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Email: tcdhsg@sgu.edu.vn ; Website: http://sj.sgu.edu.vn/

INSIGHTS OF CHEMICAL CONSTITUENTS OF THE MOSS *ERYTHRODONTIUM JULACEUM* PARIS

Nghiên cứu thành phần hóa học của rêu Erythrodontium julaceum Paris

Phạm Nguyễn Kim Tuyến[(1)*], Nguyễn Thị Quỳnh Trang[(1)], Nguyễn Thị Ngọc Thảo[(1)]
[(1)]Trường ĐH Sài Gòn

ABSTRACT

The moss *Erythrodontium julaceum* Paris is widely distributed in Vietnam. Phytochemical data of this moss are scarce. From the moss growing in Lam Dong, the ethyl acetate extract was chemically investigated using multiple chromatographic method. NMR spectroscopic method was exploited to conclude the chemical structures of compounds. Four compounds vicanicin (**1**), ursolic acid (**2**), *β*-amyrin (**3**), and *β*-stigmast-4-en-3-one (**4**) were isolated and structurally elucidated. All isolated compounds **1**-**4** are firstly reported in the genus *Erythrodontium*.

Keywords: *Erythrodontium julaceum, vicanicin, ursolic acid, triterpene.*

TÓM TẮT

Loài rêu *Erythrodontium julaceum* Paris sinh trưởng phổ biến ở Việt Nam. Tuy nhiên, các nghiên cứu hóa thực vật trên loài này ít được thực hiện. Từ loài rêu mọc ở Lâm Đồng, cao ethyl acetate của *Erythrodontium julaceum* được thực hiện phân tích hóa học. Các phương pháp hoá lý hiện đại được thực hiện để xác định cấu trúc của các hợp chất. Bốn hợp chất được xác định cấu trúc gồm vicanicin (**1**), ursolic acid (**2**), *β*-amyrin (**3**), và *β*-stigmast-4-en-3-one (**4**). Các hợp chất vicanicin (**1**), ursolic acid (**2**), *β*-amyrin (**3**), và *β*-stigmast-4-en-3-one (**4**) lần đầu được tìm thấy trong chi *Erythrodontium.*

Từ khoá: *Erythrodontium julaceum, vicanicin, ursolic acid, triterpene.*

1. Introduction

Approximately 14,000 species of mosses were detected and widely distributed in the world [1]. However, the moss *Erythrodontium julaceum* Paris (Entodontaceae) was rarely reported in both chemical and biological perspectives. They are thought to be traditional medicines [1]. *E. julaceum* is native to Vietnam [2]. Chemical data of this moss is little. In 2006, a Chinese research group investigated the antibacterial activity of *E. julaceum* against several Gram-positive bacteria [3] but all of the prepared extracts showed weak activity. To date, there have been two chemical investigations on the moss *E. julaceum* with the reports of 11 components. In 2016, Gao et al. reported six known compounds from the Chinese moss [4]. In 2023, a new compound julacelide together with four known ones were isolated from the Vietnamese bio-source [5].

In the continuation of the chemical investigation of this moss, fractionation of the

*Corresponding author: phngktuyen@sgu.edu.vn

remaining fractions was conducted. Four compounds vicanicin (**1**), ursolic acid (**2**), *β*-amyrin (**3**), and *β*-stigmast-4-en-3-one (**4**) were obtained, and their structure were defined.

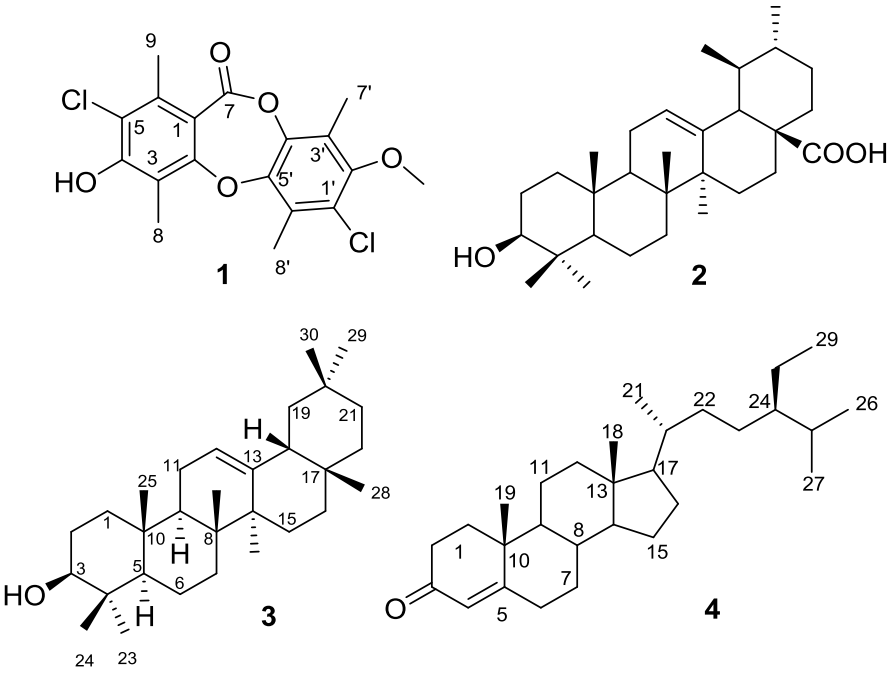


Figure 1. *Chemical structures of isolated compounds* ***1-4***

2. Experimental

2.1. General experimental procedures

Bruker Avance III and MicrOTOF–Q mass spectrometers were chosen for NMR and HRESIMS spectra. NMR (^{1}H, 500 MHz and ^{13}C, 125 MHz) spectra were recorded using deuterated solvents: $CDCl_3$ and acetone-d_6. Normal phase and reverse phase silica gel (Merck) were used for TLC (thin-layer chromatography) method. Silica gel 60 (Himedia) was used for CC (column chromatography) method.

2.2. Plant Material

In *Pinus* trees growing in Lam Dong Province, *Erythrodontium julaceum* Paris was gathered (September 2022). The botanical name (registration No. US-MUE01) was identified by Dr. Tram Vo Khanh Trinh, University of Sciences, Ho Chi Minh City, Vietnam.

2.3. Extraction and Isolation of Compounds

890 g of *E. julaceum* material (dried and ground) was macerated with EtOAc (2 x 10 L) then concentrated using evaporator to provide an EtOAc extract (98 g). A silica gel column chromatography (CC) eluted with *n*-hexane-EtOAc-acetone (10:1:0.2, v/v/v) was conducted on this extract to give ten fractions EA1-EA10. Fraction EA3 (351 mg) was purified by silica gel CC and eluted with *n*-hexane-EtOAc (8:1, v/v) to give fractions EA3.1-3.5. Fraction EA3.1 (34 mg) was rechromatographed by C_{18} silica gel CC, eluted with acetone-H_2O (10:1, v/v) to give compounds **1** (6.3 mg) and **4** (1.7 mg). Fraction EA3.2 (101 mg) was further analyzed by silica gel CC [eluent: *n*-hexane-EtOAc (5:1, v/v)] to give three subfractions EA3.2.1-3.2.3. Purification of

subfraction EA3.2.3 (19 mg) by silica gel CC [mobile phase: *n*-hexane-EtOAc (3:1, v/v)] to give compounds **2** (4.6 mg) and **4** (3.5 mg).

Vicanicin (1)

Colorless oil. ^{1}H NMR and ^{13}C NMR data recorded in $CDCl_3$: see Table 1. The NMR data were consistent with those reported in the literature [6].

Ursolic acid (2)

White amorphous powder. ^{1}H NMR data recorded in $CDCl_3$: 3.11 (1H, dd, J= 10.0, 5.0, H-3), 5.16 (1H, t, J=3.5, H-12), 2.11 (1H, d, J=11.8, H-18), 0.90 (3H, s, H-23), 0.74 (3H, s, H-24), 0.70 (3H, s, H-25), 0.84 (3H, s, H-26), 1.01 (3H, s, H-27), 0.86 (3H, d, J= 6.1, H-29), 0.78 (3H, d, J= 6.5, H-30). ^{13}C NMR data recorded in $CDCl_3$: see Table 2. The NMR data were consistent with those reported in the literature [7].

***β*-Amyrin (3)**

White amorphous powder. ^{1}H NMR data recorded in $CDCl_3$: 3.22 (1H, dd, J= 11.5, 5.0, H-3), 5.25 (1H, brs, H-12), 0.98 (3H, s, H-23), 0.78 (3H, s, H-24), 0.77 (3H, s, H-25), 0.85 (3H, s, H-26), 1.08 (3H, s, H-27), 0.86 (3H, s, H-28), 0.94 (3H, s, H-29), 0.90 (3H, s, H-30). ^{13}C NMR data recorded in $CDCl_3$: see Table 2. The NMR data were consistent with those reported in the literature [8].

***β*-Stigmast-4-en-3-one (4)**

White amorphous powder. ^{1}H NMR data recorded in acetone-d_6: 5.61 (1H, brs, H-4), 0.76 (3H, s, H-18), 1.18 (3H, s, H-19), 0.96 (1H, d, J = 6.5 Hz, H-21), 0.84 (3H, d, J = 6.5 Hz, H-26), 0.81 (3H, d, J = 6.5 Hz, H-27), 0.88 (3H, t, J_1 = 7.0 Hz, J_2 = 7.5 Hz, H-28). ^{13}C NMR data recorded in acetone-d_6: see Tables 2 and 3. The NMR data were consistent with those reported in the literature [9].

3. Results and discussion

Compound **1**, a white amorphous powder was defined its structure using 1D and 2D NMR. In particular, the ^{1}H-NMR and HSQC spectra revealed one methoxy group (δ_H 3.77, 3H, s), four methyl groups (δ_H 2.50, 2.49, 2.42, 2.30, each 3H, s). The ^{13}C spectrum and HMBC correlation indicated the complete ^{13}C assignments of **1** (Table 1). The methyl at δ_H 2.49 gave HMBC correlations to three quaternary carbons at δ_C 137.9 (C-6), 118.6 (C-5), and 115.5 (C-1) and the methyl at δ_H 2.42 gave HMBC correlations to three quaternary carbons at δ_C 159.4 (C-4), 153.4 (C-2), and 114.3 (C-3), indicating the connectivity in the A-ring (Figure 2). The chemical shifts of carbons in the A-ring (especially C-1, C-2, and C-4) were highly similar to those of the A-ring of depsidones, supporting the chemical structure of the A-ring [10]. In the B-ring, the methoxy group at δ_H 4.00 and the methyl at δ_H 2.30 showed a HMBC correlation to carbon at δ_C 152.0 (C-2'), indicating their neighboring positions. This methyl also showed HMBC correlations to carbons at δ_C 142.0 (C-4') and 122.3 (C-3'), defining the chemical shifts of C-3' and C-4'. The methyl at δ_H 2.50 gave HMBC correlations to carbons at δ_C 146.6 (C-5'), 126.4 (C-6'), and 125.5 (C-1'). The chemical shifts of C-4' and C-5' were characteristic of lichen-derived depsidones, supporting the depsidone skeleton of **1** [10]. NMR data of vicanicin and **1** gave high consistency [6], indicating that **1** was vicanicin. Although this compound was isolated previously, the complete ^{13}C NMR data of this compound has not been reported.

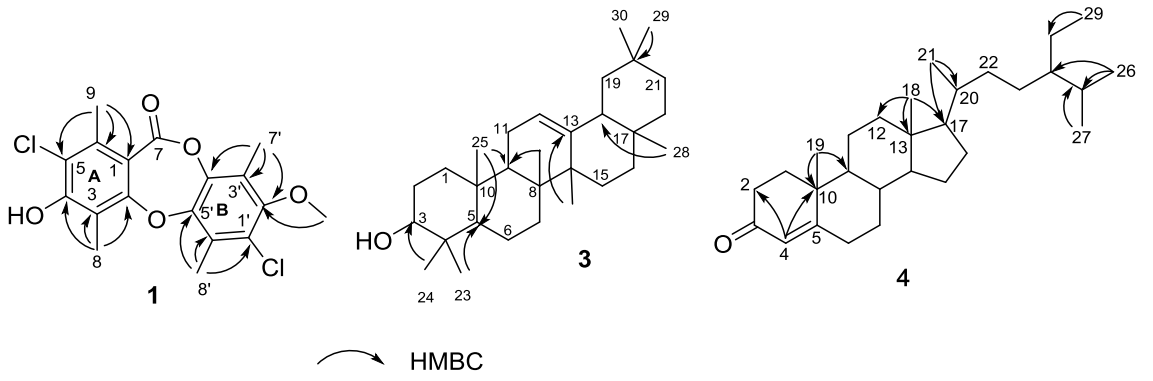


Figure 2. Selected HMBC correlations of **1**, **3**, and **4**

Table 1. ^{1}H NMR and ^{13}C NMR data of compound **1** and **Vicanisin**

No.	**1**		**Vicanisin** [6]
	δ_H (multi, *J* in Hz) ($CDCl_3$, 500MHz)	δ_C ($CDCl_3$, 125MH)	δ_H (multi, *J* in Hz) (DMSO-d_6, 500 MHz)
1		115.5	
2		153.4	
3		114.3	
4		159.4	
5		118.6	
6		137.9	
7		Not determined	
8	2.42 (s)	10.8	2.41 (s)
9	2.49 (s)	18.7	2.45 (s)
1'		125.5	
2'		152.0	
3'		122.3	
4'		142.0	
5'		146.6	
6'		126.4	
7'	2.30 (s)	10.4	2.24 (s)
8'	2.50 (s)	14.3	2.59 (s)
2'-OMe	3.77 (s)	60.6	3.75 (s)

Compound **2** was isolated as a white amorphous powder. The ^{1}H NMR spectrum showed an olefinic proton (δ_H 5.16, H-12), an oxygenated methine (δ_H 3.11, dd, 10.0, 5.0, H-3), together with seven methyls [δ_H 1.01, 0.90, 0.86, 0.84, 0.78, 0.74, 0.70], indicating **2** was a pentacylic-triterpene. The JMOD spectrum exhibited 30 carbon signals, including one carbonyl carbon (δ_C 180.8), two olefinic carbons (δ_C 125.8 and 138.5), one oxygenated methine carbon (δ_C 79.0), seven methyl carbons, nine methylene carbons, five sp^3 methine carbon, five quaternary carbons (see Table 2). The NMR data of **2** were highly compatible to ursolic acid (see Table 2) [7], determining that **2** was ursolic acid.

Compound **3** was isolated as a white amorphous powder. The ^{1}H NMR spectrum showed an olefinic proton (δ_H 5.25, H-12), an oxygenated methine (δ_H 3.22, dd, 11.5, 5.0, H-3), together with eight singlet methyls [δ_H 1.08, 0.98, 0.92, 0.90, 0.86, 0.85, 0.78, 0.77], indicating that **3** was a triterpenoid. The JMOD and HSQC spectra exhibited 30 carbon signals, including two olefinic carbons (δ_C 125.8 and 148.7), one oxygenated methine carbon (δ_C 79.1), eight methyl carbons (δ_C 33.1, 28.1, 28.0, 25.9, 23.6, 17.0, 15.6, 15.5), ten methylene carbons, three sp^3 methine carbon (δ_C 56.9, 56.7, 54.5), six quaternary carbons (48.1, 45.5, 42.2, 39.8, 39.2, and 39.0) (see Table 2). Key HMBC correlations were observed to confirm the assignments of methyl groups. In particular, HMBC cross-peaks of both H_3-23 (δ_H 0.98) and H_3-24 (δ_H 0.78) to C-3 (79.1), of three methyls H_3-23 (δ_H 0.98), H_3-24 (δ_H 0.78), and H_3-25 (δ_H 0.77) to C-5 (δ_C 55.2), of H_3-25 and H_3-26 (δ_H 0.85) to C-9, of H_3-27 (δ_H 1.08) to C-13 (δ_C 148.7), of H_3-28 (δ_H 0.86) to C-18, and of H_3-29 (δ_H 0.94) and H_3-30 (δ_H 0.90) to C-20 confirmed all positions of methyl groups. The NMR data of **3** were highly compatible to β-amyrin (see Table 2) [8], determining that **3** was *β*-amyrin.

Compound **4** was isolated as a white amorphous powder. The ^{1}H NMR spectrum showed an olefinic proton (δ_H 5.61, H-4), together with two singlet methyls [δ_H 1.18, 0.76]. The JMOD and HSQC spectra exhibited 29 carbon signals, including one carbonyl carbon (δ_C 198.3), two olefinic carbons (δ_C 124.2 and 171.2), six methyl carbons (δ_C 20.2, 19.3, 19.3, 17.7, 12.4, 12.3), eleven methylene carbons, seven sp^3 methine carbon (δ_C 57.1, 54.8, 46.7, 36.8, 35.2, 30.1, 21.3), two quaternary carbons (43.2, 39.4) (see Tables 2 and 3). Key HMBC correlations were observed to confirm the assignments of methyl groups. In particular, HMBC cross-peaks of H-4 (δ_H 5.61) to C-2 (δ_C 33.7) and C-10 (δ_C 39.4), of methyls H_3-19 (δ_H 1.18 to C-9 (δ_C 54.8) and C-10 (δ_C 39.4), of methyl H_3-18 (δ_H 12.4) to C-12 (δ_C 40.7), C-13 (δ_C 43.2) and C-17 (δ_C 57.1), of H_3-21 (δ_H 0.96) to C-17 (δ_C 57.1) and C-20 (δ_C 36.8), of H_3-27 (δ_H 0.81) to C-25 (δ_C 30.1), of H_3-26 (δ_H 0.84) to C-24 (δ_C 46.7) and C-25 (δ_C 30.1), and of H_3-29 (δ_H 0.86) to C-28 (δ_C 23.4) confirmed all positions of methyl groups. The NMR data of **4** were highly compatible to β-stigmast-4-en-3-one (see Table 2) [9], determining that **4** was β-stigmast-4-en-3-one.

In this work, three types of skeletons were reported, including a depsidone (**1**), triterpenes (**2** and **3**), and a sterol. To the best of our knowledge, these scaffolds were known to be presented for the first time in the genus *Erythrodontium*.

Table 2. ^{13}C NMR data of compounds **2-4** and β-amyrin

No	**2**	**3**	**4**	β-Amyrin [8]
	(Chloroform-*d*, 125 MHz)	(Chloroform-*d*, 125 MHz)	(Acetone-d_6, 125 MHz)	(Chloroform-*d*, 125 MHz)
1	38.9	39.5	35.2	39.5
2	27.1	27.2	33.7	27.5
3	79.0	79.1	198.3	78.5
4	39.0	39.2	124.2	39.9
5	55.6	55.2	171.2	54.8
6	18.6	18.3	32.7	18.0
7	33.4	33.0	31.9	33.9
8	39.8	38.8	35.2	40.1
9	47.9	47.6	54.8	47.4
10	37.2	37.0	39.4	37.5
11	23.6	23.3	21.3	23.0
12	125.8	125.8	40.7	124.3
13	138.5	148.7	43.2	144.1
14	42.4	41.9	56.4	42.0
15	29.9	28.1	24.9	26.2
16	24.5	27.2	29.0	26.8
17	48.1	31.1	57.1	34.3
18	53.2	52.8	12.4	47.6
19	39.4	47.9	17.7	47.5
20	39.2	31.3	36.8	31.4
21	30.9	35.6	19.3	32.9
22	37.1	36.7	34.9	41.9
23	28.3	28.1	26.2	28.6
24	15.6	15.5	46.7	15.1
25	15.8	15.6	30.1	15.7
26	17.2	17.0	20.2	16.4
27	23.7	25.9	19.3	26.5
28	180.8	28.0	23.4	28.2
29	17.1	33.1	12.3	33.9
30	21.3	23.6		23.8

Table 3. NMR data of **stigmast-4-en-3-one** and **4**

No.	**4**		**Stigmast-4-en-3-one** [9]	
	δ_H (multi, *J* in Hz) (Acetone-d_6, 500 MHz)	**δ_C (Acetone-d_6, 125 MHz)**	**δ_H (multi, *J* in Hz) ($CDCl_3$, 400 MHz)**	**δ_C ($CDCl_3$, 100 MHz**
1	-	35.2	-	35.7
2	-	33.7	-	33.9
3	-	198.3	-	198.9
4	5.61 (1H, *s*)	124.2	5.74 (1H, *d*, *J* = 2.2 *Hz*)	123.6
5	-	171.2	-	171.0
6	-	32.7	-	32.7
7	-	31.9	-	32.1
8	-	35.2	-	35.7
9	-	54.8	-	53.8
10	-	39.4	-	38.6
11	-	21.3	-	21.0
12	-	40.7	-	39.5
13	-	43.2	-	42.4
14	-	56.4	-	55.9
15	-	24.9	-	24.1
16	-	29.0	-	28.1
17	-	57.1	-	56.1
18	0.76 (3H, *s*)	12.4	0.72 (3H, *s*)	12.0
19	1.18 (3H, *s*)	17.7	1.19 (3H, *s*)	17.4
20	-	36.8	-	36.1
21	0.96 (1H, *d*, *J* = 6.5 *Hz*)	19.3	0.93 (1H, *d*, *J* = 6.6 *Hz*)	18.7
22	-	34.9	-	34.0
23	-	26.2	-	26.0
24	-	46.7	-	45.8
25	-	30.1	-	29.1
26	0.84 (3H, *d*, *J* = 3.5 *Hz*)	20.2	0.84 (3H, *d*, *J* = 6.8 *Hz*)	19.8
27	0.81 (3H, *d*, *J* = 3.5 *Hz*)	19.3	0.82 (3H, *d*, *J* = 6.8 *Hz*)	19.2
28	-	23.4	-	23.1
29	0.88 (3H, *t*, J_1 = 7.0 *Hz*, J_2 = 7.5 *Hz*)	12.3	0.85 (3H, *t*, *J* = 7.2 *Hz*)	11.1

4. Conclusions

From the moss *Erythrodontium julaceum* growing in Vietnam, four compounds vicanicin (**1**), ursolic acid (**2**), β-amyrin (**3**), and β-stigmast-4-en-3-one (**4**) were isolated and structurally elucidated. This is the first time that all isolated compounds have been reported in the genus *Erythrodontium.*

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