JST: Engineering and Technology for Sustainable Development
Volume 35, Issue 2, April 2025, 009-017

Flavonoids with Their Anti-Melanogenic Activity from *Glycyrrhiza glabra* L. in Vietnam

Nguyen Thi Viet Thanh*, Hoang Tran Nhu, Tran Minh Anh
Hanoi University of Science and Technology, Ha Noi, Vietnam
Corresponding author email: thanh.nguyenthiviet@hust.edu.vn

Abstract

Glycyrrhiza glabra L. has a long-standing history in traditional medicine across both Eastern and Western areas. The plant's phytochemials, such as glycyrrhizin, glycyrrhetic acid, and various flavonoids, offer significant therapeutic potential. Further research could explore its application in modern drug development for the treatment of systemic and non-systemic diseases. In the course of study on the chemical composition of *Glycyrrhiza glabra* L. in Vietnam, this paper described the extraction and structure evaluation of four compounds, including glabridin (**1**); 4'-*O*-methylglabridin (**2**); glabrol (**3**); kanzonol Y (**4**) as well as the melanin inhibitory activity of these compounds. The stems of this plant were collected, identified, dried and extracted in different polarity solvents. These substances were isolated from the ethyl acetate extract on the basis of column chromatography combined with thin layer chromatography. Their structures were identified based on spectroscopic evaluation and comparison of corresponding authentic compounds.

Keywords: *Glycyrrhiza glabra*, glabridin, flavonoid, anti-melanogenic.

1. Introduction

Glycyrrhiza glabra L. (*G. glabra*), commonly known as licorice root, is a perennial shrub from the Fabaceae family. The plant is native to China but is now widely cultivated in various regions of Vietnam, such as Tuyen Quang, Ha Giang, Dien Bien, and Son La. In Europe and Asia, it has been used as both a natural sweetener and a pharmaceutical [1]. According to previous phytochemical reports, *G. glabra* contains numerous bioactive compounds, including triterpenes, saponins, flavonoids, polysaccharides, and glycyrrhizin [2, 3]. Glycyrrhizin, a triterpenoid glycoside, is responsible for the sweet taste of licorice root. It consists of calcium, potassium, and magnesium salts of glycyrrhizic acid [4]. In addition, many saponins, such as oleanane triterpenoid saponin, have also been isolated. Flavonoids, including flavanones, flavonols, flavones, isoflavones, isoflavones, and chalcones, are abundant and contribute to the yellow color of the plant [2, 4].

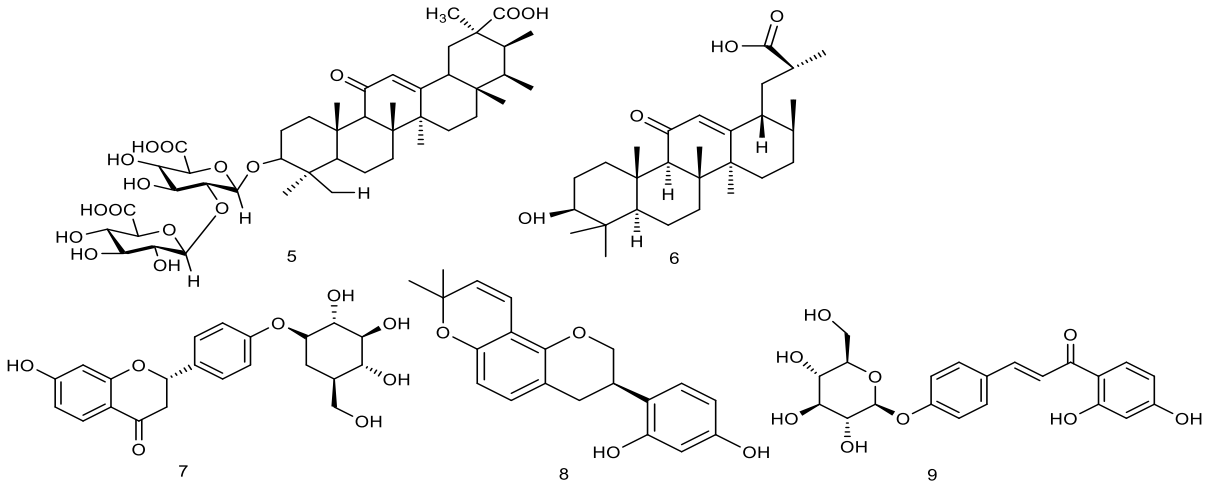

Fig. 1. Some chemical constituents from *Glycyrrhiza glabra* L.

ISSN 2734-9381
https://doi.org/10.51316/jst.181.etsd.2025.35.2.2
Received: Jan 17, 2025; revised: Feb 12, 2025
accepted: Feb 20, 2025

The root of *G. glabra* possesses diverse biological activities, including antioxidant, anti-inflammatory, expectorant, detoxifying, and hepatoprotective properties. It is used in the treatment of various conditions, such as peptic ulcers, bronchitis, eczema, herpes, and immune-related diseases. *G. glabra* also shows potential in cancer prevention, antioxidation, antibacterial action, and hormonal regulation. Extracts from the root have estrogenic and anti-estrogenic activities and can reduce serum testosterone levels in women. It is beneficial in managing autoimmune conditions and immune deficiencies such as AIDS [5, 6].

Melanin protects the skin against harmful stimuli, while its overproduction leads to skin hyperpigmentation, which can result in diseases such as lentigines, melasma, freckles, and skin cancer. Currently, the roots of *G. glabra* are commercially used as a natural treatment for skin whitening. Several compounds and extracts from *G. glabra* have been proven to inhibit melanogenesis [7]. However, limited research on chemical properties and melanin inhibition has been conducted in Vietnam. In this study, glabridin (1), 4'-*O*-methylglabridin (2), glabrol (3), and kanzonol Y (4), along with their melanin synthesis inhibitory activity, were investigated for the first time from *G. glabra* in Vietnam.

2. Materials and Methods

2.1. Plant Materials

Roots of *G.glabra* (Fig. 2) were collected in Kim Phu, Yen Son, Tuyen Quang province in September 2023. This plant was identified as *Glycyrrhiza glabra* L., belongs to genus *Glycyrrhiza*, family Fabaceae.

Fig. 2. Dry root of *Glycyrrhiza glabra* L.

2.2. General Experimental Procedures

The method of analysis and separation of the extracted residues was thin layer and column chromatography. The isolated compounds were identified by modern analytical methods such as 1D, 2D-NMR, and HR-MS.

Preparative HPLC was carried out using an AGILENT 1200 HPLC system, HPLC column YMC J'sphere ODS-H80 (4 µm, 20 × 250 mm) of brand YMC. Column chromatography was performed using either silica gel (Kieselgel 60, 70-230 mesh, and 230- 400 mesh, Merck) or RP-18 resin (150 µm, Fuji Silysia Chemical Ltd.) as the reversed-phase. Thin layer chromatography (TLC) was performed using 60 F254 (0.25 mm, Merck) and RP-18 F254S (0.25 mm, Merck) plates.

NMR spectra (including ^{1}H-NMR - Proton nuclear magnetic resonance, ^{13}C-NMR – carbon-13 nuclear magnetic resonance, HMBC - Heteronuclear Multiple Bond Correlation, HSQC - Heteronuclear Single Quantum Coherence) were recorded on an Agilent 600 NMR spectrometer (600 MHz for ^{1}H-NMR and 150 MHz for ^{13}C-NMR). Chemical shift (δ) was reported in parts per million (ppm) and abbreviations such as s (single), d (double), t (triplet), q (quadruplet), m (multiple), and br. (extensive) were used to report data. HR-MS mass spectra were recorded on an Agilent 1200 series LC-MSD Ion Trap. The melting point was measured on a Cole-Parmer Instrument electrothermal melting point instrument with serial number R216000334. Plant samples were extracted with methanol using Vevor Ultrasonic model JPS-100A.

2.3. Extraction and Isolation

The cleaned *G. glabra* roots were chopped, dried, and crushed. The resulting dry powder (12.0 kg) was extracted with methanol at room temperature (e times × 15 L, each extraction for 1 hour). The extracts were filtered and then distilled under reduced pressure to recover the solvent, yielding 400.0 g of methanol extract. The extract residue was dissolved in 2 liters of distilled water and then extracted with ethyl acetate (EtOAc), resulting in both EtOAc and aqueous extracts. These two extracts were concentrated under reduced pressure at 40-50 °C to recover the solvents. This process yielded an ethyl acetate residue (E, 158.78 g) and an aqueous residue (W).

The ethyl acetate extract (E, 158.78 g) of *G. glabra* powder was subjected to column chromatography, using silica gel as the stationary phase. The elution solvent system consisted of a dichloromethane/methanol (100/0, 50/1, 25/1, 10/1, 5/1, 2.5/1, v/v). The resulting fractions were evaporated under reduced pressure, yielding six fractions: E1, E2, E3, E4, E5, and E6.

Fraction E1 (23.71 g) was separated using silica gel as the adsorbent and eluted with a gradient solvent system of n-hexane/ethyl acetate (from 100/0 to 1/1, v/v), yielding seven fractions: E1A, E1B, E1C, E1D, E1E, E1F, and E1G. Fraction E1D (3.57 g) was impregnated with silica gel, evaporated under reduced pressure until the powder became loose and dry, and then subjected to column chromatography using silica

gel as the stationary phase with dichloromethane/acetone (100/1, 50/1, v/v) elution solvent system. Fractions were collected in 20 mL test tubes, and the elution was monitored by TLC using appropriate solvent systems and silica gel plates. Fractions displaying similar spots on TLC were combined into larger fractions, and the solvent was evaporated under reduced pressure to obtain eight subfractions (E1D1 to E1D8).

Fraction E1D5 (0.69 g) was further purified by column chromatography with silica gel as the stationary phase, using a solvent system of n-hexane/acetone/methanol (4/1/0.1, v/v/v), yielding three fractions: E1D5', E1D5'', and E1D5'''. Fraction E1D5''' (0.36 g) was further purified by column chromatography with silica gel as the stationary phase, using a solvent system of n-hexane/acetone/methanol (7/1/0.1, v/v/v), resulting in the isolation of compound **2** (70.08 mg).

Fraction E2 (33.83 g) was further separated by silica gel column chromatography using a gradient solvent system, yielding six fractions: E2A to E2F. Fraction E2B (3.79 g) was separated using silica gel as the stationary phase and dichloromethane:acetone (15/1, v/v) as the eluent, yielding three fractions: E2B1, E2B2, and E2B3. Fraction E2B2 was further purified by column chromatography using n-hexane/acetone/methanol (3/1/0.1, v/v/v), resulting in compound **1** (100.05 mg).

Fraction E2B1 (0.96 g) was subjected to column chromatography using silica gel as the stationary phase and n-hexane/dichloromethane/methanol (1/1/0.2, v/v/v) as the eluent system. The eluates were collected in 20 mL test tubes, and the progress of the elution was monitored by TLC on both normal- and reverse-phase plates with appropriate solvent systems. Tubes with similar TLC profiles were pooled into larger fractions, which were then evaporated under reduced pressure to yield six main fractions, E2B1A to E2B1F. Fraction E2B1B was then subjected to reverse-phase YMC column chromatography with acetone/water (2/1, v/v) as the eluent, resulting in four fractions: E2B1B', E2B1B", E2B1B"', and E2B1B"". Finally, fraction E2B1B" was purified by high-performance liquid chromatography (HPLC) using an acetonitrile 65% in water as the solvent system with a retention time of 45.25 minutes, yielding compound **3** (12.62 mg), while fraction E2B1B"' was purified by HPLC using an acetonitrile 65% in water as the solvent system with a retention time of 48.54 minutes, yielding compound **4** (22.17 mg).

2.4. Anti-Melanogenic Assay

The melanin synthesis inhibitory activity of compounds **1** and **2** was evaluated based on the melanin content in cultured cells. Prior to cellular melanin measurement, B16.F10 cells were cultured in 6-well plates at a concentration of 1×10^5 cells/mL and stabilized overnight. The cells were then incubated with different concentrations of the sample in the presence of α-MSH (10 nM) for 48 hours. Kojic acid (HiMedia) was used as a reference control. After 48 hours of incubation at 37 °C and 5% CO_2, the cells were harvested and washed with phosphate buffered saline. The cell residue was then dissolved in 1N NaOH solution containing 10% dimethyl sulfoxide (DMSO) and incubated at 80 °C for 1 hour. The absorbance of the extracted melanin was then measured at wavelengths 405/450 nm [8].

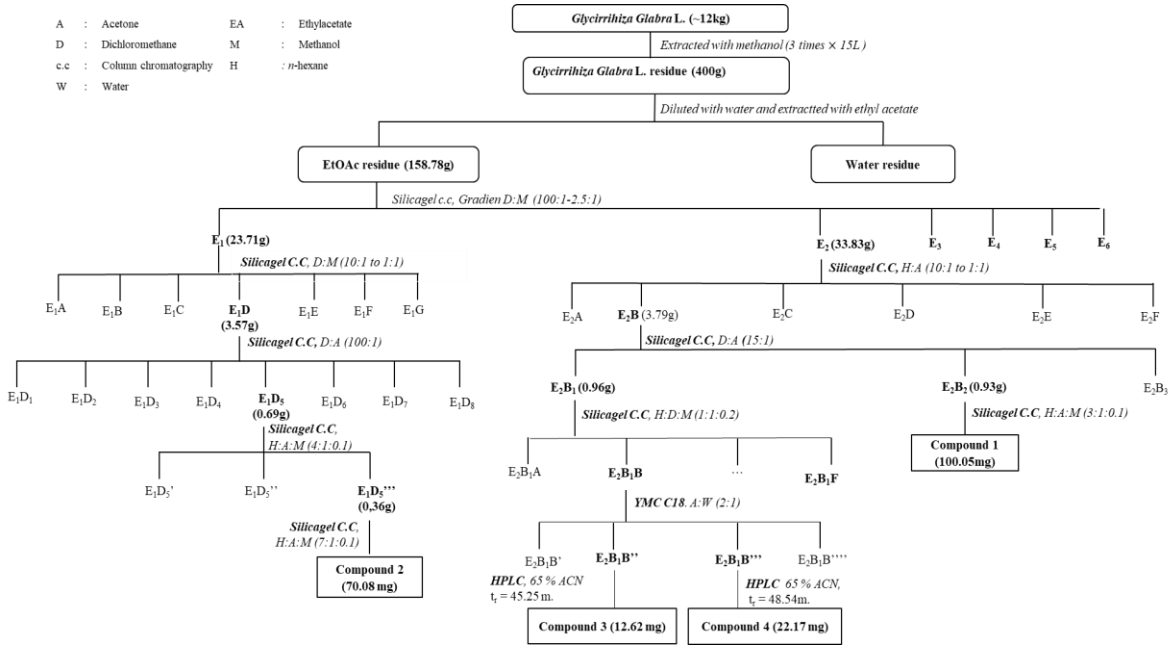

Fig. 3. Diagram of isolation of compounds **1-4** from *G.glabra*

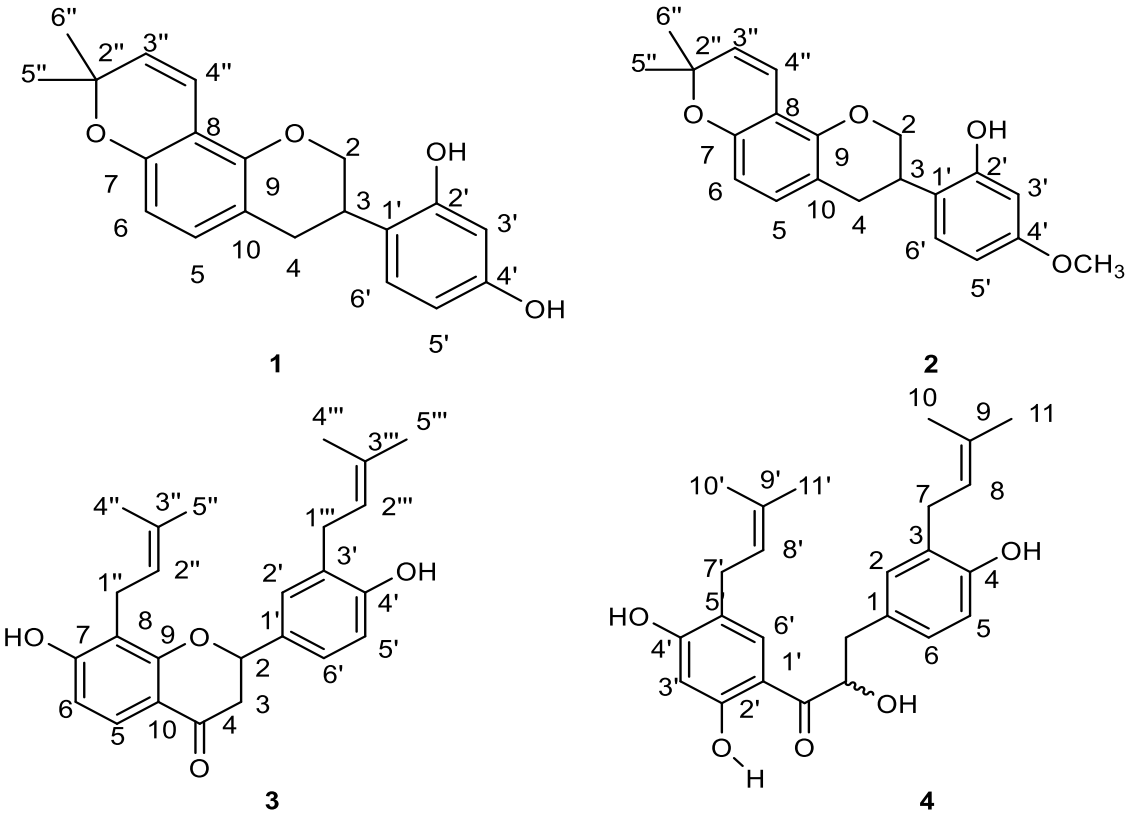

Fig. 4. The chemical structures of compounds **1-4**

Glabrindin (1): HR-MS: *m/z*= 325.1421[M+H]$^+$, calcd. for $[C_{20}H_{21}O_4]^+$ = 325.1434**.**

^{1}H-NMR (600 MHz, $CDCl_3$): δ_H (ppm) 7.29 (s, -OH); 7.26 (s, -OH); 6.90 (1H, d, *J* = 8.4 Hz, H-6'); 6.80 (1H, d, *J* = 8.4 Hz, H-5); 6.64 (1H, d, *J* = 9.6 Hz, H-4"); 6.41 (1H, d, *J* = 2.4 Hz, H-3'); 6.37 (1H, dd, *J* = 8.4, 2.4 Hz, H-5'); 6.35 (1H, d, *J* = 8.4 Hz, H-6); 5.54 (1H, d, *J* = 9,6 Hz, H-3"); 4.36 (1H, ddd, *J* = 10.2, 3.6, 1.8 Hz, H-2eq); 4.00 (1H, dd, *J* = 10.2 Hz, 10.2, H-2ax); 3.50 (1H, m, H-3ax); 2.96 (1H, dd, *J* = 15.6, 11.4 Hz, H-4ax), 2.82 (1H, ddd, *J* = 15.6, 4.8, 1.8 Hz, H-4eq); 1.42 (3H, s, H-5") and 1.40 (3H, s, H-6")

^{13}C-NMR (150 MHz, $CDCl_3$): δ_C (ppm) 155.8 (C-4'); 155.2 (C-2'); 151.7 (C-7); 149.8 (C-9); 129.2 (C-5); 128.9 (C-3"); 128.1 (C-6'); 119.5 (C-1'); 117.0 (C-4"); 114.7 (C-10); 109.9 (C-8); 108.6 (C-6); 107.5 (C-5'); 103.2 (C-3'); 75.6 (C-2"); 70.1 (C-2); 30.9 (C-3); 30.6 (C-4); 27.7 (C-5"); 27.5 (C-6").

4'-*O*-methylglabridin (2): HR-MS: m/z = 339.1580 [M+H]$^+$**,** calcd. for $[C_{21}H_{23}O_4]^+$, M = 339.1591**.**

^{1}H-NMR (600 MHz; $CDCl_3$): δ_H (ppm) 6.99 (1H, d, *J* = 8.4 Hz, H-6); 6.81 (1H, d, *J* = 8.4 Hz, H-6'); 6.64 (1H, d, *J* = 10.2 Hz, H-4"); 6.45 (1H, dd, *J* = 8.4, 2.4 Hz, H-5'); 6.37 (1H, d, *J* = 8.4 Hz, H-5); 6.34 (1H, d, *J* = 2.4 Hz, H-3'); 5.76 (1H, s, -OH); 5.55 (1H, d, *J* = 10.2 Hz, H-3"); 4.37 (1H, ddd, *J* = 10.2, 3.6, 1.8 Hz, H-2eq); 4.01 (1H, dd, *J* = 10.2, 10.2 Hz, H-2ax); 3.73 (3H, s, -OCH_3); 3.49 (1H, m, H-3ax); 2.96 (1H, dd, *J* = 15.6, 11.4 Hz, H-4ax H-4a); 2.84 (1H, ddd, *J* = 15.6, 4.8, 1.8 Hz, H-4eq); 1.42 (3H, s, H-5"); 1.41 (3H, s, H-6").

^{13}C-NMR (150MHz, $CDCl_3$): δ_C (ppm) 159.3 (C-4'); 154.6 (C-2'); 151.8 (C-7); 149.8 (C-9); 129.3 (C-5); 129.0 (C-3"); 128.2 (C-6'); 120.1 (C-4"); 117.0 (C-1'); 114.6 (C-10); 110.0 (C-8); 108.7 (C-6); 105.9 (C-5'); 102.2 (C-3'); 75.7 (C-2"); 70.1 (C-2); 31.7 (C-4), 30.6 (C-3); 27.8 (C-5"); 27.5 (C-4") and 55.4 (-OCH_3).

Glabrol (3): HR-MS: m/z = 391.1905 [M]$^+$, calcd. for $[C_{25}H_{26}O_4]^+$, M = 391.1904.

^{1}H- NMR (600 MHz; MeOD): δ_H (ppm) 7.59 (1H, d, *J* = 8.4 Hz, H-5); 7.20 (1H, d, *J* = 2.4 Hz, H-2'); 7.12 (1H, d, *J* = 8.4, 2.4 Hz, H-6'); 6.80 (1H, d, *J* = 8.4 Hz, H-5'); 6.52 (1H, d, *J* = 8.4 Hz, H-6); 5.33 (1H, m, H-2); 5.29 (1H, m, H-2"); 5.20 (1H, m, H-2'"); 3.33 (2H, m, H-1"); 3.30 (2H, m, H-1'"); 2.97 (1H, m, H-3a); 2,70 (1H, m, H-3b); 1.73 (3H x2, s, H-4'", H-5'") and 1,62 (3H x2, s, H-4", H-5").

^{13}C-NMR (150 MHz; MeOD): δ_C (ppm) 194.3 (C-4); 163.9 (C-7); 163.0 (C-9); 156.3 (C-4'); 133.1 (C-3'"); 132.1 (C-3"); 131.4 (C-1'); 129.4 (C-3'); 128.9 (C-2'); 126.7 (C-5); 125.9 (C-6'); 123.8 (C-2"); 123.3

(C-2'"); 117.1 (C-8); 115.6 (C-5'); 115.1 (C-10); 110.7 (C-6); 80.8 (C-2); 44.7 (C-3); 29.2 (C-1'"); 26.0 (C-4", C4'"); 23.1 (C-1"); 18.0 (C-5'") and 17.9 (C-5").

Kanzonol Y (4): Amorphous powder, HR-MS: *m/z* = 411.2153 $[M+H]^+$, calcd. for $[C_{25}H_{31}O_5]^+$, M = 411.2166

^{1}H-NMR (600 MHz, MeOD): δ_H (ppm) 7.36 (1H, s, H-6'); 6.87 (1H, dd, *J* = 8.4, 2.4 Hz, H-6); 6.79 (1H, d, *J* = 2.4 Hz, H-2); 6.67 (1H, d, *J* = 8.4 Hz, H-5); 6.30 (1H, s, H-3'); 5.28 (1H, m, H-8'); 5.21 (1H, m, H-8); 5.10 (1H, dd, J= 7.2, 5.4, H-α); 3.22 (2H, d, *J* = 7.2 Hz, H_2-7); 3.18 (2H, d, *J* = 7.2 Hz, H_2-7'); 2.98 (1H, dd, J_1 = 13.8, 5.4 Hz, H-β); 2.84 (1H, dd, J_1 = 13.8, 7.2 Hz, H-β); 1.78 (3H, s, H_3-11'); 1.72 (3H, s, H_3-10'), 1.71 (3H, s, H_3-11) and 1.67 (3H, s, H_3-10).

^{13}C-NMR (150 MHz, MeOD): δ_C (ppm) 205.0 (C=O); 165.1 (C-2'), 165.0 (C-4'); 154.8 (C-4); 133.9 (C-9); 132.8 (C-9'); 131. 7 (C-6'), 131.5 (C-2); 129.1 (C-1); 129.0 (C-3); 128.6 (C-6); 124. 0 (C-8); 123.4 (C-8'); 122.1 (C-5'), 111.8 (C-1'); 115.7 (C-5); 103.2 (C-3'); 74.5 (C-α); 42.9 (C-β); 29.1 (C-7); 28.4 (C-7'); 26.0 (C-11); 25.9 (C-11'); 17.9 (C-10) and 17.8 (C-10').

3. Results and Discussions

Four compounds were isolated from ethyl acetate extract of *G.glabra*. The chemical structures of the isolated compounds were determined based on modern spectroscopic methods such as one- and two-dimensional nuclear magnetic resonance and mass spectroscopy.

Compound **1** was isolated as an amorphous powder. ^{1}H-NMR spectrum of **1** showed oxymethylene group signals at δ_H 4.36 (1H, ddd, *J* = 10.2, 3.6, 1.8 Hz, H-2eq); 4.00 (1H, dd, *J* = 10.2, 10.2 Hz, H-2ax), one proton signal of methylene group at 3.50 (1H, m, H-3ax); 2 proton signals of methylene group at δ_H 2.96 (1H, dd, *J* = 15.6, 11.4 Hz, H-4ax), 2.82 (1H, ddd, *J* = 15.6, 4.8, 1.8 Hz, H-4eq). These data revealed an structure of an isoflavan [9]. Additionally, the spectrum presented signals of *ortho*-coupled aromatic protons and ABX spin coupling systems: [δ_H 6.80 (1H, d, *J* = 8.4 Hz, H-5), 6.35 (1H, d, *J* = 8.4 Hz, H-6) and δ_H 6.90 (1H, d, *J* = 8.4 Hz, H-6'), 6.37 (1H, dd, *J* = 8.4, 2.4 Hz, H-5'), 6.41 (1H, d, *J* = 2.4 Hz, H-3')], characteristic of two aromatic rings.

Furthermore, ^{1}H-NMR spectrum showed the proton signals assignable to one γ,γ-dimethylallyl δ_H 6.64 (1H, d, *J* = 9.6 Hz, H-4"), 5.54 (1H, d, *J* = 9.6 Hz, H-3"), 1.42 (3H, s, H-5"), 1.40 (3H, s, H-6").

Based on the above spectral data, compound **1** was suggested as an isoflavan skeleton, conjugating with a γ,γ-dimethylallyl group.

The ^{13}C-NMR spectrum combined with the HSQC spectrum showed the appearances of 20 carbon signals: 12 sp^2 aromatic carbon signals including: quaternary carbons bearing oxygen signals δ_C 155.8 (C-4'), 155.2 (C-2'), 151.7 (C-7), 149.8 (C-9); one oxymethylen at δ_C 70.1 (C-2); 1 CH group at δ_C 30.9 (C-3) suggesting a CO bond in the pyran ring. Combined with the ^{1}H-NMR spectrum, it could be confirmed that this compound is composed of an isoflavan framework. In addition, 3 carbon signals of CH group were dedermined as: 1 double bond C=C at δ_C 128.9 (C-3"), 117.0 (C-4"), one quaternary carbon signal attached to oxygen element at δ_C 75.6 (C-2"); 2 carbon signals of dimethylallyl group at δ_C 27.7 (C-5"), 27.5 (C-6").

HMBC spectrum allowed the confirmation of the position of groups in the molecule. The correlations between H-2 (δ_H 4.3, 4.0)/H-3 (δ_H 3.5)/H-4 (δ_H 2.96, 2.82)/H-5' (δ_H 6.37)/H-6' (δ_H 6.90) with C-1' and the correlations between H-6' (δ_H 6.90)/H-3 (δ_H 3.5)/H-4 (δ_H 2.96, 2.82) with C-3 (δ_C 30.9) indicated the linkage between the pyran ring and the aromatic ring at C-3, C-1'. The interactions between H-4" (δ_H 6.64) with C-7 (δ_C 151.7), C-9 (δ_C 149.8), C-8 (δ_C 109.9), C-2" (δ_C 75.6), between H-3" (δ_H 5.54) with C-8 (δ_C 109.9), C-2" (δ_C 75.6) established the pyran ring position at C-7 and C-8; The interaction of protons in the pyran ring with other carbons were implied based on the interaction between H-2 (δ_H 4.3, 4.0) with C-9 (δ_C 149.8)/C-3 (δ_C 30.9)/C-4 (δ_C 30.6); between H-4 (δ_H 2.96, 2.82) with C-9 (δ_C 149.9)/C-10 (δ_C 114.7)/ C-2 (δ_C 70.1)/C-3 (δ_C 30.9). These data were closely resembling those of glabrindin suggesting that **1** was glabridin (Table 1)

Moreover, the HR–MS spectrum of compound 1 showed an ion peak *m/z*= 325.1421 $[M+H]^+$, which suggested the molecular formula of $C_{20}H_{20}O_4$. From the above evidence, compound **1** was identified as glabridin.

Compound **2** was obtained as yellow solid**.** The NMR spectra of compound **2** was similar to NMR spectrum of **1** exception for the presence of proton signals of -OCH_3 at 3.73 ppm (3H, s) in ^{1}H-NMR and the signal of -OCH_3 (55.4 ppm) presented in the ^{13}C-NMR spectra. These data suggested that compound **2** was the oxmethyl derivative of glabridin. HSQC and HMBC spectra confirmed the structure of **2**. Especially the interaction of C-4' (δ_C 155.8) and -OCH_3 (δ_H 3.73) revealed the position of -OCH_3 at C-4'. Moreover, the molecular formula of **2** was $C_{20}H_{20}O_4$ as determined from its HR-MS (found *m/z*= 325.1421 $[M+H]^+$, calcd. for $[C_{20}H_{21}O_4]^+$ = 325.1434.

In addition, The NMR data of **2** were perfectly equivalent to those of 4'-*O*-methylglabridin (Table 1). From the above data, combined with the reference documents, it can be confirmed that compound **2** is 4'-*O*-methylglabridin.

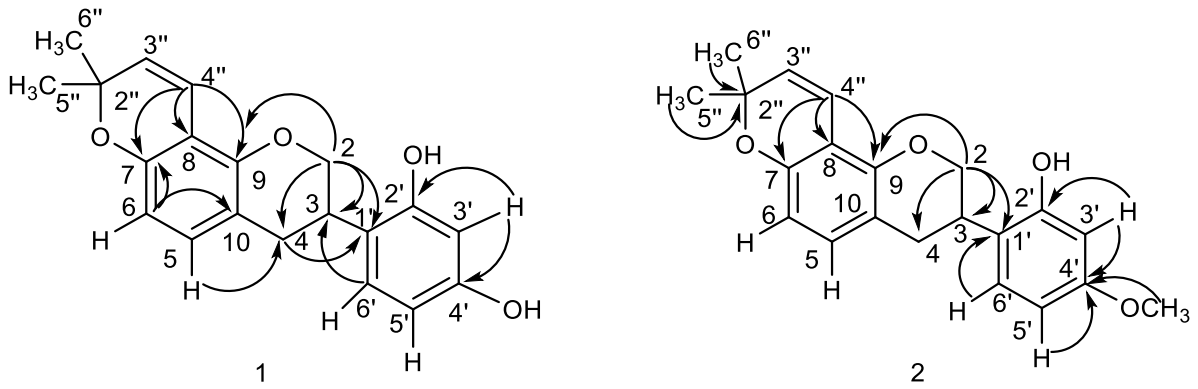

Fig. 5: The key HMBC correlations of compounds **1** and **2**

Table 1: ^{13}C-NMR spectral data of compound **1, 2** and reference compounds

C	**$\delta C^{a,b}$ (ppm)**	**$^{\#}\delta C^{a,b}$ (ppm)**	**$\delta C^{a,b}$ (ppm)**	**$^{\&}\delta C^{a,c}$ (ppm)**
1			-	-
2	70.1	69.8	70.1	70.0
3	30.9	31.0	30.6	30.5
4	30.6	30.9	31.7	31.8
5	129.2	129.4	129.3	129.2
6	108.6	108.2	108.7	108.7
7	151.7	151.3	151.8	151.7
8	109.9	109.2	110.0	109.9
9	149.8	149.4	149.8	149.7
10	114.7	114.8	114.6	114.5
1'	119.5	117.6	117.0	117.0
2'	155.2	156.0	154.6	154.4
3'	103.2	102.6	102.2	102.0
4'	155.8	157.0	159.3	159.2
5'	107.5	106.4	105.9	105.9
6'	128.1	127.7	128.2	128.1
1"	-		-	-
2"	75.6	75.3	75.7	75.7
3"	128.9	129.3	129.0	129.2
4"	117.0	116.5	120.1	119.9
5"	27.7	27.5	27.8	27.7
6"	27.5	27.3	27.5	27.4
-OH			-	-
-OCH_3			55.4	55.3

Recorded in a) $CDCl_3$, b)150MHz, c) 125 MHz, $^{\#}\delta C^{a,b}$ of glabridin measured in $CDCl_3$ [10], $^{\&}\delta C^{a,c}$ of 4'-O-methylglabridin measured in $CDCl_3$ [11].

Compound **3** was isolated as an amorphous compound. The ^{1}H-NMR spectrum of the compound **3** indicated a non- equivalent methylene at δ_H 2.97 (1H, m, H-3a); 2,70 (1H, m, H-3b), 1 signal of methin group at δ_H 5.33 (1H, m, H-2). Besides, NMR spectrum of **3** suggested two aromatic rings including one ABX spin coupling systems [δ_H 7.20 (1H, d, J = 2.4 Hz, H-2'), 7.12 (1H, d, J = 8.4, 2.4 Hz, H-6'); 6.80 (1H, d, J = 8.4 Hz, H-5')] and *ortho*-coupled aromatic protons δ_H 7.59 (1H, d, J = 8.4 Hz, H-5), 6.52 (1H, d, J = 8.4 Hz, H-6). This evidence suggested the framework structure of flavanone [12].

Furthermore two signals of methin olefin group at δ_H 5.29 (1H, m, H-2''), 5.20 (1H, m, H-2'''); 2 signals of methylene group at δ_H 3.33 (2H, m, H_2-1''), 3.30 (2H, m, H_2-1'''); 2 signals of methyl group δ_H 1.73 (3H x2, s, H_3-4''', H_3-5'''), 1.62 (3H x2, s, H_3-4'', H_3-5'') indicated the presence of two γ,γ-dimethylallyl.

The ^{13}C-NMR and HSQC spectra revealed 25 carbon signals: 1 carbonyl signal (C=O) at δ_C 194.3 (C-4); 1 signal for C-2 at δ_C 80.8, indicating a C=O bond in the pyran group; 12 sp² carbon signals from the aromatic ring. Among them, there were 2 signals for the quaternary carbons linked to an OH group at δ_C 163.9 (C-7), 156.3 (C-4') along with 5 signals for quaternary carbons carbons and 5 signals of CH groups characteristic of the flavanone framework. Additionally, 2 methyl olefin signals at δ_C 123.8 (C-2''), 123.3 (C-2''') as well as 4 signals of metyl group at δ_C 26.0 (C-4'', C4'''), 18.0 (C-5'''), 17.9 (C-5'') corresponded to prenyl groups.

The two-dimensional HMBC spectrum of confirmed structure of compound **3** such as the interactions H-1'' (δ_H 3.33) with C-7 (δ_C 163.9)/C-8 (δ_C 117.1)/C-9 (δ_C 163.0) and H_2-1''' (δ_H 3.30) with C-3' (δ_C 129.4)/C-2' (δ_C 128.9) confirmed positions of 2 prenyl groups. The piran ring position was indicated based on the correlations between H-2 and C-4 (δ_C 194.3/C-6' (δ_C 125.9)/C-1' (δ_C 131.4). Moreover, the HR-MS mass spectrometry showed an ion peak m/z 393.2953 $[M+H]^+$. The mass calculated for $[C_{25}H_{26}O_4]^+$ = 391.1904, which suggested the molecular formula of $C_{25}H_{28}O_4$. From the above spectral data and comparison with reference (Table 2), compound **3** was identified as glabrol (Table 2)

Compound **4** was obtained as a yellow solid. ^{1}H-NMR spectrum of compound **4** showed 16 proton signals. In there, 5 proton signals of the aromatic ring were at δ_H 7.36 (1H, s, H-6'), 6.87 (1H, dd, J = 8.4, 2.4 Hz, H-6), 6.79 (1H, d, J = 2.4 Hz, H-2), 6.67 (1H, d, J = 8.4 Hz, H-5); 6.30 (1H, s, H-3'); the hydroxymethine and methylene groups appeared at α and β position consequently*:* hydroxymethine group at δ_H 5.10 (1H, dd, J = 7.2, 5.4 Hz, H-α) and one methylene group signal at δ_H 2.98 (1H, dd, J = 13.8, 5.4 Hz, H-β), 2.84 (1H, dd, J = 13.8, 7.2 Hz, H-β) predicted a dihydrochalcone skeleton structure. In addition, there are 2 olefin proton signal H at δ_H 5.28 (1H, m, H-8'), 5.21 (1H, m, H-8), 2 proton signals of CH_2 group at δ_H 3.22 (2H, d, J = 7.2 Hz, H-7), 3.18 (2H, d, J = 7.2 Hz, H-7'); 4 proton signals of CH_3 group at δ_H 1.78 (3H, s, H-11'); 1.72 (3H, s, H-10'), 1.71 (3H, s, H-11), 1.67 (3H, s, H-10).

The ^{13}C-NMR spectrum combined with the HSQC spectrum showed the appearance of 25 carbon signals including: 1 signal of the C=O group at δ_C 205.0 representing the dihydrochalcone framework; 12 sp^2 aromatic carbons of which 3 carbons attached to OH groups at δ_C 165.1 (C-2'), 165.0 (C-4'), 154.8 (C-4); 1 carbon signal of methine group attached to hydroxy group at δ_C 74.5 (C - α); 1 carbon signal of methylene group at δ_C 42.9 (C-β) and 10 carbon signals belonging to 2 prenyl groups. Combined with the ^{1}H-NMR spectrum, compound **4** was implied as dihydrochalcon frame linked to 2 prenyl frames.

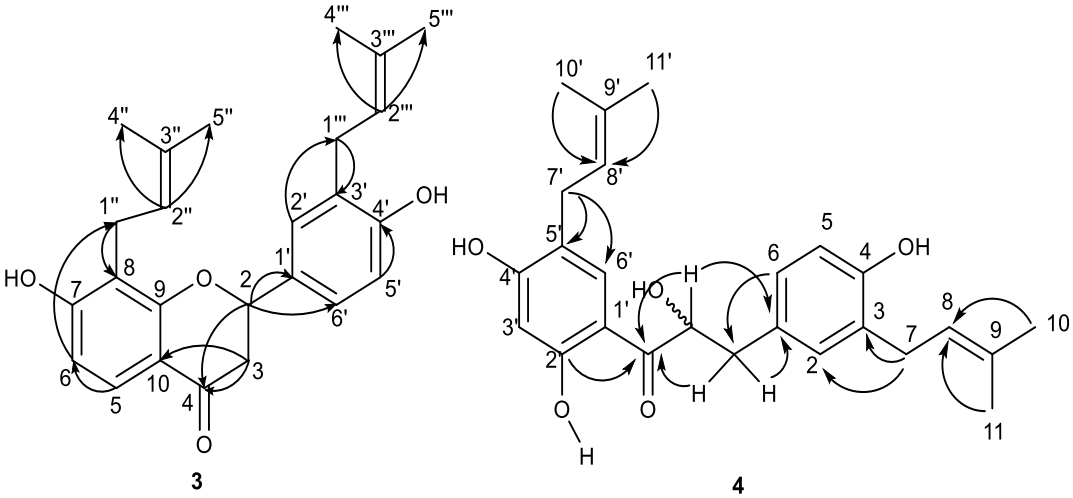

Fig. 6. The key HMBC correlations of compound **3** and **4**

HMBC spectrum confirmed the structure of **4.** The correlations from protons to carbons in 2 aromatic rings such as: from H-2 (δ_H 6.79)/H-5 (δ_H 6.67) to C-4 (δ_C 154.8)/C-1 (δ_C 129.1)/C-3 (δ_C 129.0); from H-6 (δ_H 6.87) to C-4 (δ_C 154.8)/C-2 (δ_C 131.5); from H-3' (δ_H 6.30) to C-4' (δ_C 165.0)/C-1' (δ_C 111.8)/C-2' (δ_C 103.2). The interaction between H-7 (δ_H 29.1) and C-2 (δ_C 131.5)/C–3 (δ_C 129.0)/C-8 (δ_C 124.0); between H-7' (δ_H 28.3) and C-6' (δ_C 132.0)/C-8' (δ_C 123.4)/C-5' (δ_C 122.1) indicated the position of 2 prenyl rings with aromatic rings at positions C-5' and C-3. The interactions between H-6' (δ_H 7.36) and C=O (δ_C 205.0); between H-α (δ_H 5.10) and C=O (δ_C 205.0)/C-1 (δ_C 129.1)/C-β (δ_C 42.9); between H-β (δ_H 2.98, 2.84) with C=O (δ_C 205.0)/C-2 (δ_C 131.5)/ C-1 (δ_C 129.1)/C- α (δ_C 74.5) confirmed the positions of C=O, α and β in the dihydrochalcone framework.

The NMR data of **4** were perfectly matching with those of kanzonol Y (Table 2). Besides, its HR-MS proved the molecular formula of $C_{25}H_{30}O_5$ (found *m/z* 411.2153 $[M+H]^+$, calculated for $[C_{25}H_{31}O_5]^+$ = 411.2166). From the above evidence, compound **4** was identified as kanzonol Y.

Table 2: ^{13}C-NMR spectral data for compound **3, 4** and reference compounds

C	$\delta C^{e,b}$ (ppm)	$^{\Omega}\delta C^{e,d}$ (ppm)	C	$\delta C^{e,b}$ (ppm)	$^{\epsilon}\delta C^{f,d}$ (ppm)
1	-	-	**1**	129.1	129.0
2	80.8	80.5	**2**	131.5	131.5
3	44.7	44.6	**3**	129.0	128.3
4	194.3	191.0	**4**	154.8	154.4
5	126.7	126.3	**5**	115.7	115.5
6	110.7	110.4	**6**	128.6	128.5
7	163.9	162.2	**7**	29.1	29.0
8	117.1	116.5	**8**	124.0	123.8
9	163.0	161.9	**9**	133.9	133.2
10	115.1	115.5	**10**	17.9	17.83
1'	131.4	131.6	**11**	26.0	25.9
2'	128.9	128.8	**1'**	111.8	111.4
3'	129.4	128.8	**2'**	165.1	164.9
4'	156.3	155.9	**3'**	103.2	103.3
5'	115.4	115.6	**4'**	165.0	163.9
6'	125.9	125.9	**5'**	122.1	121.5
1"	23.0	22.8	**6'**	132.0	132.1
2"	123.8	123.2	**7'**	28.3	28.2
3"	132.1	132.6	**8'**	123.4	123.1
4"	26.0	25.9	**9'**	132.8	132.1
5"	17.9	17.9	**10'**	17.8	17.79
1'"	29.2	29.1	**11'**	25.9	25.8
2'"	123.3	123.5	**C = O**	205.0	204.9
3'"	133.1	132.6	***α***	74.5	74.1
4'"	26.0	25.9	***β***	42.9	42.6
5'"	18.0	18.0			

Recorded in e) MeOD, f) Me_2CO-d_6, b)150MHz, d) 100 MHz, $^{\Omega}\delta C^{e,d}$ of glabrol measured in MeOD [13], $^{\epsilon}\delta C^{f,d}$ of kanzonol Y *measured in Me_2CO-d_6 [9].*

The anti-melanogenic activities of the biggest yelded compound, **1** and **2,** were evaluated. As the results (Table 3), compounds **1** and **2** inbibited melanogenic activities with IC_{50} values of 11.59 and 3.39 µg/mL, respectively. Both compounds exhibited better activity than Kojic Acid as reference control. This result suggested that glabrindin and 4'-*O*-methylglabridin could be potential melanogenic inhibitors from the *G.glabra.*

Table 3: Anti-melanogenic activities of **1**-**2**

Compounds	IC_{50} (µg/mL)
1	11.59
2	3.39
Kojic Acid*	15.88

*Positive control compound

4. Conclusion

This research was completed in the frame work of phytochemical study of *G.glabra*. Four compounds were isolated based on column chromatography methods. Their structures were identified on a basis of modern spectroscopy methods such as 1D, 2D-NMR, HR-MS. These components and their anti-melanogenic activity were firstly extracted and evaluated from *G.glabra* in Vietnam. This study contributed to clarify the chemical composition of *G.glabra* species and scientific information to the treasure of natural compounds in Vietnam.

References:

[1] Do Tat Loi, Vietnamese Medicinal Plants and Herbs, vol. 1, Hong Duc Publishng House, Hanoi, 2004, pp. 863-868.

[2] Wei Li, Yoshihisa Asada, Takafumi Yoshikawa, Flavonoid constituents from *Glycyrrhiza glabra* hairy root cultures, Phytochemistry, vol. 55, iss. 5, pp. 447-456, Nov. 2000. https://doi.org/10.1016/S0031-9422(00)00337-X

[3] Gaber El-Saber Batiha, Amany Magdy Beshbishy, Amany El-Mleeh, Mohamed M.Abdel-Daim and Hari Prasad Devkota, Traditional uses, bioactive chemical constituents, and pharmacological and toxicological activities of *Glycyrrhiza glabra* L. (Fabaceae), Biomolecules, vol. 10, iss. 3, Feb. 2020. https://doi.org/10.3390/biom10030352

[4] Yoshikazu Yamamura, Junichi Kawakami, Tomofumi Santa, Hajime Kotaki, Katsuyoshi Uchino, Yasufumi Sawada, Naomi Tanaka, Tatsuji Iga, Pharmacokinetic profile of glycerrhizin in healthy volunteers by a new high-performance liquid chromatographic method, Journal of Pharmaceutical Sciences, vol. 81, iss. 10, pp. 1042–1046, Oct. 1992. https://doi.org/10.1002/jps.2600811018

[5] Rajandeep Kaur, Harpreet Kaur and Ajaib Singh Dhindsa, *Glycyrrhiza glabra*: a phytocharmacologycal review, International Journal of Pharmaceutical Sciences and Research, vol. 4, pp. 2470-2477, 2013. http://dx.doi.org/10.13040/IJPSR.0975-8232.4(7).2470-77

[6] Giulia Pastorino, Laura Cornara, M. Beatriz P.P. Oliveira Sónia Soares, Francisca Rodrigues, Liquorice (Glycyrrhiza glabra): a phytochemical and pharmacological review, Phytotherapy Research, vol. 32, pp. 2323–2339, Aug. 2018. https://doi.org/10.1002/ptr.6178

[7] Antonietta Cerulli, Milena Masullo, Paola Montoro and Sonia Piacente, Licorice (*Glycyrrhiza glabra, G. uralensis*, and *G. inflata*) and their constituents as active cosmeceutical ingredients, Cosmetics, vol. 9, iss. 1, pp. 1-19, Jan. 2022. https://doi.org/10.3390/cosmetics9010007

[8] Hyun Ji Kim, Hye Ja Lee, Mi Kyung Park, Kyung Jin Gang, Hyun Jung Byun, Jeong Ho Park, Mi Kyung Kim, Soo Youl Kim, and Chang Hoon Lee, Involvement of transglutaminase-2 in α-MSH-induced melanogenesis in SK-MEL-2 human nelanoma cells, Biomolecules & Therapeutics, vol. 22, pp. 207-212, May. 2014. https://doi.org/10.4062/biomolther.2014.031

[9] Toshio Fukai, Cai Bao Sheng, Tukasa Horikoshi, Taro Nomura, Isoprenylated flavonoids from underground parts of *Glycyrriza glabra*, Phytochemistry, vol. 43, iss. 5, pp. 1119-1124, Nov. 1996. https://doi.org/10.1016/S0031-9422(96)00391-3

[10] Simmler Charlotte, Pauli Guido F., Chen Shao-Nong, Phytochemistry and biological properties of glabridin, Fitoterapia, vol. 90, pp. 160-184, Oct. 2013. https://doi.org/10.1016/j.fitote.2013.07.003

[11] Sejal Patel and Dr. Rakesh Jat, Isolation and characterization of some natural compound from liquorice, Journal of Pharmacognosy and Phytochemistry, vol. 6, iss. 3, pp. 210-220, 2017.

[12] Wen-Li Lo, Fang-Rong Chang, Tian-Jye Hsieh and Yang-Chang Wu, The Constituents of *Euchresta formosana*, Journal of the Chinese Chemical Society, vol. 49, iss. 3, pp. 421-426, Sep. 2013. https://doi.org/10.1002/jccs.200200066

[13] Yoshihisa Asada, Wei Li, Takafumi Yoshikawa, Biosynthesis of the dimethylallyl moiety of glabrol in *Glycyrrhiza glabra* L. hairy root cultures via a non-mevalonate pathway, Phytochemistry, vol. 55, iss. 4, pp. 323-326, Oct. 2000. https://doi.org/10.1016/S0031-9422(00)00327-7