ISOLATION AND STRUCTURE DETERMINATION OF COMPOUNDS FROM ETHYL ACETATE EXTRACT OF *Chukrasia tabularis*

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Abstract: From an ethyl acetate extract of the barks of *Chukrasia tabularis*, three compounds including one limonoid (tabularisin A), one flavonoid (quercetin), and one coumarin (scopoletin) were isolated by chromatographic methods. Their structures were comfirmed by analysis of spectroscopic data (ESI-MS, ^{1}H-NMR, ^{13}C-NMR, DEPT, HMBC, HSQC và COSY) and by comparison with the literature reports.

Keywords: *Chukrasia tabularis*; limonoid; tabularisin A; quercetin; scopoletin.

1. Introduction

Chukrasia tabularis A. Juss belongs to *Chukrasia* genus, *Meliaceae* family. The trees are tall with a cylindrical bole and spreading crown. *C. velutina* leaves are abruptly pinnate or bipinnate with leaflets that alternate or are subopposite, entire and unequal at the base. The erect, oblong flowers, which are rather large and born in terminal panicles, possess four to five petals. Mature fruits are a septifragal three to five valved capsules [1]. *Chukrasia tabularis* is widely distributed in Asian tropical areas including the South of China and India [2]. In Vietnam, it can be found in Tuyen Quang, Lang Son, Vinh Phu, Hoa Binh, Nghe An, and Ha Tinh. In Chinese traditional medicine, the root barks of *Chukrasia tabularis* A. Juss have been used to treat flu, diarrhea, and some other diseases [3]. Previous studies reveal that *Chukrasia tabularis* trees contain limonoids, triterpenoids, steroids, and flavonoids [4]-[7].

In this paper, we demonstrated the isolation and structure determination of three compounds from an ethyl acetate extract of the barks of *Chukrasia tabularis* collected in Nghe An, Vietnam.

2. Experiment

2.1. Equipment

NMR spectra (^{1}H-NMR, ^{13}C-NMR, DEPT, HMBC, HSQC, COSY) were recorded on a Bruker Avance 500 spectrometer (Vietnam Academy of Science and Technology)) using tetramethyl silane (TMS) as the standard. ESI-MS spectra were obtained on a MS 6420 Agilent spectrometer (Vinh University). Column chromatography was performed using Silica gel (40-60 μm, Merck) or Sephadex LH-20 (Sigma-Aldrich). TLC analyses were performed using aluminium backed Merk silica gel TLC plates (silica gel 60 F_{254}). HPLC was performed on PrepStar 218 Agilent equipment (Vinh University).

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2.2. Material

The barks of *Chukrasia tabularis* were collected in Quy Hop District, Nghe An Province, Viet Nam in July 2020. The sample was identified by Dr. Nguyen Quoc Binh (Viet Nam Natural Museum) and stored at Chemistry-Biology and Environment Institute, Vinh University.

2.3. Isolation

The barks of *Chukrasia tabularis* was sliced and dried at 45^{o}C in 48 h, then powdered (8.0 kg) and extracted with ethanol under ultrasonic conditions (15 L x 3). The solvent was removed by an evaporator to obtain the residue (685 g). The residue was partioned in water and then extracted with hexane, ethyl acetate, and butanol, respectively. Solvents then were removed by an evaporator to afford the corresponding hexane residue (157.5 g), ethyl acetate residue (238.9 g), and butanol residue (64.6 g).

The ethyl acetate residue (238.9 g) was purified by column chromatography using chloroform/methanol eluent (ratio 100:1; 70:1; 60:1; 40:1; 20:1; 10:1; 5:1; 1:1) to get eight fractions (CTE1-CTE8). The fraction CTE3 (16.4 g) was purified by column chromatography using hexane/acetone eluent (ratio: 10:1; 5:1; 3:1; 1:1) to afford compound **CT2** (22.4 mg). The same procedure was performed with fraction CTE5 (31.5 g), using hexane/ethyl acetate eluent to furnish five subfractions (CTE5-1 to CTE5-5). Column chromatography of CTE5-2 on Sephadex LH-20 column using chloroform/methanol eluent (1:1) generated compound **CT3** (13.7 mg). The subfraction CTE6 (12.6 g) was purified by HPLC using methanol/water (2:3) eluent to afford compound **CT1** (14.8 mg).

Compound 1 (CT1): Colorless powder; ESI-MS *m/z* 860 $[M]^+$; ^{1}H-NMR (500 MHz, *CDCl$_3$*) δ_H (ppm): 7.49 (1H, *s*, H-21), 7.39 (1H, *t*, *J* = 2.0 Hz, H-23), 7.10 (1H, *d*, *J* = 2.5 Hz, H-15), 6.50 (1H, *d*, *J* = 1.5 Hz, H-22), 6.43 (1H, *s*, H-17), 5.91 (1H, *s*, H-6), 5.48 (1H, *s*, H-3), 5.38 (1H, *s*, H-30), 5.33 (1H, *dd*, *J* = 3.5, 1.0 Hz, H-12), 4.23 (1H, *d*, *J* = 3.0 Hz, H-11), 3.80 (3H, *s*, 7-OMe), 3.33 (1H, *s*, 2-OH), 2.89 (1H, *s*, H-5), 2,86 (1H, *s*, 1-OH), 2.67 (1H, *dd*, *J* = 7.0, 3.0 Hz, H-18a), 2.53 (1H, *m*, H-2'), 2.33 (3H, *s*, 15-OAc), 2.22 (3H, *s*, 3-OAc), 2.19 (3H, *s*, 6-OAc), 2.16 (1H, *d*, *J* = 10.5 Hz, H-29a), 1.96 (1H, *d*, *J* = 12.0 Hz, H-29b), 1.67 (6H, *s*, 12-OAc and H-32), 1.42 (1H, *d*, *J* = 6.5 Hz, H-18b), 1.35 (3H, *s*, H-19), 1.22 (3H, *d*, *J* = 7.0 Hz, H-3'), 1.18 (3H, *d*, *J* = 7.0 Hz, H-4'), 0.99 (3H, *s*, H-28); ^{13}C-NMR (125 MHz, *CDCl$_3$*) δ_C (ppm): 173.4 (C-1'), 172.2 (15-OAc), 171.5 (C-7), 170.6 (12-OAc), 169.0 (3/6-OAc), 166.8 (C-16), 143.4 (C-23), 141.9 (C-21), 122.1 (C-20), 119.5 (C-32), 109.7 (C-22), 90.7 (C-9), 85.9 (C-3), 82.9 (C-1), 78.1 (C-8), 76.5 (C-2), 74.8 (C-11), 71.4 (C-17), 70.7 (C-6), 70.0 (C-30), 69.7 (C-15), 66.3 (C-12), 53.6 (7-OMe), 45.1 (C-10), 44.7 (C-4), 43.0 (C-5), 39.9 (C-29), 33.9 (C-2'), 30.9 (C-13), 30.6 (C-14), 21.5 (15-OAc), 21.0 (3/6-OAc), 19.6 (12-OAc), 19.4 (C-3'), 18.8 (C-4'), 18.5 (C-18), 16.2 (C-32), 15.2 (C-28), 15.1 (C-19).

Compound 2 (CT2): Yellow powder; ESI-MS *m/z* 303 $[M+H]^+$; ^{1}H-NMR (500 MHz, *CD$_3$OD*) δ_H (ppm): 7.75 (1H, *s*, H-2'), 7.66 (1H, *d*, *J* = 8.5 Hz, H-6'), 6.91 (1H, *d*, *J* = 8.5 Hz, H-5'), 6.41 (1H, *s*, H-8), 6.21 (1H, *s*, H-6);^{13}C-NMR (125 MHz, *CD$_3$OD*) δ_C (ppm): 177.4 (C-4), 165.6 (C-7), 162.5 (C-5), 158.3 (C-9), 148.8 (C-4'), 146.3 (C-2), 146.2

(C-3'), 137.2 (C-3), 124.2 (C-1'), 121.7 (C-6'), 116.3 (C-5'), 116.0 (C-2'), 104.5 (C-10), 99.3 (C-6), 94.4 (C-8).

Compound 3 (CT3): Colorless needle crystal; ESI-MS *m/z* 193 $[M+H]^+$; ^{1}H-NMR (500 MHz, *CD_3OD*) δ_H (ppm): 8,84 (1H, *br.s*), 7,58 (1H, *d*, *J* = 9,6 Hz), 6,88 (1H, *s*), 6,82 (1H, *s*), 6,27 (1H, *d*, *J* = 9,6, Hz), 3,90 (3H, *s*). ^{13}C-NMR (125 MHz, *CD_3OD*) δ_C (ppm): 161,3 (C-2), 151,8 (C-6), 151,1 (C-8a), 145,9 (C-7), 144,6 (C-4), 113,8 (C-5), 112,3 (C-4a), 109,9 (C-3), 103,7 (C-8), 56,7 (6-OCH_3).

3. Result and discussion

From an ethyl acetate extract of the barks of *Chukrasia tabularis*, three pure compounds labeled as **CT1, CT2** và **CT3** were obtained by column chromatography and HPLC.

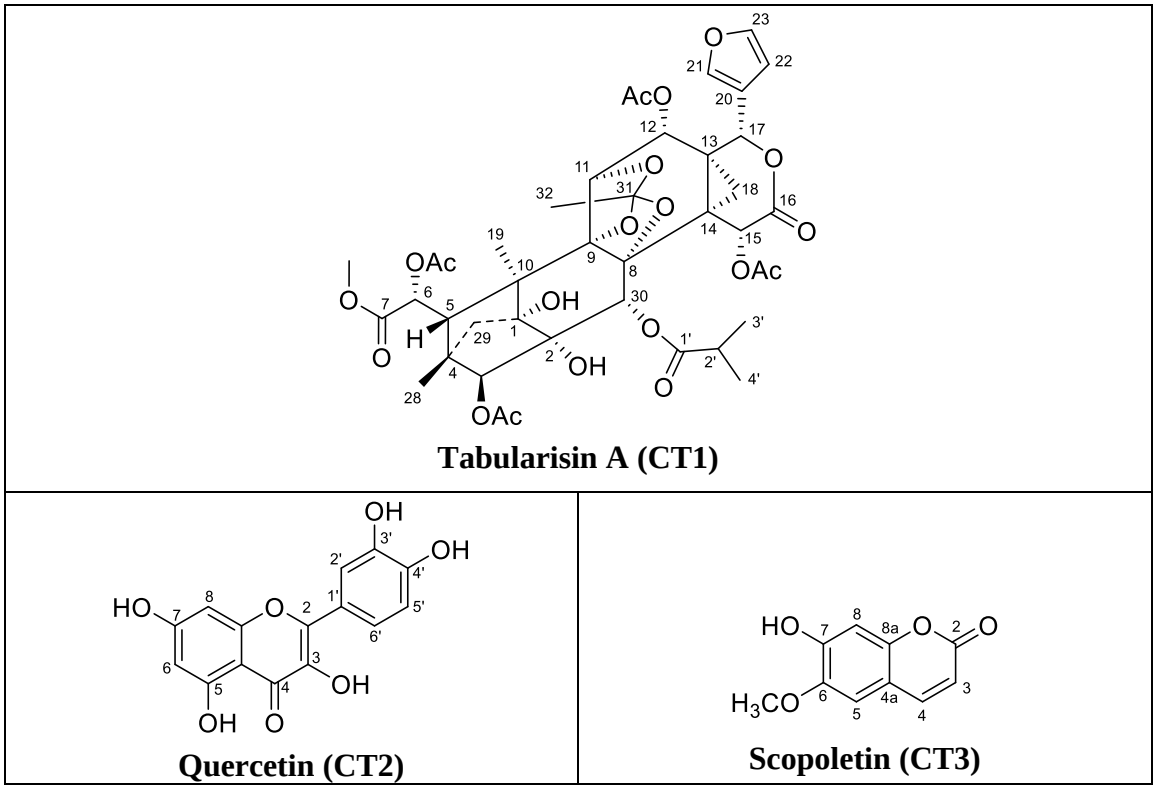

Figure 1: *Structures of compounds **CT1, CT2**,* and ***CT3***.

Compound **CT1** was isolated as a colorless powder. The ESI-MS spectrum shows the molecular ion of *m/z* 860 $[M]^+$ which is consistent with the molecular formula of $C_{41}H_{48}O_{20}$ (18 degrees of unsaturation). Analyses of ^{1}H-NMR, ^{13}C-NMR, DEPT, HSQC spectra revealed that **CT1** contains a methoxyl group (7-OMe, δ_C 53.6 and δ_H 3.80), an isobutyryl group, four acetoxyl group (3-OAc, 6-OAc, 12-OAc, and 15-OAc), a β-furyl (δ_C 122.1, δ_C 141.9, δ_C 109.7, and δ_C 143.4; δ_H 7.49, δ_H 6.50, and δ_H 7.39). Positions of these groups were deduced from interactions between proton and carbon in HMBC spectra. ^{13}C-NMR, DEPT spectra indicate signals of 22 carbon atoms including two ester carbons, eight quaternary carons (four of them linked to oxygen), 8 methine carbons (seven of them

linked to oxygen), two methylene carbon, and two tertiary methyl carbons. The aforementioned structural characteristic suggested **CT1** is a phragmalin-type limonoid. Comparison spectroscopic data of **CT1** with literature data [8,9] confirmed that **CT1** is tabularisin A.

Table 1: *^{1}H-, ^{13}C-NMR data of compound **CT1***

#	δ_C[a]	δ_C[b] [9]	δ_H[c]	δ_H[d] [9]
1	82,9	82,9	-	-
2	76,5	76,4	-	-
3	85,9	85,8	5,48, *s*	5,46, *s*
4	44,7	44,6	-	-
5	43,0	42,9	2,89, *s*	2,87, *s*
6	70,7	70,6	5,91, *s*	5,89, *s*
7	171,5	171,4	-	-
8	78,1	78,0	-	-
9	90,7	90,6	-	-
10	45,1	44,9	-	-
11	74,8	74,7	4,23, *d*, 3,0	4,21, *d*, 2,9
12	66,3	66,2	5,33, *dd*, 3,5, 1,0	5,30, *br d*, 2,9
13	30,9	30,8	-	-
14	30,6	30,5	-	-
15	69,7	69,6	7,10, *d*, 2,5	7,09, *d*, 3,0
16	166,8	166,7	-	-
17	71,4	71,3	6,43, *s*	6,42, *s*
18	18,5	18,4	2,67, *dd*, 7,0, 3,0 1,42, *d*, 6,5	2,65, *dd*, 7,0, 3,0 1,42, *br d*, 7,0
19	15,1	15,0	1,35, *s*	1,34, *s*
20	122,1	121,9	-	-
21	141,9	141,9	7,49, *s*	7,47, *br s*
22	109,7	109,6	6,50, *d*, 1,5	6,49, *d*, 0,8
23	143,4	143,9	7,39, *t*, 2,0	7,38, *br s*
28	15,2	15,1	0,99, *s*	0,99, *s*
29	39,9	39,8	2,16, *d*, 10,5 1,96, *d*, 12,0	2,05, *d*, 10,8 1,94, *d*, 10,8
30	70,0	69,9	5,38, *s*	5,37, *s*
31	119,5	119,4	-	-
32	16,2	16,1	1,67, *s*	1,66, *s*
7-OMe	53,6	53,6	3,80, *s*	3,78, *s*
1’	173,4	173,4	-	-
2’	33,9	33,8	2,53, *m*	2,50-2,55, *m*
3’	19,4	19,3	1,22, *d*, 7,0	1,21, *d*, 7,2
4’	18,8	18,7	1,18, *d*, 7,0	1,19, *d*, 7,2
3-OAc	169,0 21,0	169,0 20,9	- 2,22, *s*	- 2,11, *s*

#	δ_C[a]	δ_C[b] [9]	δ_H[c]	δ_H[d] [9]
6-OAc	169,0 21,0	169,0 20,9	- 2,19, *s*	- 2,08, *s*
12-OAc	170,6 19,6	170,5 19,5	- 1,67, *s*	- 1,66, *s*
15-OAc	172,2 21,5	172,2 21,4	- 2,33, *s*	- 2,32, *s*
1-OH	-		2,86, *s*	2,84, *s*
2-OH	-		3,33, *s*	3,33, *s*

[a] *Measured in $CDCl_3$, 125 MHz.*
[b] *Measured in $CDCl_3$, 100 MHz [9].*
[c] *Measured in $CDCl_3$, 500 MHz.*
[d] *Measured in $CDCl_3$, 400 MHz [9].*

Compound **CT2** was obtained as a yellow powder. ESI-MS spectrum of **CT3** showed $[M+H]^+$ ion peak at *m/z* 193, corresponding to the molecular formula of $C_{15}H_{10}O_7$. Analyses of NMR data of **CT2** suggested that it is a flavonoid. ^{1}H-NMR spectrum shows signals of aromatic protons at δ_H 6.21 (s, H-6), δ_H 6.41 (s, H-8), δ_H 7.75 (s, H-2'), δ_H 6.91 (d, 8.5, H-5'), and δ_H 7.66 (d, 8.5, H-6'). Additional information from ^{13}C-NMR and DEPT data of **CT2** indicated signals of 15 carbons including five tertiary carbons and ten quaternary carbon, especially one carbonyl carbon at δ_C 177.4 (C-4). Comparison spectroscopic data of **CT2** with literature data [10] confirmed that **CT1** is quercetin.

Compound **CT3** was isolated as a colorless needle crystal. ESI-MS spectrum of **CT3** showed $[M+H]^+$ ion peak at *m/z* 193, corresponding to the molecular formula of $C_{10}H_9O_4$. ^{1}H-NMR spectra of **CT3** show signals of C3-H and C4-H of the coumarin skeleton at δ_H 6.24 ppm and δ_H 7.60 ppm, respectively. Protons at C-5, C-8, and OCH_3 resonate at δ_H 6.91 ppm, δ 6.84 ppm, and δ_H 3.94 ppm (3H, *s*), respectively. ^{13}C-NMR and DEPT spectra of **CT3** shows signals of ten carbons including five methine group, five quaternary carbons, and one -OCH_3. Analyses of MS, ^{1}H-NMR, and ^{13}C-NMR data and comparison with the literature report [11] concluded that **CT3** is scopoletin.

4. Conclusion

The barks of *Chukrasia tabularis*) extracted with ethanol and the resulting was fractionally extracted with ethyl acetate to afford the ethyl acetate extract and then the ethyl acetate residue by evaporation. Three compounds labelled as **CT1**, **CT2**, and **CT3** were obtained by using chromatography. The structures of these compounds were confirmed by analyses of NMR data and by comparison with literature data. They are tabularisin A (**CT1**), quercetin (**CT2**), and scopoletin (**CT3**).

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TÓM TẮT

PHÂN LẬP, XÁC ĐỊNH CẤU TRÚC CÁC HỢP CHẤT TỪ CAO ETHYL ACETATE CỦA CÂY LÁT HOA (*Chukrasia tabularis*)

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Ngày nhận bài 31/8/2022, ngày nhận đăng 12/10/2022

Từ cao chiết ethyl acetate của vỏ cây Lát hoa (*Chukrasia tabularis*), sử dụng kết hợp các phương pháp sắc kí, chúng tôi đã phân lập được 3 hợp chất sạch gồm 1 hợp chất limonoid (tabularisin A), 1 hợp chất flavonoid (quercetin) và 1 hợp chất coumarin (scopoletin). Cấu trúc của các hợp chất này đã được xác định bằng các phương pháp phổ (ESI-MS, ^{1}H-NMR, ^{13}C-NMR, DEPT, HMBC, HSQC và COSY) và so sánh với các dữ liệu phổ của các chất đã biết.

Từ khóa: *Chukrasia tabularis*; limonoid; tabularisin A; quercetin; scopoletin.