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Research Article

Isolation and Structural Characterization of Compounds from *Blumea lacera*

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ABSTRACT

Background: The medicinal plants consider as a rich resource of ingredients which can be used in drug development and synthesis. Blumea lacera (Burm. f.) DC. is generally used in traditional medicine for the treatment of cough, bronchitis, dysentery, wound healing. The aim of this study is to isolate and identify the compounds from the aerial parts of Blumea lacera. **Methods:** The aerial parts of B. lacera were dried, powdered and extracted using EtOH, and the concentrated extract was partitioned in succession with n-hexane, CH_2Cl_2, and EtOAc. From the EtOAc fraction, the compounds were isolated through column chromatography and their chemical structures were elucidated by NMR spectroscopy and confirmed by comparison of their NMR data with literature data. **Results:** Repeated column chromatography of the EtOAc-soluble fraction from the aerial parts of B. lacera resulted in the isolation of β-sitosterol (1), campesterol (2), artemetin (3) and acid paracatechuic (4).

Key words: Blumea lacera, Asteraceae, Flavonoid, Column chromatography.

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INTRODUCTION

The genus *Blumea*, belonging to the family Asteraceae, comprises 80 species of small annual weeds. The plants of this genus are widely distributed in tropical and subtropical Asia, Africa, and Oceania. The plants of this genus are mostly small annual weeds and are of great medicinal value. Some of these species are used as folk medicines for treating colds, fevers, blood diseases, dysentery, gynecological diseases.[1,2] These have provided a variety of constituents, including flavonoids, monoterpenes, sesquiterpenes, acetylenic thiophenes, triterpenoids, xanthenes, diterpenes, and essential oils.[1] *Blumea lacera* (Burn. f.) DC, a herbaceous weed named "Cai troi" in Vietnam, is mainly distributed in Lao Cai, Vinh Phuc and Ha Giang provinces in Vietnam.[3] It is also commonly found in China, India, Bangladesh, Australia and tropical Africa.[4] The plant has long been used in traditional medicine as expectorant, diuretic, astringent, antispasmodic, antipyretic, antioxidant, antidiarrheal, liver tonic and stimulant.[5,6] Previous biological studies have shown that extracts of *B. lacera* exhibit antiviral,[7] anti-leukemic[7], antiulcer[6] and cytotoxic activities against several human cancer cell lines.[8,9] In addition, it has been reported that essential oil from this herb exhibit analgesic, hypothermic, and tranquilizing activities and cytotoxic activities against breast cancer cells and healing cuts.[6,10] Several investigations into the secondary metabolites of *B. lacera* have revealed the presence of flavonoids, terpene glycosides, phenol glycosides, sterols, essential oils, coniferyl alcohol derivatives, terpenoid ketones and steroidal glycoalkaloids.[4,8-14] To increase the value of this herb in terms of its potential application for medicinal purposes, it was considered necessary to investigate its chemical constituents and to understand their biological properties.

MATERIALS AND METHODS

General experimental procedure

The NMR spectra were measured using a Varian Unity-Inova 400 MHz spectrometer. The solvents used for extraction and isolation were of analytical grade solvents. Silica gel (63–200 mm; Merck, Darmstadt, Germany) and RP-18 (75 mm; Merck) were used for column chromatography. Thin layer chromatography was carried out on pre-coated silica gel 60 F254 plates and RP-18 F254 plates (both from Merck) and the plates were visualized by spraying with 10% H_2SO_4 /EtOH solution followed by warming.

Sample collection

The aerial parts of *Blumea lacera* were collected in April 2019 at Sapa, Lao Cai Province, Vietnam. The plant was authenticated by Dr. V. H. Do from Department of Plant Resources, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology. A voucher specimen (no. NVT12042019) was deposited at Vietnam Academy of Science and Technology.

Extraction and isolation of compounds from the aerial parts of *B. lacera*

The dried aerial parts of *B.lacera* (0.2 kg) were extracted three times with 95% EtOH (3 × 2.0 L) under reflux. The filtrate was evaporated under reduced pressure to afford a crude extract. That extract (27.5 g) was suspended in H_2O and then partitioned using *n*-hexane, CH_2Cl_2, and EtOAc successively. The EtOAc fraction (6.2 g) was subjected to silica gel column chromatography (100–200 mesh), eluting with a CH_2Cl_2:EtOAc by gradient system (from 30:1 to 0:1, v/v) to afford five fractions (E1–E6) according their TLC profiles.

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Fraction E1 was subjected to silica gel column chromatography and eluted with *n*-hexane:Me_2CO (from 15:1 to 0:1, v/v) to afford four subfractions (E1-1 to E1-4). Further purification of E1-1 by silica gel column chromatography eluted with CH_2Cl_2:EtOAc (20:1, v/v) to yield compound **1** (17.2 mg) and compound **2**. Fraction E4 was subjected to silica gel column chromatography eluted with CH_2Cl_2:MeOH (from 20:1 to 0:1, v/v) to give three subfractions (E4-1 to E4-3). Fraction E4-3 was further separated by MPLC (octadecylsilane, ODS) and eluted with a stepwise gradient of MeOH:H_2O (from 1:1 to 1:0, v/v) to yield two subfractions (E4-3a and E4-3b). Further purification of E4-3b by a silica gel column chromatography eluted with CH_2Cl_2:MeOH:H_2O (from 15:2:1 to 6:4:1, v/v) to obtained compound **3** and compound **4**.

RESULTS AND DISCUSSION

Phytochemical study on the aerial parts of *B. lacera* has led to the isolation of β-sitosterol (1), campesterol (**2**), artemetin (**3**), and acid protocatechuic (**4**). The NMR data of **1** are in accordance with the data reported in the literature for β-sitosterol[15], **2** for campesterol[16], **3** for artemetin[17], and **4** for acid protocatechuic.[18]

Compound **1** was isolated as white powder. The ^{1}H-NMR spectrum of **1** showed six methyl signals that appeared as two methyl singlets at δ_H 0.68 (3H, s, H-18), and 1.00 (3H, s, H-19) confirming the presence of two methyl groups attached to quaternary carbons; three methyl doublets that appeared at δ_H 0.81 (3H, d, J = 6.4 Hz, H-26), 0.82 (3H, d, J = 7.2 Hz, H-27), , and 0.92 (3H, t, J = 6.6 Hz, H-21); and a methyl triplet δ_H 0.84 (3H, overlapping, H-29). The multiplet at δ_H 3.52 (1H, m, H-3) is due to a proton connected to the carbon which attached with -OH group. Moreover, the double doublet signal also appeared for –CH at δ_H 5.35 (1H, d, J = 5.2 Hz, H-6) indicated that the presence of one olefinic proton. The ^{13}C-NMR spectrum of **1** revealed 29 carbon signals, including a pair of olefinic carbons at δ_C 121.7 (C-6) and δ_C 140.8 (C-5), an oxygenated carbon at δ_C 71.8 (C-3). Based on the NMR data and comparison of the data given in the literature, the structure of compound **1** was identified as β-sitosterol.[15]

Compound **2** was also obtained as white powder. The ^{1}H-NMR spectrum of **2** showed six methyl signals [δ_H 0.73 (3H, s, H-18), 0.81 (3H, d, J = 7.0 Hz, H-28), 0.83 (3H, d, J = 7.2 Hz, H-26), 0.87 (3H, d, J = 6.5 Hz, H-27), 0.95 (3H, d, J = 6.0 Hz, H-21), and 1.02 (3H, s, H-19)], one oxygenated methine at δ_H 3.40 (1H, m, H-3), and an olefinic proton at δ_H 5.31 (1H, dd, J = 5.0 Hz, H-5). The ^{13}C-NMR spectrum of **1** revealed 28 carbon signals, including a pair of olefinic carbons at δ_C 121.5 (C-6) and δ_C 142.4 (C-5), an oxygenated carbon at δ_C 71.7 (C-3). The NMR data (Tables 1 and 2) for **2** were almost superimposable on those of **1**, except for the signals from the 24-methyl group (C-28) instead of the 24-ethyl group (C-28–C-29) in **1**. Based on the above data, compound **2** was elucidated to be campesterol by the comparison of spectral data with the literature.[15]

Compound **3** was purified as a white amorphous powder. The ^{1}H-NMR spectrum of **3** displayed a singlet resonance of a chelated hydroxyl proton at δ_H 12.60 (5-OH); four aromatic protons signals, including an ABX spin coupled system at δ_H 7.73 (1H, dd, J = 1.5,8.5 Hz), 7.69 (1H, d, J = 1.5 Hz) and 6.69 (1H, d, J = 8.5 Hz) was assigned to H-6′, H-2′ and H-5′. Further it also revealed the presence of an aromatic proton at δ_H 6.50 (1H, s), which was assigned (Chart 1) to the H-8. In addition, the appearance of the five singlet signals each integrated of three protons related to the aryl methoxyl groups at δ_H 3.97 (3H, s), 3.97 (3H, s), 3.97 (3H, s), 3.93 (3H, s), and 3.87 (3H, s). The ^{13}C NMR and HSQC spectra of **3** showed 20 carbon signals comprising 14 aromatic or olefinic carbons, a carbonyl carbon, and five methoxy carbons. The signal of the conjugated carbonyl at δ_C 178.9 and further signals for conjugated olefinic carbons at δ_C 138.9 and 155.9 were typical of flavone. The overall structure of **3** was deduced mainly by HMBC

Table 1: ^{1}H NMR and ^{13}C NMR data of compounds (1 and 2) (δ values).

Position	Compound			
	1[a]		2[b]	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	37.3		37.3	
2	31.9		28.9	
3	71.8	3,50 (1H, m)	71.7	3.40, m
4	42.3		40.7	
5	140.8		142.4	
6	121.7	5.33, d (3.0)	121.5	5.31, d (5.0)
7	31.7		28.9	
8	31.9		31.0	
9	50.2		51.2	
10	36.5		38.2	
11	21.1		19.8	
12	39.8		39.6	
13	42.3		43.1	
14	56.8		57.7	
15	26.2		21.8	
16	28.5		25.0	
17	56.1		57.0	
18	11.9	0.68, s	19.8	0.73, s
19	19.4	1.00, s	12.2	1.02, s
20	34.0		32.5	
21	18.8	0.92, d (6.6)	19.1	0.95, d (6.0)
22	45.9		34.5	
23	23.1		20.5	
24	45.8		43.3	
25	29.2		36.7	
26	19.8	0.81, d (6.4)	18.5	0.83, d (7.2)
27	19.1	0.82, d (7.2)	19.1	0.87, d (6.5)
28	23.1		24.9	0.81, d (7.0)
29	12.0	0.84, d (6.6)		

[a] Measured in $CDCl_3$-d_3.
[b] Measured in $CDCOCD_3$-d_6.

Table 2: ^{1}H NMR and ^{13}C NMR data of compounds (3 and 4) (δ values).

Position	Compound			
	3[a]		4[b]	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1				123.0
2		155.9	7.43, *d* (1.5)	117.8
3		138.9		146.0
4		178.9		151.5
5		152.8	6.83, *d* (8.0)	123.9
6		132.4	7.46, *dd* (1.5, 8.0)	115.8
7		158.8		
8	6.50, s	90.4		
9		152.4		
10		106.7		
1′		123.0		170.3
2′	7.69, *d* (1.5)	111.4		
3′		148.9		
4′		151.5		
5′	6.99, *d* (8.5)	111.0		
6′	7.73, *dd* (1.5, 8.5)	122.2		
3-OCH_3	3.87, *s*	60.2		
6-OCH_3	3.93, *s*	60.9		
7-OCH_3	3.97, s	56.4		
3′ -OCH_3	3.97, *s*	56.0		
4′ -OCH_3	3.97, *s*	56.1		

[a] Measured $CDCl_3$-d_3
[b] Measured CD_3OD-d_4

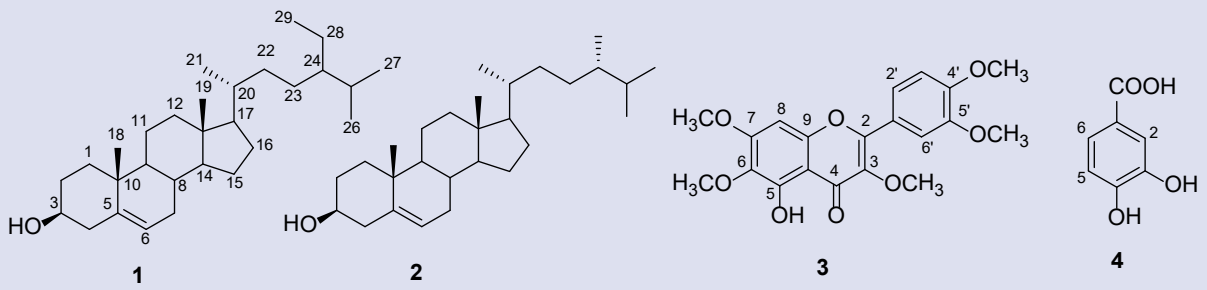

Chart 1: **Chemical structure of isolated compounds (1–4) from B. lacera.**

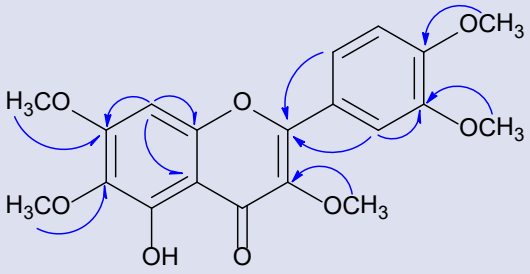

Figure 1: **Selected HMBC correlations of compound 3.**

data. The correlations from H-2′ (δ_H 7.69) and H-6′ (δ_H 7.73) to C-2 (δ_C 155.9) confirmed the attachment of B benzene ring to C-2. HMBC correlations from H-8 (δ_H 6.50) to C-6 (δ_C 132.4), C-7 (δ_C 158.8), C-9 (δ_C 152.4), C-10 (δ_C 106.7), 6-OCH_3 to C-6 (δ_C 132.4), and 7-OCH_3/C-7 (δ_C 158.8) proved the positions of protons of A ring. Two methoxyl groups were linked to the B ring at C-3′ and C-4′ as indicated by the HMBC correlations of –OCH_3 (δ_H 3.97) to C-3′(δ_C 148.9) and –OCH_3 (δ_H 3.97) to C-4′(δ_C 151.5), respectively. The cross peak of the methoxyl group –OCH_3 (δ_H 3.87) with C-3 (δ_C 60.2), indicated that the methoxyl group was linked to C-3 of the aglycone. On the basis of the above analysis, compound **3** was assigned structurally as artemetin (Figure 1). Direct comparison of spectroscopic data from this compound displayed a high similarity with those previously described for 5-hydroxy-3,6,7,3',4'-pentamethoxyflavone or named artemetin.[17]

Compound **4** was obtained as reddish-brown powder. The ^{1}H NMR spectrum of **4** showed an ABX spin coupled system at δ_H 6.83 (1H, d, J = 8.0 Hz, H-5), 7.43 (1H, d, J = 1.5 Hz, H-2), and 7.46 (1H, dd, J = 1.5 & 8.0 Hz, H-6) was assigned to H-5, H-2 and H-6. The ^{13}C NMR spectrum of **4** showed the presence of 7 carbon signals comprising 6 aromatic carbons, and a carbonyl carbon at δ_H 170.3 (C-1'). On the basis of ^{1}H, ^{13}C NMR data and by comparison with those reported in the literature, the compound **4** is identified as acid protocatechuic.[18]

Isolation, identification and characterization of the compounds isolated from aerial parts of *Blumea lacera* yielded four known compounds. They are β-sitosterol (**1**), campesterol (**2**), artemetin (**3**), and acid protocatechuic (**4**). To the best of our knowledge, this is the first report on the isolation of acid protocatechuic (**4**) from the aerial parts of *B. lacera*.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ABBREVIATIONS

NMR: Nuclear Magnetic Resonance; EtOAc: Ethyl acetate; EtOH: Ethanol; CH_2Cl_2: Dichloromethane; Me_2CO: Acetone; CH_3OH: Methanol, H_2O: Water.

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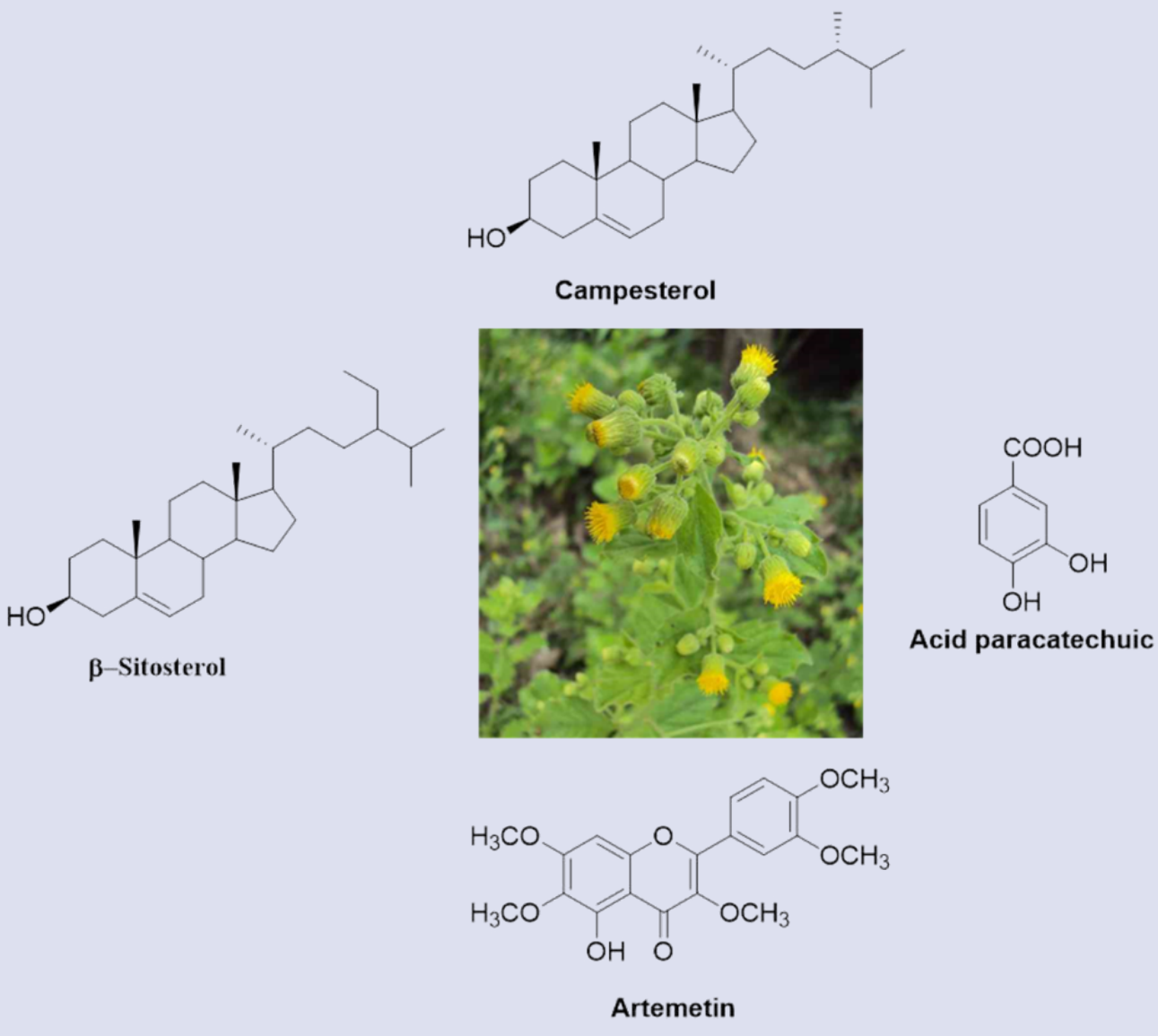
GRAPHICAL ABSTRACT



SUMMARY

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