 Hz, H-6″a), 3.74 (1H, dd, *J* = 12.0, 6.6 Hz, H-6″b); ^{13}C NMR (methanol-d_4, 150 MHz) δ_C ppm: 154.9 (C-2), 126.1 (C-3), 177.7 (C-4), 128.3 (C-5), 117.0 (C-6), 163.4 (C-7), 105.0 (C-8), 159.1 (C-9), 120.2 (C-10), 124.1 (C-1′), 131.5 (C-2′/C-6′), 116.3 (C-3′/C-5′), 158.8 (C-4′), 101.8 (C-1″), 74.8 (C-2″), 77.9 (C-3″), 78.5 (C-4″), 71.2 (C-5″), 62.4 (C-6″).

2.3. α-Glucosidase inhibitory assay

The inhibition of *α*-glucosidase enzyme of the isolated compounds (**1**–**4**) was carried out using previous described method [23].

3. Results and Discussion

Compound **1** was purified as a white powder. Its ^{1}H NMR spectrum showed eight aromatic protons resonating from 6.87 ppm to 8.15 ppm, indicating two protons for *ortho* position at 6.87 ppm (2H, d, *J* = 8.4 Hz) and 7.39 ppm (2H, d, *J* = 8.4 Hz). These NMR spectral data revealed a flavanoid structure for compound **1**. Characterization of ^{13}C NMR spectrum of compound **1** revealed 15 carbon signals, including one conjugated carbonyl at 178.2 ppm and four aromatic carbons at 154.6, 158.7, 159.8, and 164.6 ppm. Consequently, compound **1** was charaterized as daidzein, this compound was found in soybean with strong cytotoxicity against breast cancer cells [24].

Table 1. *^{1}H NMR data of compounds **1**–**3** measured in methanol-d_4 at 600 MHz*

Position	**1** δ_H (ppm, *J* in Hz)	**2** δ_H (ppm, *J* in Hz)	**3** δ_H (ppm, *J* in Hz)
2	8.15 (s)	8.07 (s)	8.15 (s)
3			
4			
5	8.08 (d, 9.0)		7.55 (s)
6	6.96 (dd, 8.4, 1.8)	6.24 (d, 1.8)	
7			
8	6.87 (d, 1.8)	6.35 (d, 2.4)	6.94 (s)
9			
10			
1′			
2′	7.39 (d, 8.4)	7.38 (d, 7.5)	7.40 (d, 9.0)
3′	6.87 (d, 8.4)	6.87 (d, 7.0)	6.88 (d, 9.0)
4′			
5′	6.87 (d, 8.4)	6.87 (d, 7.0)	6.88 (d, 9.0)
6′	7.39 (d, 8.4)	7.38 (d, 7.5)	7.40 (d, 9.0)
OCH_3			3.99 (s)

Compound **2** had similar NMR spectra with those of compound **1** except for the disappearance of a signal asignable for H-5. Analysis of its ^{13}C NMR spectrum revealed an additional aromatic carbon signal at 163.9 ppm. Thus, compound **2** was identified as genistein [25].

Compound **3** was obtained as a white powder. It had similar NMR spectral data with those of compound **1**. The signal assigned for H-5 was resonated at 7.55 ppm as singlet and a methoxyl signal appeared at 3.99 ppm. This methoxyl group was elucidated to attach to C-6 position by comparison of NMR spectral data of compound **3** with published values [26]. Thus, compound **3** was identified as glycitein.

Table 2. *^{13}C NMR data of compounds **1**–**3** measured in methanol-d_4 at 150 MHz*

Position	**1** δ_C (ppm)	**2** δ_C (ppm)	**3** δ_C (ppm)
2	154.6	154.8	154.3
3	126.0	123.3	125.3
4	178.2	182.3	177.6
5	128.5	163.9	105.6
6	116.2	100.1	148.7
7	164.6	166.0	154.3
8	103.2	94.8	104.0
9	158.7	159.7	154.3
10	118.2	106.3	117.9
1′	124.3	124.8	124.6
2′	131.4	131.4	131.5
3′	116.4	116.3	116.3
4′	159.8	158.8	158.6
5′	116.4	116.3	116.3
6′	131.4	131.4	131.5
OCH_3			56.7

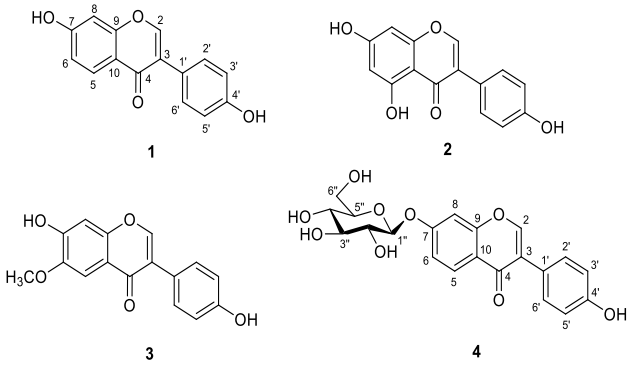

Figure 2. *Chemical structures of compounds (**1**–**4**) isolated from Erythrina variegata* L.

The MS of compound **4** indicated a molecular ion peak at *m/z* 416.8 $[M]^+$. 1D NMR spectra of compound **4** revealed an isoflavone glycoside with an aglycone as daidzein (**1**) and a monosacharide. This monosacharide was identified as *β*-D-glucose due to the presence of an anomeric proton signal at 5.12 (d, J = 6.6 Hz, H-1″). This sugar moiety was detected attaching to the daidzein structure at C-7 position based on a correlation between H-1″ and C-7 found in its HMBC spectrum (Figure 3). Thus, compound **4** was identified as daidzin [27].

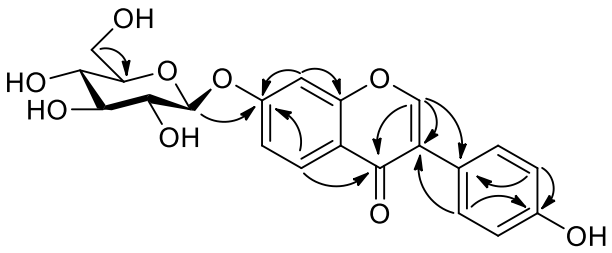

Figure 3. *Selected key HMBC correlations for compound **4***

Compounds (**1**–**4**) were tested for their *α*-glucosidase inhibitory activity *in vitro* using acarbose as a positive control. The results (listed in Table 3) showed that all compounds exhibited inhibitory activity, in which glycitein (**3**) showed strongest activity with an IC_{50} value of 34.3 ± 0.9 µM, followed by daidzein (**1**) with an IC_{50} value of 97.6 ± 0.6 µM. Genistein (**2**) and daidzin (**4**) displayed moderate inhibitory effects with IC_{50} values of 230.4 ± 0.4 and 217.2 ± 0.2 µM, respectively, these were a half less than that of acarbose (IC_{50} 125.8 ± 1.2 µM).

Table 3. *Inhibitory activity of compounds **1**–**4** against α-glucosidase*

Compounds	Inhibitory activity (IC_{50}, µM)[a]
daidzein (**1**)	97.6 ± 0.6
genistein (**2**)	230.4 ± 0.4
glycitein (**3**)	34.3 ± 0.9
daidzin (**4**)	217.2 ± 0.2
acarbose[b]	125.8 ± 1.2

[a] *IC_{50} values expressed as µM with duplicated experiment;* [b] *the positive control.*

All isolated compounds are isoflavone type compounds. Of these, daidzein (**1**) is a basic isoflavone structure containing 7,4′-dihydroxy groups, genistein (**2**) is also a basic isoflavone structure with an additional hydroxyl group substituted at C-5 position. Compound **1** showed potential inhibition toward *α*-glucosidase with two times stronger than compound **2**. Similar to **2**, compound **4** bearing a sugar unit linked at 7-O position showed less activity (IC_{50} value of 217.2 ± 0.2 µM) than compound **1** (Figure 2 and Table 1). These obtained data might suggest that hydroxylation at C-5 and/or substitution of sugar moiety at 7-O position may be reduced the inhibitory effect of these isoflavones on *α*-glucosidase enzyme. However, methoxylation at C-6 (compound **3**) could be enhanced the activity of this isoflavone (Table 3).

4. Conclusion

Four natural compounds including daidzein (**1**), genistein (**2**), glycitein (**3**) and daidzin (**4**) were successfully isolated and structurally characterized from stems and leaves of *E. variegata* L. All compounds showed strong inhibition against *α*-glucosidase enzyme activity as compared with positive control acarbose. Daidzein (**1**) and glycitein (**3**) exhibited greatest inhibition with IC_{50} values of 97.6 ± 0.6 and 34.3 ± 0.9 µM, respectively. Compounds **2** and **4** showed similar inhibitory activity with IC_{50} values of 230.4 ± 0.4 and 217.2 ± 0.2 µM, while acarbose exhibited an IC_{50} value of 125.8 ± 1.2 µM. This result may reveal the potential application of these active ingredients as well as the plant material in the development of medicinal plant-derived product for the diabetic and obese patients.

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