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CHEMICAL CONSTITUENTS FROM THE AERIAL PARTS OF *BARLERIA LUPULINA* (ACANTHACEAE)

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Summary

Phytochemical studies of the *n*-hexane and ethyl acetate fractions from aerial parts of *Barleria lupulina* L. (Acanthaceae) collected in Cu Chi, Ho Chi Minh city, Vietnam, led to the isolation and identification of six known compounds, including (+)-lyoniresinol (**1**), (+)-lyoniresinol 9′-*O*-*β*-D-glucopyranoside (**2**), 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone 3-*O*-*β*-D-glucopyranoside (**3**), (3*S*)-l-octene-3-ol 3-*O*-*β*-D-glucopyranoside (**4**), uracil (**5**) and stigmasterol (**6**). The structures of the isolated compounds were determined on the basis of detailed spectroscopic (MS and NMR) analysis and comparison with reported literature. Compounds **1**, **3**, **4**, and **5** were found for the first time from the *Barleria* genus.

Keywords: *Barleria lupulina; Lignan; Anthraquinone; Sterol.*

1. Introduction

Genus *Barleria* (Acanthaceae), which comprises over 300 species of herbs and shrubs, has been traditionally utilized across various countries for treating a wide range of ailments [1]. *Barleria lupulina* L., commonly known as hop-headed barleria, is one species recognized for its decorative and medicinal value. Traditionally, different parts of the plant have been employed in Asian countries, such as India, Thailand, and Indonesia, for the treatment of diabetes, rheumatoid arthritis, inflammation, skin infections, and mental strain [2]. In Vietnam, the leaves of *B. lupulina* have been applied in case of snakebites and fever [3]. Recent phytochemical studies have revealed that *B. lupulina* is a rich source of diverse bioactive compounds, predominantly iridoid glycosides, phenylethanoid glycosides, phenylpropanoid glycosides, and phenolic compounds [2],[4],[5],[6]. These isolated constituents are associated with a broad spectrum of pharmacological activities, including antibacterial, anti-inflammatory, anti-arthritic, anti-diabetic, antioxidant, anti-osteoporotic, anti-ulcer, and neuroprotective effects [2],[7],[8],[9].

Despite promising traditional uses and preliminary scientific validation, the chemical investigations of *B. lupulina* in Vietnam are still limited [5],[6],[7]. Preliminary biological screening results showed that the *n*-hexane and ethyl acetate extracts from the *B. lupulina* sample collected in Ho Chi Minh city displayed potent anti-inflammatory activity, inhibiting 72-87% of NO-production in LPS-induced RAW264.7 cells at the concentration of 50 μg/mL. Hence, our research paper aims to provide an isolation and identification of chemical constituents from *n*-hexane and ethyl acetate extracts, including (+)-lyoniresinol (**1**), (+)-lyoniresinol 9′-*O*-*β*-D-glucopyranoside (**2**), 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone 3-*O*-*β*-D-glucopyranoside (**3**), 3-*O*-*β*-D-glucopyranosyl (3*S*)-l-octene-3-ol (**4**), uracil (**5**), and stigmasterol (**6**).

2. Materials and methods

2.1. Materials

The aerial parts of *Barleria lupulina* L. (Acanthaceae) were collected in Cu Chi, Ho Chi Minh City, in 2024. The plant species was identified by MSc Nguyen Quynh Nga and BS. Dao Viet Quoc, Center of Medicinal Material Resources (CMMR), National Institute of Medicinal Materials. The voucher specimen (NIMM-20133) was deposited at the National Institute of Medicinal Materials.

2.2. General experimental procedures

ESI-MS spectra were acquired on an Agilent

1260 series single quadrupole LC/MS system. NMR spectra (^{1}H, ^{13}C, HSQC, HMBC, and NOESY) were obtained on Bruker AVANCE III HD 500 MHz or Bruker AVANCE NEO 600 MHz spectrometers with tetramethylsilane as the internal standard. Column chromatography (CC) was performed on normal phase *silica gel* 60 (230-400 mesh, Merck), reversed phase (RP-18) resin (YMC), and Sephadex® LH20. Preparative HPLC was conducted by Shimadzu HPLC (LC-20AP), SunFire® C18 OBD™ Prep Column (100 Å, 10 μm, 10 × 250 mm). Thin-layer chromatography has been performed on *silica gel* 60 GF_{254} and RP-18 G $F_{254}S$. Compounds were visualized by spraying with 10% sulfuric acid in EtOH and heating.

2.3. Extraction and isolation

The dried aerial parts (28 kg) were macerated and extracted with 70% EtOH (3 times × 220 L for 24 h) at room temperature. The combined extracts were concentrated under reduced pressure to yield the EtOH extract (4.3 kg). The EtOH extract (4 kg) was suspended in 4 L of distilled water and then successively extracted with *n*-hexane, dichloromethane (DCM), and EtOAc (each 3 × 4 L). The organic solvents were then removed *in vacuo* to afford *n*-hexane (95 g), DCM (265 g), and EtOAc (300 g) extracts, respectively.

The *n*-hexane extract (90 g) was chromatographed on a *silica gel* CC, eluted with a solvent gradient of *n*-hexane–acetone (20:1 to 1:1, *v/v*), DCM–MeOH (20:1 → 2:1, v/v), and MeOH to give 11 fractions (H1-H11). Fraction H1 (2.8 g) was chromatographed by *silica gel* CC, eluted with a gradient solvent of *n*-hexane–acetone (15:1 → 1:1, v/v) to afford 3 fractions H1.1–H1.3. Compound **6** (50 mg) was obtained by recrystallization in MeOH from fraction H1.3.

The EtOAc extract (240 g) was applied to *silica gel* CC, eluted with a solvent gradient of *n*-hexane–acetone (20:1 to 1:1, *v/v*), DCM–MeOH (20:1 → 2:1, v/v), and MeOH to give 10 fractions (E1–E10). Fraction E6 (3.0 g) was chromatographed by Sephadex® LH20 CC (MeOH–H_2O 1:1, v/v), yielding 5 fractions E6.1–E6.5. Fraction E6.2 (1.8 g) was separated by *silica gel* CC eluted with a solvent gradient of DCM–MeOH (20:1 → 2:1, v/v) to afford ten fractions E6.2.1–E6.2.10. Fraction E6.2.4 (55 mg) was then purified by reversed-phase *silica gel* CC (MeOH–H_2O 1:1, v/v) to afford **1** (3.0 mg). Fraction 6.2.7 (89 mg) was subjected to a reversed-phase *silica gel* CC (MeOH–H_2O 2:3, v/v), yielding 2 sub-fractions E6.2.7.1 and E6.2.7.2. Fraction 6.2.7.1 (32 mg) was further purified by *silica gel* CC (EtOAc–MeOH 35:1, *v/v*) to give **5** (4.1 mg). Fraction E6.2.8 (138 mg) was chromatographed by reversed-phase *silica gel* CC (MeOH–H_2O 1:1, v/v) to afford **3** (9.0 mg). Fraction E8 (23.4 g) was subjected to *silica gel* CC, eluted with gradient solvents of DCM–MeOH (10:1 - 100% MeOH, v/v), yielding 12 fractions E8.1–E8.12. Fraction E8.9 (6.2 g) was purified by reversed-phase *silica gel* CC (MeOH–H_2O 1:2, v/v) to give 12 sub-fractions E8.9.1–E8.9.12. Fraction E8.9.2 (3.2 g) was separated by reversed-phase *silica gel* CC (*n*-hexane–acetone *v/v*, 19:1), followed by Sephadex LH-20 to afford 3 fractions (E8.9.2.1–E8.9.2.3). Fraction 8.9.2.1 (170 mg) was purified by preparative HPLC (acetonitrile–H_2O 13:87 (v/v), flow rate: 2.5 mL/min, injection volume 65 μL, detection wavelength 210 nm) to yield compound **2** (2.6 mg, t_R = 45 min). Fraction 8.9.2.3 (55 mg) was purified by preparative HPLC (MeOH–H_2O 43:57 (v/v), flow rate: 2.5 mL/min, injection volume 49 μL, detection wavelength 254 nm) to give **4** (14 mg, t_R = 15 min).

(+)-Lyoniresinol (1): White solid; ESI-MS: *m/z* 419 $[M-H]^-$; $[\alpha]_D^{20}$ = +17.9 (MeOH, *c* = 0.56). ^{1}H-NMR (600 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD): See **Table 1**.

(+)-Lyoniresinol 9'-O-β-D-glucopyranoside (2): White solid; $[\alpha]_D^{20}$ = +60.1 (MeOH, *c* = 0.33). ESI-MS *m/z* 581 $[M-H]^-$. ^{1}H-NMR (600 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD): See **Table 1**.

2-Methyl-1,3,6-trihydroxy-9,10-anthraquinone 3-O-β-D-glucopyranoside (3): Yellow solid, ESI-MS: *m/z* 291 $[M+H]^+$. ^{1}H-NMR (600 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD): See **Table 2**.

3-O-β-D-Glucopyranosyl (3S)-l-octene-3-ol (4): Yellow solid, $[\alpha]_D^{20}$ = -7,9 (MeOH, *c* = 1,27). ^{1}H-NMR (600 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD): See **Table 2**.

Uracil (5): Yellow solid. ESI-MS *m/z* 111.2 $[M–H]^-$. ^{1}H-NMR (500 MHz, CD_3OD): δ_H 5.61 (1H, d, *J* = 7.5 Hz, H-5), 7.38 (1H, d, *J* = 7.5 Hz, H-6). ^{13}C-NMR (125 MHz, CD_3OD): δ_C 151.5 (C-2), 167.3 (C-4), 101.7 (C-5), 143.5 (C-6).

Stigmasterol (6): White solid, ESI-MS *m/z* 413 $[M+H]^+$. ^{1}H-NMR (600 MHz, $CDCl_3$): δ_H 3.52 (1H, m, H-3), 5.34 (1H, m, H-6), 0.70 (3H, s, H-18), 1.01 (3H, s, H-19), 1.03 (3H, d, *J* = 6.6 Hz, H-21), 5.15 (1H, dd, *J* = 15.0; 8.4 Hz, H-22), 5.02 (1H, dd, *J* = 15.0; 8.4 Hz, H-23), 0.84 (3H, d, *J* = 6.6 Hz, H-26), 0.81 (3H, d, *J* = 6.6 Hz, H-27), 0.80 (3H, t, *J* = 7.2 Hz, H-29). ^{13}C-NMR (125 MHz, $CDCl_3$): δ_C 37.3 (C-1), 31.7 (C-2), 71.8 (C-3), 42.3 (C-4), 140.8 (C-5), 121.7 (C-6), 31.9 (C-7), 31.9 (C-8), 50.2 (C-9), 36.5 (C-10), 21.1 (C-11), 39.7 (C-12), 42.3 (C-13), 56.9 (C-14), 24.4 (C-15), 28.9 (C-16), 56.0 (C-17), 12.1 (C-18), 19.4 (C-19), 40.5 (C-20), 21.1 (C-21), 138.3 (C-22), 129.3 (C-23), 51.3 (C-24), 31.9 (C-25), 21.2 (C-26), 19.0 (C-27), 25.4 (C-28), 12.2 (C-29).

3. Results and discussion

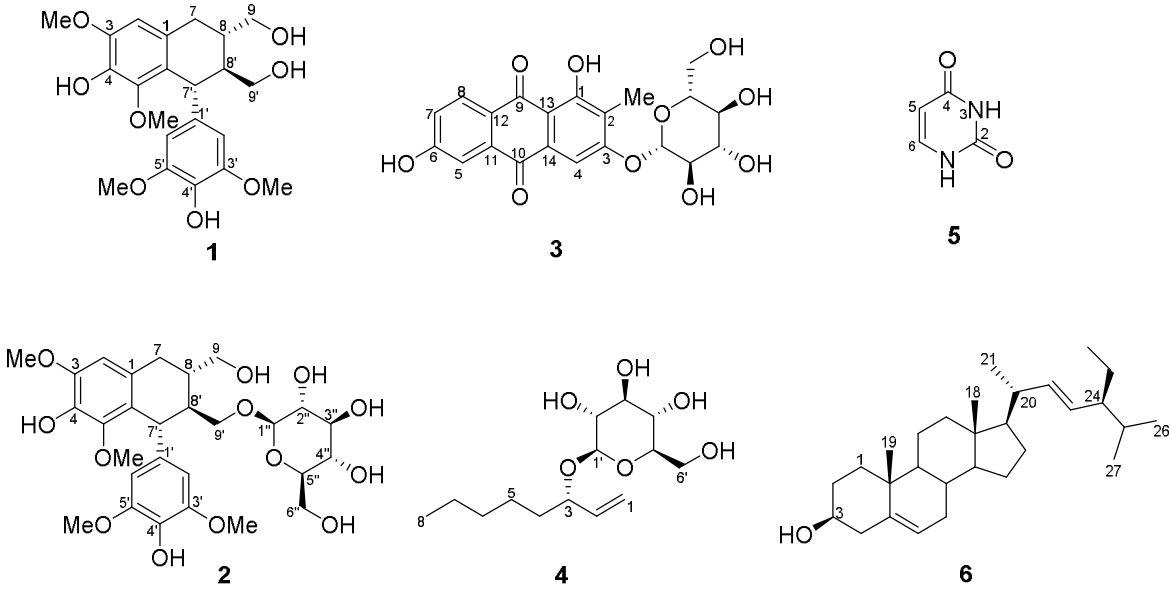
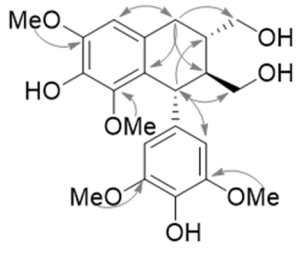


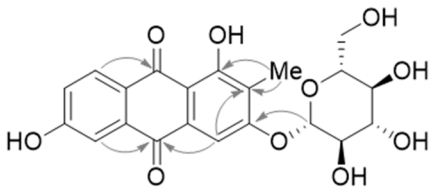
Fig. 1. Chemical structures of compounds **1**–**6** from *B. lupulina*

Compound **1** was isolated as a white solid. The ^{1}H-, ^{13}C-NMR and HSQC spectra of **1** exhibited signals of 3 aromatic protons at δ_H 6.61 (1H, s, H-2)/δ_C 107.8 (C-2), δ_H 6.40 (2H, s, H-2′ and H-6′)/δ_C 106.98 (C-2); 2 oxymethylene groups at δ_H 3.61-3.51 (2H, H-9)/δ_C 66.8 (C-9) and δ_H 3.51 (2H, d, *J* = 5.4 Hz, H-9′)/δ_C 64.2 (C-9′), 4 methoxy groups at δ_H 3.40 (3H, s, 3-OMe)/δ_C 56.6; δ_H 3.88 (3H, s, 5-OMe)/δ_C 60.2 and 3.76 (6H, s, 3′-OMe, 5′-OMe)/δ_C 56.8; one methylene group at δ_H 2.72-2.59 (2H, H-7)/δ_C 33.6 (C-7), 3 methine groups at δ_H 1.64 (1H, m, H-8)/δ_C 40.9 (C-8), δ_H 4.33 (1H, d, *J* = 5.4 Hz, H-7′)/δ_C 42.3 (C-7′) and δ_H 1.99 (1H, m, H-8′)/δ_C 49.9 (C-8′). The negative ESI-MS spectrum of **1** showed a deprotonated molecular ion peak at *m/z* 419 $[M–H]^-$, which gave evidence of its molecular formula as $C_{22}H_{28}O_8$ (M = 420). The NMR and MS spectra suggested that **1** was an aryltetralin lignan. The aromatic proton at C-2 was confirmed by the HMBC correlations of H-2 (δ_H 6.61) to C-7 (δ_C 33.6) and of H-7 (δ_H 2.72 and 2.59) to C-2 (δ_C 107.8). The symmetrical 3′,5′-dimethoxy-4′-hydroxy phenyl group substituted at C-7′ was confirmed by the HMBC correlations of H-2′ and H-6′ (δ_H 6.40) to C-7′ (δ_C 42.3) and C-4′ (δ_C 134.6), of methoxy protons (δ_H 3.76) to C-3′ and C-5′ (δ_C 149.0). In addition, the methoxy groups at C-3 and C-5 were assigned by HMBC cross-peaks of methoxy protons at δ_H 3.40 and 3.88 to C-3 (δ_C 56.6) and C-5 (δ_C 60.2), respectively. In addition, HMBC cross-peaks of H-7 (δ_H 2.72 and 2.51) to C-8′ (δ_C 49.9) and C-9 (δ_C 66.8), of H-7′ (δ_H 4.33) to C-9′ (δ_C 64.2) and C-8 (δ_C 40.9) confirmed the aryltetralin skeleton (Fig. 2). The relative configuration of **1** was

assigned based on the NOESY spectrum. The cross-peaks of H-8′ (δ_H 1.99) with H-2′ (δ_H 6.40) and H-9 (δ_H 3.51 and 3.61); of H-7′ (δ_H 4.33) with H-8 (δ_H 1.64) and H-9′ (δ_H 3.51) suggested H-7′, H_2-9′ and H-8 are on the same side, while the phenyl ring, H-8′ and H_2-9 are on the other side. Based on spectral analysis and the specific optical rotation value of +17.9 (ref [10]: $[\alpha]_D^{25}$ = +65.1 (MeOH, *c* 0.10)), **1** was identified as (+)-lyoniresinol [11].



1



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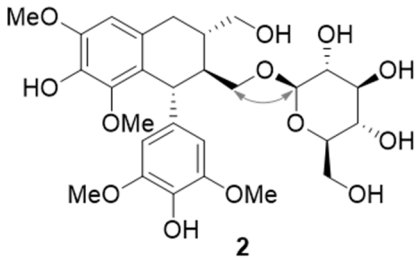

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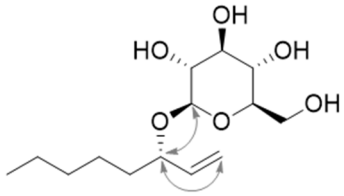

4

Fig. 2. Key HMBC (⟶) correlations of compounds **1**, **3**, and **4**

Compound **2** was isolated as a white solid. The molecular formula of **2** was determined as $C_{28}H_{38}O_{13}$ based on a deprotonated molecular ion peak at *m/z* 581 $[M–H]^-$ in the ESI-MS spectrum and NMR data. The ^{1}H-, ^{13}C-NMR and HSQC spectra of **2** revealed similar signals as those of **1** including 3 aromatic protons (δ_H 6.58 (1H, s, H-2)/δ_C 107.9 (C-2), δ_H 6.43 (2H, s, H-2′ and H-6′)/δ_C 107.0 (C-2′, C-6′)); 2 oxymethylene groups (δ_H 3.64-3.53 (2H, H-9)/δ_C 66.3 (C-9) and δ_H 3.88-3.45 (2H, H-9′)/δ_C 71.5 (C-9′)), one methylene (δ_H 2.71-2.61 (2H, H-7)/δ_C 33.8 (C-7)) and 3 methine groups (δ_H 1.70 (1H, m, H-8)/δ_C 40.6 (C-8), δ_H 4.41 (1H, d, *J* = 6.6 Hz, H-7′)/δ_C 42.8 (C-7′) and δ_H 2.08 (1H, m, H-8′)/δ_C 46.7 (C-8′)). The NMR spectra of 2 showed additional signals of a *β*-glucopyranosyl moiety at δ_H 4.28 (1H, d, *J* = 7.8 Hz, H-1″), δ_H 3.24-3.83 (5H, H-2″-H-6″)/δ_C 104.8 (C-1″), 75.2 (C-2″), 78.0 (C-3″), 71.7 (C-4″), 78.3 (C-5″), 62.9 (C-6″). The HMBC correlations of H-1″ (δ_H 4.28) with C-9′ (δ_C 71.5) indicated that the *β*-D-glucopyranoside moiety is linked at C-9′. Compound **2** was assigned as (+)-lyoniresinol 9′-*O*-*β*-D-glucopyranoside by comparison of specific optical rotation (+60.1, Ref. [12] $[\alpha]_D^{20}$ = + 47 (MeOH, *c* = 0.8)) and NMR data with previous literature [11].

Table 1. ^{1}H- and ^{13}C-NMR spectroscopic data of **1**–**2**

Pos.	**1**		**Ref [11]**	**2**		**Ref [11]**
	$\delta_H^{a,b}$ (mult., *J* in Hz)	$\delta_C^{a,c}$	$\delta_C^{a,c}$	$\delta_H^{a,b}$ (mult., *J* in Hz)	$\delta_C^{a,c}$	$\delta_C^{a,c}$
1		130.2	130.2		130.2	130.2
2	6.61 (1H, s)	107.8	107.8	6.58 (1H, s)	107.9	107.9
3		148.7	148.7		148.7	148.7
4		138.9	138.9		138.9	138.9
5		147.7	147.7		147.6	147.6
6		126.3	126.3		126.4	126.4

Pos.	1		Ref [11]	2		Ref [11]
	δ_H [a,b] (mult., *J* in Hz)	δ_C [a,c]	δ_C [a,c]	δ_H [a,b] (mult., *J* in Hz)	δ_C [a,c]	δ_C [a,c]
7	2.72 (1H, dd, 15.0, 4.8) 2.59 (1H, dd, 15.0, 11.4)	33.6	33.6	2.71 (1H, dd, 15.0, 4.8) 2.61 (1H, dd, 15.0, 12.0)	33.8	33.8
8	1.64 (1H, m)	40.9	40.9	1.70 (1H, m)	40.6	40.6
9	3.61 (1H, dd, 9.0, 5.4) 3.51 (1H, m)	66.8	66.8	3.64 (1H, m) 3.53 (1H, dd, 10.8, 6.6)	66.3	66.2
3-OCH_3	3.88 (3H, s)	56.6	56.6	3.86 (3H, s)	56.6	56.6
5-OCH_3	3.40 (3H, s)	60.2	60.2	3.35 (3H, s)	60.2	60.2
1′	-	139.3	139.3		139.3	139.3
2′, 6′	6.40 (2H, s)	106.9	106.9	6.43 (2H, s)	107.0	107.0
3′, 5′	-	149.0	149.0		149.0	149.0
4′	-	134.6	134.5		134.5	134.5
7′	4.33 (1H, d, 5.4)	42.3	42.3	4.41 (1H, d, 6.6)	42.8	42.8
8′	1.99 (1H, dq, 9.0, 5.4)	49.9	49.9	2.08 (1H, m)	46.7	46.7
9′	3.51 (2H, d, 5.4)	64.2	64.2	3.88 (1H, dd, 9.6, 5.4). 3.45 (1H, dd, 9.6, 4.2)	71.5	71.5
3′,5′-OCH_3	3,76 (6H, s)	56.8	56.8	3.75 (6H, s)	56.9	56.9
1′				4.28 (1H, d, 7.8)	104.8	104.8
2′				3.24 (1H, m)	75.2	75.2
3′				3.24 (1H, m)	78.0	77.9
4′				3.28 (1H, m)	71.7	71.7
5′				3.36 (1H, m)	78.3	78.2
6′				3.83 (1H, dd, 12.0, 2.4) 3.64 (1H, m)	62.9	62.8

[a:] CD_3OD, [b] 600 MHz, [c:] 125 Mz.

Compound **3** was isolated as a pale yellow solid. The negative ESI-MS spectrum showed the peak *m/z* 323 $[M–H]^-$, suggesting the molecular formula $C_{21}H_{20}O_{10}$. The ^{1}H-NMR spectrum of **3** displayed 4 aromatic signals including a singlet at δ_H 7.41 (1H, s) and 3 protons of an ABX system at δ_H 7.39 (1H, d, *J* = 2.4 Hz, H-5), 7.03 (1H, dd, *J* = 2.4, 8.4 Hz, H-7), and 8.03 (1H, d, *J* = 8.4 Hz, H-8); a methyl singlet at δ_H 2.18 (3H, s, 2-Me); and signals of a sugar at δ_H 5.07 (1H, d, *J* = 2.4 Hz, H-1′), 3.37-3.43 (4H, m, H-2′, H-3′, H-4′, H-5′), and 3.82 (1H, dd, *J* = 12.0; 2.4 Hz, H-6a), 3.67 (1H, dd, *J* = 12.0, 6.0 Hz, H-6b). The ^{13}C NMR and HSQC spectra showed signals for 21 carbons, including 2 ketone groups at δ_C 188.1 (C-9) and 183.8 (C-10); 12 aromatic carbons, a methyl carbon at δ_C 8.6 and carbon signals of a *β*-D-glucopyranosyl part at δ_C 101.6 (C-1′), 74.8 (C-2′), 78.1 (C-3′), 71.1 (C-4′), 78.3 (C-5′), and 62.3 (C-6′). The NMR and MS data suggested that **3** was an anthraquinone glucoside. HMBC correlations of methyl protons to C-1 (δ_C 163.2), C-2 (δ_C 122.5), and C-3 (δ_C 162.1); of H-4 (δ_H 7.41) to C-10 (δ_C 183.8) and C-2; of H-8 (δ_H 8.03) to C-9 (δ_C 188.1) allowed the assignment of substitutions at the anthraquinone skeleton. Comparing the NMR data with reference [13], compound **3** was determined as 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone 3-*O*-*β*-D-glucopyranoside.

Table 2. ^{13}C-NMR spectroscopic data of **3**–**4**

Pos.	3		Ref [13]	4		Ref [14]
	δ_H [a,b] (mult., *J* in Hz)	δ_C [a,c]	δ_C [a,d]	δ_H [a,b] (mult., *J* in Hz)	δ_C [a,c]	δ_C
1	-	163.2	163.4	5.19 (1H, d, 16.5) 5.08 (1H, d, 11.0)	141.0	141.0
2	-	122.5	120.7	5.87 (1H, ddd, 16.5, 11.0, 7.0)	116.0	116.0
3	-	162.1	160.7	4.12 (1H, q, 7.0)	82.8	82.8
4	7.41 (1H, s)	106.9	105.6	1.68 (1H, m) 1.52 (1H, m)	33.0	33.0
5	7.39 (1H, d, 2,4)	114.1	112.5	1.40−1.29 (6H, m)	25.6	25.6
6	-	165.8	161.2		35.6	35.6
7	7.03 (1H, dd, 8.4, 2.4)	122.5	121.3		23.6	23.6
8	8.03 (1H, d, 8.4)	130.7	129.5	0.90 (3H, t, 7.0)	14.4	14.4

Pos.	3		Ref [13]	4		Ref [14]
	δ_H[a,b] (mult., *J* in Hz)	δ_C[a,c]	δ_C[a,d]	δ_H[a,b] (mult., *J* in Hz)	δ_C[a,c]	δ_C
9	-	188.1	186.3			
10	-	183.8	181.5			
11	-	137.0	135.2			
12	-	126.0	124.4			
13	-	112.0	110.5			
14	-	133.7	131.9			
2-Me	2.18 (3H, s)	8.6	8.3			
1′	5.07 (1H, d, 7.2)	101.6	100.3	4.32 (1H, d, 7.5)	103.2	103.2
2′	3.37−3.47 (4H, m)	74.8	73.1	3.19 (1H, m)	75.3	75.3
3′		78.1	76.2	3.32 (1H, m)	78.2	78.2
4′		71.1	69.3	3.29 (1H, m)	71.7	71.6
5′		78.3	72.2	3.19 (1H, m)	77.8	77.8
6′	3.82 (1H, dd, 12.0, 2.4) 3.67 (1H, dd, 12.0, 6.0)	62.3	60.4	3.80 (1H, dd, 12.0, 2.4) 3.65 (1H, dd, 12.0, 6.0)	62.8	62.8

[a:] $CDCl_3$, [b:] 600 MHz, [c:] 125 MHz, [d:] 100 MHz.

Compound **4** was isolated as a yellow solid. The ^{1}H-NMR spectrum of **4** shows signals of allyl group at δ_H 5.19 (1H, d, J = 16.5 Hz, H-1a), 5.08 (1H, d, J = 11.0 Hz, H-1b), 5.87 (1H, ddd, J = 16.5, 11.0, 7.0 Hz, H-2), an oxymethine group at δ_H 4.12 (1H, q, J = 7.0 Hz, H-3), 4 methylene groups at δ_H 1.68-1.52 (2H, m, H-4), 1.40-1.29 (6H, m, H-5, H-6, H-7), and a methyl group at δ_H 0.90 (3H, t, J = 7.0 Hz). In addition, a β-glucose moiety was observed with signals at δ_H 4.32 (1H, d, J = 7.5 Hz, H-1), 3.19-3.32 (4H, m, H-2, H-3, H-4, H-5), 3.80 (1H, dd, J = 12,0, 2.4 Hz, H-6a) and 3.65 (1H, dd, J = 12.0, 6.0 Hz, H-6b). The HMBC correlations of H-1′ (δ_H 4.32) and H-1 (δ_H 5.19 and 5.08) to C-3 (δ_C 82.8) indicated the sugar linked to the carbon chain at C-3. Compound **4** was identified as (-)-3-*O*-β-D-glucopyranosyl (3*S*)-l-octen-3-ol by comparison of specific optical rotation (-7.9) ($[\alpha]_D^{20}$ = -37.2 (MeOH, c = 0.17) and NMR data with previous literature [14].

Finally, compounds **5** and **6** were determined as uracil and stigmasterol, respectively, based on the comparison of NMR data with those in the previous literature [15], [16].

(+)-Lyoniresinol 9′-*O*-β-glucoside **2** has already been identified from *B. lupulina* [1], but its aglycone (**1**) was found in the genus *Barleria* for the first time. (+)-Lyoniresinol (**1**) was identified in various plants and displayed antioxidant, anti-inflammatory, and cytoprotective activities [17], while (+)-lyoniresinol 9′-*O*-β-glucoside (**2**) exhibited antimicrobial and antidiabetic effects [18]. In the assay, (+)-lyoniresinol showed weak NO-production inhibitory activity with an IC_{50} value of 238 μM [19]. Compound **3** was only reported from *Rubia cordifolia* (Rubiacea family); it was first identified from the *Barleria* genus. Compound **4** might be a hydrolyzed product from 1-octen-3-yl β–primeveroside, which was found in this plant [1]. Uridine (**5**) is an essential nucleoside that plays a pivotal role in various biological processes, including macromolecule synthesis, circadian rhythms, inflammatory response, and antioxidant process [20]. Uridine was reported to reduce oxidative stress and inflammation by inhibiting the MAPK and NF-kB signaling pathways under pathological conditions [21]. Stigmasterol (**6**) is a common sterol in many plants and possesses anti-inflammatory, antioxidant, and neuroprotective activities [22]. Studies revealed that stigmasterol exhibited substantial anti-inflammatory and immune regulatory effects and has potential for the prevention and treatment of inflammation-related diseases like arthritis [23],[24]. The anti-inflammatory effect of these isolated compounds may make contributions to the activity of *n*-hexane and ethyl acetate extracts from *B. lupulina.*

4. Conclusions

Six chemical constituents including (+)-lyoniresinol (**1**), (+)-lyoniresinol 9-*O*-β-D-glucopyranoside (**2**), 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone 3-*O*-β-D- glucopyranoside (**3**), 3-*O*-β-D-glucopyranosyl (3*S*)-l-octen-3-ol (**4**), uracil (**5**), and stigmasterol (**6**) were identified from *n*-hexane and ethyl acetate

extracts of *B. lupulina* aerial parts collected in Ho Chi Minh city. To the best of our knowledge, compounds **1**, **3**, **4**, and **5** were identified for the first time from the *Barleria* genus.

References

1. Gangaram S., Naidoo Y., Dewir Y. H., El-Hendawy S. (2021), Phytochemicals and biological activities of *Barleria* (Acanthaceae), *Plants*, 11(1), 82.

2. Sawarkar H. A., Pandey A. K., Biyani K. R. (2023), Phytochemicals and therapeutic potentials of *Barleria lupulina*, *Bangladesh Journal of Pharmacology*, 18(4), 119-129.

3. Do T. L. (2004), *Vietnamese medicinal plants and materials*, Medical Publishing House, Hanoi.

4. Keomanykham O., Anh H. T., Duyen N. T., Giang T. N., Kim N. Q., Hoa T. T., Hoa L. T. P., Quang D. N. (2025), Iridoid glucosides from *Barleria lupulina* LINDL, *HNUE Journal of Science Natural Science*, 70(1), 53-60.

5. Lee S. R., Clardy J., Senger D. R., Cao S., Kim K. H. (2016), Iridoid and phenylethanoid glycosides from the aerial part of *Barleria lupulina*, *Revista Brasileira de Farmacognosia*, 26(3), 281-284.

6. Kim K. H., Park Y. J., Chung K. H., Yip M. R., Clardy J., Senger D., Cao S. (2015), Iridoid glycosides from *Barleria lupulina*, *Journal of Natural Products*, *78*(2), 320-324.

7. Suba V., Murugesan T., Arunachalam G., Mandal S. C., Saha B. P. (2004), Anti-diabetic potential of *Barleria lupulina* extract in rats, *Phytomedicine*, 11(2-3), 202-205.

8. Ilori N. T. O., Liew C. X. Q., Fang C. M. (2020), The anti-inflammatory properties of *Acanthus ebracteatus*, *Barleria lupulina* and *Clinacanthus nutans*: A systematic review, *Molecular Biology Reports*, 47(12), 9883-9894.

9. Kumari R., Kumar S., Kumar A., Goel K. K., Dubey R. C. (2017), Antibacterial, antioxidant and immuno-modulatory properties in extracts of *Barleria lupulina* Lindl, *BMC Complementary and Alternative Medicine*, 17(1), 484.

10. Nguyen N. T., Dang P. H., Nguyen H. X., Le T. H., Do T. N. V., Nguyen M. T. T. (2022), A new lignan from the stems of *Buchanania lucida* Blume (Anacardiaceae), *Natural Product Research*, 36(14), 3737-3740.

11. Pham H. Y., Dan T. T. H., Nguyen H. H., Ngo A. B., Duong T. H. Y., Nguyen A. H., Nguyen V. T., Bui H. T., Nguyen X. N., Phan V. K. (2022), Lignans from *Gymnema sylvestre*, *VNU Journal of Science: Natural Sciences and Technology*, 38(4), 18-24.

12. Yuasa K., Ide T., Otsuka H., Ogimi C., Hirata E., Takushi A., Takeda Y. (1997), Lignan and neolignan glycosides from stems of *Alangium premnifolium*, *Phytochemistry*, 45(3), 611-615

13. Itokawa H., Qiao Y., Takeya K. (1989), Anthraquinones and naphthohydroquinones from *Rubia cordifolia*, *Phytochemistry*, 28(12), 3465-3468.

14. Yamamura S., Ozawa K., Ohtani K., Kasai R., Yamasaki K. (1998), Antihistaminic flavones and aliphatic glycosides from *Mentha spicata*, *Phytochemistry*, 48(1), 131-136.

15. Ha N. T. T., Tra N. T., Ninh B. H., Tiep N. K. (2022), Chemical constituents from the stem barks of *Polyscias serrata* Balf, *Vietnam Journal of Science and Technology*, 60(6), 966-973.

16. Khoi N. M., Luc T. M. (2016), Isolation of stigmasterol from methanolic extract of stem and foliage of *Abrus precatorius* Linn, *Journal of Science and Technology, the University of Danang*, 12(109), 39-42.

17. Guo X. H., Pang L., Gao C. Y., Meng F. L., Jin W. (2023), Lyoniresinol attenuates cerebral ischemic stroke injury in MCAO rat based on oxidative stress suppression via regulation of Akt/GSK-3β/Nrf2 signaling, *Biomedicine & Pharmacotherapy*, 167, 115543.

18. Lu C. L., Zhu W., Wang D. M., Chen W. L., Hu M. M., Wang M., Lu C. J. (2015), Inhibitory effects of chemical compounds isolated from the rhizome of *Smilax glabra* on nitric oxide and tumor necrosis factor-α production in lipopolysaccharide-induced RAW264.7 cell, *Evidence-Based Complementary and Alternative Medicine*, 2015(1), 602425.

19. Lee D. G., Jung H. J., Woo E. R. (2025), Antimicrobial property of (+)-lyoniresinol-3*α*-*O*-*β*-D-glucopyranoside isolated from the root bark of *Lycium chinense* Miller against human pathogenic microorganisms, *Archives of Pharmacal Research*, 28, 1031-1036

20. Yang Y., Ye Y., Deng Y., Gao L. (2024), Uridine and its role in metabolic diseases, tumors, and neurodegenerative diseases, *Frontiers in Physiology*, 15, 1360891.

21. Luo Y., Chen H., Huang R., Wu Q., Li Y., He Y. (2021), Guanosine and uridine alleviate airway inflammation via inhibition of the MAPK and NF-κB signals in OVA-induced asthmatic mice, *Pulmonary Pharmacology & Therapeutics*, 69, 102049.

22. Li J., Zheng X., Qi J. (2025), Research progress on the therapeutic mechanisms of stigmasterol for multiple diseases, *Molecules*, 30(9), 1874.

23. Xie W., Yang H., Guo C., Xie R., Yu G., Li Y. (2023), Integrated network pharmacology and experimental validation approach to investigate the mechanisms of stigmasterol in the treatment of rheumatoid arthritis, *Drug Design, Development and Therapy*, 17, 691–706.

24. Ahmad-Khan M., Sarwar A., Rahat R., Ahmed R. S., Umar S. (2020), Stigmasterol protects rats from collagen induced arthritis by inhibiting proinflammatory cytokines, *International Immunopharmacology*, 85, 106642.