Continuous Research on the Extraction of Stilbenes from Gnetum montanum Markgr. in Vietnam

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Abstract

Gnetum montanum Markgr. belongs to the Gnetaceae family. Gnetum montanum Markgr. is distributed in the northern mountainous parts of Vietnam and is used as a Vietnamese herbal medicine for the treatment of rheumatoid arthritis, muscular strain and malaria. Previous phytochemical investigations on Gnetum plants have revealed an abundance of flavonoids and stilbenoids. With the goal of isolating compounds from this species in Vietnam, in the continuous phytochemical investigation of stem of Gnetum montanum Markgr., we used extraction methods with increasing polar solvents and modern chromatography methods such as thin-layer chromatography (TLC), column chromatography (CC), and high-performance liquid chromatography (HPLC). They were two dimeric stilbenes gneafricanin F (1), gnetuhainin Q (2) together with a stilbene 2,3',5'-trihydroxy-trans-stilbene (3) and a flavonoid homoeriodictyol (4). Their structures were elucidated by ESI-MS, 1D-NMR, and 2D-NMR combined with comparison with published spectral data.

Keywords: *Gnetum montanum* Markgr., stilbenoids, gneafricanin F, gnetuhainin Q, 2,3',5'-trihydroxy-*trans*-stilbene, homoeriodictyol

1. Introduction

The genus Gnetum is a unique number in the family Gnetaceae and the order Gnetales, comprising approximately 30-35 species of gymnosperms. They are usually tropical trees, shrubs, and climbers that can be found in large numbers in Africa, South America and Southeast Asia. In Vietnam, the genus Gnetum has about 6 species (Nguyen Tien Ban, 2003) [1]. Previous studies have shown that the composition of species in the genus Gnetum are abundant. They are rich in stilbenoids, oligostilbenoids, alkaloids, and flavonoids. Many stilbene derivatives, represented by resveratrol, rhapontigenin, and isorhapontigenin have been isolated from the genus Gnetum. The common stilbenoid derivatives have been found in many species in the genus Gnetum. Many different stilbene-based compounds have been classified, namely gnetifolin A, gnetifolin C, gnetifolin D, gnetifolin E, gnetifolin F, gnetifolin P, resveratrol, isorhapotigenin, lehmbachol A, and lehmbachol D (Li-Qin et al., 2008 [2], Lin et al., 1992 [3], Chun-Suo et al., 2005 [4]). The compounds in the genus Gnetum exhibit a wide range of biological activities, such as anti-inflammatory (Kaisheng et al., 2001) [5]; antibacterial, antifungal (Sakagami et al., 2007) [6]; antioxidant (Ibrahim et al., 2003) [7], and anti-cancer (Ka-Wing et al., 2008) [8]. In Vietnam, the chemical compositions of some plants belonging to the genus *Gnetum* have also been studied. In 2014, Nguyen Ba Anh and Trieu Duy Viet [9] isolated G. latifolium Blume and obtained two

stilbenoids, isorhapontin and gnetifolin E. Le Huu Tho *et al.* (2019) [10] isolated alkaloids including nicotinic acid, uracil, thymine, 2'-deoxyuridine, uridine, and thymidine. In 2022, from the stem of Gnetum latifolium, Le Thi Hong Nhung *et al.* [11] isolated (±)-bisisorhapotingenin A and gnetifolin K.

Gnetum montanum Markgr. (G. montanum) is a climbing plant, distributed in the wild at an altitude of 200-1.200 m in the mountainous region of Northern Vietnam. It is used as a herbal medicine to treat rheumatoid arthritis, arthritis, and malaria (Do Tat Loi, 2004) [12]. Previous phytochemical studies have shown that the plant contains a high amount of flavonoids and stilbenoids (Li-Qin et al., 2008) [2]. In Vietnam, some studies on the chemical composition and biological activity of this species revealed attractive results. From the stem harvested in Tuyen Quang, the compounds trans-resveratrol, resveratroloside, and isorhapontigenin-13-glucoside were classified and their structures were determined (Vu Thi Lan Phuong, 2019) [13]. In our study, many stilbenes were isolated from characterized this plant In the course of phytochemical research of this plant, four compounds including gneafricanin F (1), gnetuhainin Q (2) 2,3',5'-trihydroxy-trans-stilbene (3), and homoeriodictyol (4) from G. montanum collected in Quang Tri province, Vietnam were isolated and purified continuously. Their chemical structures determined by modern spectroscopic methods and compared with published literature.

ISSN 2734-9381

https://doi.org/10.51316/jst.183.etsd.2025.35.3.4 Received: Feb 7, 2025; revised: Mar 14, 2025;

accepted: Apr 1, 2025

Fig. 1. Some compounds from *Gnetum montanum* Markgr: lembachol (5), isorhapontin (6), gnetifolin E (7), gnetifolin K (8), gnetifolin A (9), trans-pynosylvin (10)

2. Materials and Methods

2.1. Plant Materials

The stems of *G. montanum* (Fig. 2) were collected in Vinh Trung, Vinh Linh, Quang Tri Province, Vietnam in December 2020 and identified by Dr. Nguyen The Cong, Institute of Ecology and Biological Resources.



Fig. 2. Stems of Gnetum montanum Markgr

2.2. General Experimental Procedures

The method of analysis and separation of the extracted residues was thin layer and column

chromatographies. The isolated compounds were identified by modern analytical methods such as 1D, 2D-NMR, and HR-MS. Preparative high performance liquid chromatography (HPLC) was carried out using an AGILENT 1200 HPLC system.

Column chromatography was performed using either silica gel (Kieselgel 60, 70-230 mesh, and 230-400 mesh, Merck), RP-18 resin (150 $\mu m,$ FuJi Silysia Chemical Ltd.) as the reversed-phase or Sephadex LH-20 (Amersham Biosciences, Sweden). Thin layer chromatography (TLC) was performed using 60 F254 (0.25 mm, Merck) and RP-18 F254S (0.25 mm, Merck) silica-gel thin plates.

NMR spectra were recorded on an Agilent 600 MR NMR spectrometer (600 MHz for 1 H-NMR and 150 MHz for 13 C-NMR). Chemical shift (δ) is reported in parts per million (ppm) and abbreviations such as s (single), d (double), t (triplet), q (quadruplet), m (multiple), and br (extensive) are 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. Plant samples were extracted with methanol using Vevor Ultrasonic model JPS-100A.

2.3. Extraction and Isolation

The cleaned G. montanum stems were chopped, dried, and crushed. The resulting dry powder stems (12.0 kg) were extracted with methanol at room temperature (3 times × 15 L, every 1 h). The obtained extracts were filtered and distilled under reduced pressure to recover the solvent and to yield 600.0 g of methanol extract (GM). This residue is dissolved in water and extracted with dichloromethane and ethyl acetate, respectively. The dichloromethane and ethyl acetate extracts were distilled to recover the solvent under reduced pressure to obtain the dichloromethane fraction (GM.D, 40 g), the ethyl acetate fraction (GM.E, 60 g), and water residue. The GM.E was separated on a silica gel chromatographic column with a gradient elution system of D/M $(100/0 \rightarrow 0/100, \text{ v/v})$, four segments are obtained: GM2B, GM2C, GM2D, and GM2E. The GM2C and GM2D fractions were combined and chromatographed on a silica gel column with D/M elution system (100/0-1/1, v/v) as diluent to obtain five subfractions GM4B, GM4D, GM4E, GM4F, and GM4G.

The GM4F was separated on a silica-gel column with the solvent system D/M (10/1, v/v) to produce 3 fractions: GM27A, GM27B, and GM27C. GM27C was further separated on a Sephadex column with the solvent system A/W (1/1, v/v) to obtain a fraction GM28A. GM28A was separated on HPLC using acetonitrile: water 33% volume as the solvent system at the retention time of 57 minutes resulting compound 1 (21.0 mg). The fraction GM27B was separated on a

reverse phase silica-gel column with an elution system of A/W (1/1, v/v) to obtain 3 subfractions GM29C, GM29D, and GM29F. GM29F was separated on HPLC using acetonitrile: water (35% volume) solvent system at the retention time of 44 minutes to yield 2 (6.0 mg).

The fractions GM4D and GM4E were combined and then chromatographed on a silica gel column with D/M elution system (12/1, v/v) to obtain 3 subfractions: GM13A, GM13B, and GM13C. The GM13B was loaded on the reverse phase YMC column and eluted with a A/W elution system (1/1.5, v/v) to obtain three fractions GM15A, GM15B, and GM15C. The GM15C subfraction was further separated chromatographically on an HPLC chromatograph with ACN/water elution system (38%, v/v) and at the retention time of 47 minutes to obtain compound 3 (8.2 mg).

The GM4B was separated on a silica-gel column with the solvent system H/A (2/1, v/v) to produce 4 fractions: GM21E, GM21F, GM21H, and GM21K. GM21H was further separated on a reverse phase silica-gel column with the solvent system M/W (1/1, v/v) obtains 2 fractions GM24C and GM24B. GM24C was separated on HPLC using acetonitrile: water 38% volume, at the retention time of 57 minutes resulting compound 4 (4.0 mg). Column chromatographic performance was monitored by thin-layer chromatography (see Fig. 3, Fig. 4). The separated compounds were structurally determined by 1D, 2D-NMR, and Mass spectroscopy methods.

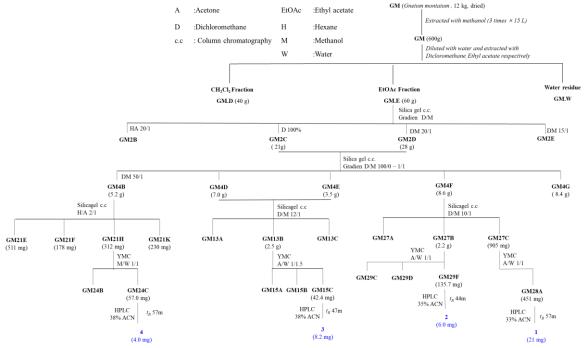


Fig. 3. Diagram of isolation of compounds 1-4 from Gnetum montanum Markgr

Fig. 4. Four compounds were isolated from Gnetum montanum Markgr

Gneafricanin F (1): White amorphous powder, The HR-ESI-MS: m/z 513.1559 [M-H]⁻, calcd. for $[C_{30}H_{25}O_8]^-$ 513.1550

¹H-NMR (600 MHz, MeOD), δ (ppm): 6.71 (2H, d, J = 7.8 Hz, H-5a, 5b), 6.69 (2H, d, J = 1.8 Hz, H-2a, 2b), 6.60 (2H, dd, J = 7.8, 1.8 Hz, H-6a, 6b), 6.58 (2H, d, J = 1.8 Hz, H-14a, 14b), 6.15 (2H, d, J = 1.8 Hz, H-12a, 12b), 4.51 (2H, br s, H-7a, 7b), 3.80 (2H, br s, H-8a, 8b), 3.77 (6H, s, 3a-OMe, s, 3b-OMe)

¹³C-NMR (150 MHz, MeOD), δ (ppm): 159.4 (2C, C-13a, C-13b), 155.6 (2C, C-11a, C-11b), 150.8 (2C, C-9a, C-9b), 148.8 (2C, C-3a, C-3b), 145.5 (2C, C-4a, C-4b), 139,2 (2C, C-1a, C-1b), 123.7 (2C, C-10a, C-10b), 120.7 (2C, C-6a, C-6b), 116.1 (2C, C-5a, C-5b), 112.1 (2C, C-2a, C-2b), 103.4 (2C, C-14a, C-14b), 102.6 (2C, C-12a, C-12b), 60.9 (2C, C-8a, C-8b), 55.2 (2C, C-7a, C-7b)

Gnetuhainin Q (2): Pale white amorphous powder; The HR-ESI-MS: m/z 485.1571 [M+H]⁺, calcd. for $[C_{29}H_{25}O_7]^+$ 485.1600.

¹H-NMR (600 MHz, MeOD), *δ* (ppm): 7.40 (1H, dd, J = 7.8, 1.8 Hz , H-6b), 7.21 (1H, br s, H-2b), 7.00 (1H, d, J = 16.2 Hz, H-7b), 6.91 (1H, d, brs, H-2a), 6.89 (1H, d, J = 7.8 Hz, H-5b), 6.80 (1H, d, J = 16.2 Hz, H-8b), 6.80 (2H, m, H-5a, 6a), 6.45 (2H, d, J = 1.8 Hz, H-10b, 14b), 6.22 (1H, t, J = 1.8 Hz, H-12b), 6.17 (2H, d, J = 1.8 Hz, H-10a, 14a), 6.17 (1H, d, J = 1.8 Hz, H-12a), 5.41 (1H, d, J = 9.0 Hz, H-7a), 4.43(1H, d, J = 9.0 Hz, H-8a), 3.89 (3H, s, 3a-OMe)

¹³C-NMR (150 MHz, MeOD), δ (ppm): 161.0 (C-4b), 160.0 (C-11a, C-13a), 159.6 (C-11b, C-13b), 149.1 (C-3a), 147.8 (C-4a), 145.3 (C-9a), 141.2 (C-9b), 132.4 (C-1b, C-3b), 129.4 (C-7b), 128.7 (C-6b), 127.5 (C-8b), 124.2 (C-2b),

120.1 (C-6a), 116.2 (C-5a), 113.5 (C-1a), 110.7 (C-2a), 110.4 (C-5b), 107.8 (C-10a, C-14a), 105.9 (C-10b, C-14b), 102.5 (C-12b), 95.0 (C-7a), 58.9 (C-8a), 56.4 (3a-OMe).

2,3',5'-trihydroxy-*trans***-stilbene (3):** White amorphous powder. The HR-ESI-MS: m/z 226.9783 [M-H]⁻, calcd. for $[C_{14}H_{11}O_{3}]^{-}$ 227.0703.

¹H-NMR (600 MHz, MeOD), *δ* (ppm): 7.51 (1H, d, J = 7.8 Hz, H-6), 7.38 (1H, d, J = 16.2 Hz, H- α), 7.07 (1H, dd, J = 7.8, 7.8, H-5), 6.99 (1H, d, J = 16.2 Hz, H- β), 6.83 (1H, dd, J = 7.8, 7.8, H-4), 6.81 (1H, d, J = 7.8 Hz, H-3), 6.51 (2H, d, J = 2.4, H-2', H-6'), 6.19 (1H, dd, J = 2.4, 2.4 Hz, H-4')

¹³C-NMR (150 MHz, MeOD), δ (ppm): 159.9 (C-3', C-5'), 156.1 (C-2), 141.6 (C-1'), 129.4 (C-4), 129.4 (C-6), 127.4 (C- β), 125.7 (C-1), 124.7 (C- α), 120.8 (C-5), 116.6 (C-3), 102.8 (C-4'), 105.9 (C-2', C-6').

Homoeriodictyol (4): white amorphous powder. The HR-ESI-MS: m/z 303.0886 [M+H]⁺, calcd. for $[C_{16}H_{15}O_6]^+$ 303.0863.

¹H-NMR (600 MHz, MeOD), δ (ppm): 7.09 (1H, d, J = 2.4 Hz, H-5'), 6.94 (1H, dd, J = 7.8, 2.4 Hz, H-6'), 6.84 (1H, d, J = 7.8, H-2'), 5.92 (1H, d, J = 1.8 Hz, H-6), 5.90 (1H, d, J = 1.8 Hz, H-8), 5.36 (1H, dd, J = 12.6, 3.0, H-2), 3.90 (3H, s, 3'-OMe), 3.15 (1H, dd, J = 16.8, 12.6, H-3), 2.73 (1H, dd, J = 16.8, 3.0 Hz, H-3).

 $^{13}\text{C-NMR}$ (150 MHz, MeOD), δ (ppm): 197.6 (C-4), 168.8 (C-7), 165.5 (C-5), 164.8 (C-9), 149.1 (C-3'), 148.1 (C-4'), 131.8 (C-1'), 120.5 (C-6'), 116.2 (C-5'), 111.3 (C-2'), 96.3 (C-6), 96.3 (C-8), 80.7 (C-2), 56.5 (3'-OMe), 44.2 (C-3).

JST: Engineering and Technology for Sustainable Development Volume 35, Issue 3, July 2025, 025-033

Table 1. ¹³C-NMR spectral data for compounds **1** and **2** and reference compounds

1			2		
C	#δ C	$\delta c^{a,b}$	C	$^{\Omega}\!\delta_{ m C}$	$\delta_{\mathrm{C}^{\mathrm{a,b}}}$
1a	138.1	139.2	1a	132.7	133.5
2a	111.6	112.1	2a	110.9	110.7
3a	147.8	148.8	3a	148.3	149.1
4a	145.3	145.5	4a	147.4	147.8
5a	115.3	116.1	5a	115.5	116.2
6a	120.1	120.7	6a	120.1	120.1
7a	54.4	55.2	7a	94.2	95.0
8a	60.6	60.9	8a	57.7	58.9
9a	150.2	150.8	9a	144.9	145.3
10a	122.9	123.7	10(14)a	107.3	107.8
11a	155.1	155.6	11(13)a	159.6	160.0
12a	102.3	102.6	12a	102.5	102.8
13a	159.1	159.4	1b	131.6	132.4
14a	103.3	103.4	2b	123.8	124.2
1b	138.1	139.2	3b	132.1	132.4
2b	111.6	112.1	4b	160.5	161.0
3b	147.8	148.8	5b	110.6	110.4
4b	145.3	145.5	6b	128.5	128.7
5b	115.3	116.1	7b	129.0	129.4
6b	120.1	120.7	8b	127.1	127.5
7b	54.4	55.2	9b	140.6	141.2
8b	60.6	60.9	10(14)b	105.5	105.9
9b	150.2	150.8	11(13)b	159.4	159.6
10b	122.9	123.7	12b	102.2	102.5
11b	155.1	155.6	MeO(C-3a)	56.2	56.4
12b	102.3	102.6			
13b	159.1	159.4			
14b	103.3	103.4			

Recorded in ^{a)} MeOD, ^{b)} 150MHz, [#] $\delta_{\rm C}$ of gneafricanin F, ${}^{\Omega}\delta_{\rm C}$ of gnetuhainin Q

3. Results and Discussions

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 from the fraction of ethyl acetate extract of G. montanum as an amorphous powder. The ¹H-NMR spectrum of compound 1 exhibited two distinct sets of proton signals corresponding to two 1,3,4-trisubstituted benzene rings. Notable signals included δ 6.60 (2H, doublet of doublets, J = 1.8, 7.8 Hz, H-6a, 6b), 6.71 (2H, doublet, J = 7.8 Hz, H-5a, 5b), and 6.83 (2H, doublet, J = 2.0 Hz, H-2a, 2b). The spectrum also displayed two sets of meta-coupled protons from a 1,2,3,5-tetrasubstituted benzene ring at δ 6.20 (2H, doublet, J = 2.0 Hz, H-12a, 12b) and 6.64 (2H, doublet, J = 2.0 Hz, H-14a, 14b). Additionally, methine-coupled protons were observed at δ 3.80 (2H, broad singlet, H-8a, 8b) and 4.51 (2H, broad singlet, H-7a, 7b). The presence of two methoxy groups was noted at δ 3.8 (6H, broad singlet, OCH₃), alongside signals indicative of six hydroxyl groups.

The combination of ¹H and ¹³C-NMR spectrum for compound **1** with the similar signals allowed for the identification of a symmetrical structure. Signals of 1,3,4-substituted rings at $\delta_{\rm C}$ 139.2 (2C, C-1a, C-1b), $\delta_{\rm C}$ 148.8 (2C, C-3a, C-3b), and $\delta_{\rm C}$ 145.5 (2C, C-4a, C-4b). The signals at $\delta_{\rm C}$ 148.8 (2C, C-3a, C-3b), 145.5 (2C, C-4a, C-4b), 155.6 (2C, C-11a, C-11b), and 159.4 (2C, C-13a, C-13b) suggested the presence of oxygen bonded to these sp^2 carbons, which are not attached to hydrogen. Furthermore, the structure of compound **1** was clearly confirmed through two-dimensional NMR spectra including HSQC and HMBC.

In the HMBC spectrum, it was established that C-1a ($\delta_{\rm C}$ 139.2) connects directly to C-7a ($\delta_{\rm C}$ 55.2) via the interaction of H-1a ($\delta_{\rm H}$ 4.51) with C-7a ($\delta_{\rm C}$ 55.2), and similarly, the interaction of H-7b ($\delta_{\rm H}$ 4.51) with C-1b ($\delta_{\rm C}$ 138.0) corroborated the direct bond between C-7b and C-1b. Proton signals from H-7a ($\delta_{\rm H}$ 4.51) to C-10b ($\delta_{\rm C}$ 123.7) and H-8b ($\delta_{\rm H}$ 3.80) to C-9b ($\delta_{\rm C}$ 150.8) indicated the presence of a ring cyclopentane, with $\delta_{\rm C}$ values of 59.9 (CH, C-2), 148.6 (C, C-3), 123 (C, C-8), 52.1 (CH, C-9), and 56.6 (CH, C-10) (Fig. 5).

Moreover, interactions involving H-7b ($\delta_{\rm H}$ 4.51) and C-10a ($\delta_{\rm C}$ 123.7), along with H-8a ($\delta_{\rm H}$ 3.80) and C-9a ($\delta_{\rm C}$ 89.4), plus long-range correlations between H-1 ($\delta_{\rm H}$ 4.71) and C-11 ($\delta_{\rm C}$ 75), and H-11 ($\delta_{\rm H}$ 4.5, $\delta_{\rm H}$ 3.57) with C-1 ($\delta_{\rm C}$ 89.4) further suggested the presence of cyclopentane. The HSQC data confirmed the positions of carbons and protons, evidenced by crossed peaks among H-2a, H-2b ($\delta_{\rm H}$ 6.69) and C-2a,

C-2b (δ C 112.1), H-5a, H-5b ($\delta_{\rm H}$ 6.71) and C-5a, C-5b ($\delta_{\rm C}$ 116.1), H-6a, H-6b ($\delta_{\rm H}$ 6.60) and C-6a, C-6b ($\delta_{\rm C}$ 120.7), H-7a, H-7b ($\delta_{\rm H}$ 4.51) and C-7a, C-7b ($\delta_{\rm C}$ 55.2), H-8a, H-8b ($\delta_{\rm H}$ 3.8) and C-8a, C-8b ($\delta_{\rm C}$ 60.9), as well as H-12a, H-12b ($\delta_{\rm H}$ 6.15) and C-12a, C-12b ($\delta_{\rm C}$ 102.6), and H-14a, H-14b ($\delta_{\rm H}$ 6.58) and C-14a, C-14b ($\delta_{\rm C}$ 103.4). All NMR data for compound 1 were consistent with those of gneafricanin F (Table 1). Additionally, high-resolution mass spectrometry (HR-MS) indicated an ion peak at m/z [M-H]⁻ 513.1559 suggesting the molecular formula C₃₀H₂₆O₈. Based on the spectral data and comparisons with literature [15], compound 1 was identified as gneafricanin F.

Compound **2** was isolated from the fraction of ethyl acetate extract of *G. montanum* as a pale white amorphous powder. The ¹H-NMR spectrum of **2** showed two proton signals at $\delta_{\rm H}$ 6.17 (2H, d, J=1.8 Hz), $\delta_{\rm H}$ 6.17 (1H, t, J=1.8 Hz), assigning to a three-substituted benzene ring, including two symmetrical carbons at positions C-11a and C-13a. Similarly, two signals at $\delta_{\rm H}$ 6.45 (2H, d, J=1.8 Hz), $\delta_{\rm H}$ 6.22 (1H, t, J=1.8 Hz) suggested the appearance of a three-substituted benzene ring, including two symmetrical carbons at positions C-11b and C-13b.

The three proton signals at $\delta_{\rm H}$ 6.91(brs), $\delta_{\rm H}$ 6.80 showed that compound 2 has a 1,3,4-substituted benzene ring. The signal at $\delta_{\rm H}$ of compound 2 also indicated two olefin protons of the double bond with a trans configuration at $\delta_{\rm H}$ 7.00 (1H, d, J=16.2 Hz), $\delta_{\rm H}$ 6.80 (1H, d, J=16.2 Hz), two doublets for two aliphatic protons on the dihydrobenzofuran moiety at $\delta_{\rm H}$ 5.41, 4.43 and a singlet at $\delta_{\rm H}$ 3.89 (3 H) for the methoxy group. Based on these evidence, it can be suggested that compound 2 had a stilbene framework, a typical skeleton of the genus Gnetum. The combination of the ¹H-NMR and ¹³C-NMR spectra of 2 allowed the determination of the binding of oxygen to the sp^2 carbon atoms not attached to hydrogen at $\delta_{\rm C}$ 160.0 (C-11a, C-13a), $\delta_{\rm C}$ 159.6 (C-11b, C-13b). In addition, the signals characteristic of a furane ring were depicted at δ_C 95.0 (C-7a), δ_C 58.9 (C-8a), δ_C 132.4 (C-3b), δ_C 161.0 (C-4b).

The structure of **2** was confirmed clearly on a basis of two-dimensional spectra HSQC and HMBC. The HMBC interaction between H-7b $\delta_{\rm H}$ 7.00 (1H, d, J=16.2 Hz) and C-2b ($\delta_{\rm C}$ 124.2)/C-6b ($\delta_{\rm C}$ 128.7), the interaction between H-8b $\delta_{\rm H}$ 6.8 (1H, d, J=16.2 Hz) and C-10b/C-14b ($\delta_{\rm C}$ 105.9) allowed to determine the position and chemical shift of the 2 carbons bearing double bonds at C-7b ($\delta_{\rm C}$ 129.4) and C-8b ($\delta_{\rm C}$ 127.5). The positions of the remaining substituent group were also confirmed based on the HMBC interaction between the MeO group ($\delta_{\rm H}$ 3.89) and C-3a ($\delta_{\rm C}$ 149.1) (Fig. 5).

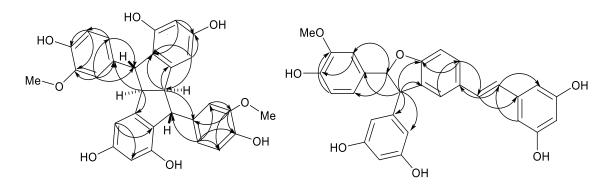


Fig. 5. The key HMBC correlations of compounds 1 and 2

Table 2. 13 C-NMR spectral data for compounds **3** and **4** and reference compounds

	3			4	
C	&∂ _C	$\delta_{\it C}^{a,b}$	C	$^\epsilon\!\delta_C$	$\delta \epsilon^{a,b}$
1	125.7	125.7	1	-	-
2	156.1	156.0	2	78.7	80.7
3	116.6	116.7	3	42.1	44.2
4	129.4	129.4	4	196.3	197.6
5	120.8	120.8	5	163.5	165.5
6	129.4	129.4	6	95.8	96.3
α	124.7	124.7	7	166.6	168.8
β	127.4	127.5	8	95.0	96.3
1'	141.6	141.5	9	162.9	164.8
2', 6'	105.9	106.0	10	101.8	103.3
3', 5'	159.6	159.4	1'	129.4	131.8
4'	102.8	102.9	2'	111.1	111.3
			4'	146.9	148.1
			5'	115.2	116.2
			6'	119.6	120.5
			3'-OMe	55.6	56.5

Recorded in ^{a)}MeOD, ^{b)}150MHz, ${}^{*}\delta_{C}$ of 2,3',5'-trihydroxy-trans-stilbene, ${}^{6}\delta_{C}$ of homoeriodictyol

The HSQC interactions approved the carbon and proton positions based on the crossed peaks between H-2a ($\delta_{\rm H}$ 6.91) and C-2a ($\delta_{\rm C}$ 110.7), H-5a ($\delta_{\rm H}$ 6.80) and C-5a (δ C 116.2), H-6a (δ _H 6.80) and C-6a (δ _C 120.1), H-7a ($\delta_{\rm H}$ 5.41) and C-7a ($\delta_{\rm C}$ 95.0), H-8a ($\delta_{\rm H}$ 4.43) and C-8a ($\delta_{\rm C}$ 58.9), H-12a ($\delta_{\rm H}$ 6.17) and C-12a ($\delta_{\rm C}$ 102.8), H-10a, H-14a ($\delta_{\rm H}$ 6.17) and C-10a, C-14a ($\delta_{\rm C}$ 107.8). All of NMR data of 2 were consistent with the corresponding data of gnetuhainin Q (Table 1). Moreover, the HR-MS mass spectrometry showed an ion peak m/z 485.1571, which suggested the molecular formula of C₂₉H₂₄O₇. From the above spectral data and comparison with reference [16], compound 2 was identified as gnetuhainin Q. Structures of compound **3-4** were elucidated successfully based on the NMR, Mass spectra combined with the comparisons with the spectroscopy data of desired compounds [17, 18] (Table 2).

4. Conclusion

This research was completed in the framework of phytochemical study of *G. montanum*. Four compounds including three stibenoids gnefricanin F (1), gnetuhainin Q (2), 2,3',5'-trihydroxy-trans-stilbene (3), and one flavonoid homoeriodictyol (4) were isolated based on column chromatographies. Their structures were identified on a basis of modern spectroscopy methods such as 1D, 2D-NMR, and HR-MS. These chemical components of *G. montanum* in Vietnam belong to main classification of *Gnetum* genus basically. This was the first time they were isolated from *G. montanum*. This study contributed to clarify the chemical composition of *G. montanum* species grown in Vietnam and scientific information to the treasure of natural compounds in Vietnam.

References

- [1] Nguyen Tien Ban, List of Plant Species Vietnam, Agricultural Publishing House, volume II, p. 284, 2003.
- [2] Li-Qin W., You-Xing Z., Jiang M., Ai-Qun J, and Jun Zhou, Stilbene derivatives from *Gnetum montanum* Markgr. f. megalocarpum Markgr, Helvetica Chimica Acta, vol. 91, pp. 159-164, Jan. 2008. https://doi.org/10.1002/hlca.200890007
- [3] Lin M., Li J. B., Li S. Z., Yu D. Q., and Liang X. T, A dimeric stilbene from *Gnetum parvifolium*, Phytochemistry, vol. 31, pp. 633-638, Feb. 1992. https://doi.org/10.1016/0031-9422(92)90050-Z
- [4] Chun-Suo Y., Mao L., Xin L., and Ying-Hong W., Stilbene derivatives from *Gnetum cleistostachyum*, Journal of Asian Natural Products Research, vol. 7, iss. 2, pp. 131-137, 2005. https://doi.org/10.1016/S0031-9422(02)00670-2
- [5] Kaisheng H., Rong-Li L., Yinghong W, and Lin M. Three New Stilbene Trimers from the Lianas of *Gnetum hainanense*, Planta Medica, vol. 67, iss. 1, pp. 61-64, 2001. https://doi.org/10.1055/s-2001-10875

- [6] Sakagami Y., Sawabe A., Komemushi S., All Z., Tanaka T., Iliya I., and Iinuma M., Antibacterial activity of stilbene oligomers against vancomycinresistant Enterococci (VRE) and methicillinresistant Staphylococcus aureus (MRSA) and their synergism with antibiotics, Biocontrol Science., vol. 12, iss. 1, pp. 7-14, 2007. https://doi.org/10.4265/bio.12.7
- [7] Ibrahim I., Toshiyuki T., Zulfiqar A., Munekazu I., Miyuki F., Ken-ichi N., Yoshiaki S., Jin M., Dedy D., Nobuyasu M., and Makoto U, Six Flavonostilbenes from *Gnetum africanum* and *Gnetum gnemon*, Heterocycles, vol. 60, pp. 159-166, 2003. https://doi.org/10.3987/com-02-9632
- [8] Ka-Wing C., Mingfu W., Feng C., and Chi-Tang H, Oligostilbenes from *Gnetum* species and anticarcinogenic and antiinflammatory activities of oligostilbenes, ACS Symposium Series, vol. 987, pp. 36-58, Sep. 2008. https://doi.org/10.1021/bk-2008-0987.ch004
- [9] Le Huu Tho, Pham Cong Trinh, Nguyen Xuan Hai, Do Van Nhat Truong, and Nguyen Thi Thanh Mai, Alkaloid compounds from *Bep* leaves, *Gnetum gnemon* L., Vietnam Journal of Chemistry, vol. 57, pp. 95-99, Aug. 2019.
- [10] Nguyen Ba Anh, Trieu Huy Diet, Hoang Viet Dung, and Nguyen Xuan Nhiem, Isolation and structural determination of two stilbenoid compounds from *Gnetum latifolium* Blume, family Gnetaceae, Journal of Pharmacy, vol. 459(54), pp. 44-48, 2014.
- [11] Le Thi Hong Nhung, Nguyen The Huu, Nguyen Thi Hoang Anh, and Ngo Anh Bang, Isolation and structural determination of some stilbenoid compounds from *Gnetum latifolium*, Journal of Science and Technology, Hanoi University of Industry, vol. 58, iss. 2, pp. 118-121, 2022.
- [12] Do Tat Loi Vietnamese medicinal plants and herbs. Medical Publishing House. pp. 662, 2004.
- [13] Vu Thi Lan Phuong, Research on chemical compositions of *Gnetum montanum* Markgr., Hai Phong University Science Journal. vol. 34, no. 5, pp. 45-50, 2019.
- [14] Nguyen Thi Viet Thanh, Dang Thanh Tung, Some Chemical Components from *Gnetum montanum Markgr*. in Vietnam, Journal of Science and Technology: Engineering and Technology for Sustainable Development, vol. 34, iss. 3, pp. 23-30, 2024.
 - https://doi.org/10.51316/jst.175.etsd.2024.34.3.4
- [15] Ibrahim Iliyaa, Toshiyuki Tanaka, Munekazu Iinuma, Zulfiqar Ali, Miyuki Furasawa, Ken-ichi Nakaya, Nobuyasu Matsuurac and Makoto Ubukata, Four dimeric stibenes in stem lianas of *Gnetum africanum*, Heterocycles, vol. 57, no. 8, pp. 1507–1512, 2002. https://doi.org/10.3987/com-02-9527
- [16] Ying-Hong Wang., Kai-Sheng Huang, and Mao Lin, Four new Stilbene Dimers from the Lianas of Gnetum Hainanense, Journal of Asian Natural

JST: Engineering and Technology for Sustainable Development

Volume 35, Issue 3, July 2025, 025-033

- Products Research, vol. 3, iss. 3, pp. 169-176, 2001. https://doi.org/10.1080/10286020108041387
- [17] Zhang, Yan, Shen, Mingyun, Cui, Sunliang, Hou, TingJun, Synthesis and antiproliferative evaluation of 2-hydroxylated (*E*)-stilbenes, Bioorganic & Medicinal Chemistry Letters, vol. 24, iss. 23, pp. 5470-5472, 2014
 - https://doi.org/10.1016/J.bmcl.2014.10.009
- [18] Wagner, H., V.M. Chari, and J. Sonnenbichler,

 13C-NMR-spektren natürlich vorkommender flavonoide, Tetrahedron Letters, vol. 17, iss. 21,
 pp. 1799-1802, May 1976.
 https://doi.org/10.1016/S0040-4039(00)93787-0