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Isolation and identification of triterpenoid compounds from *Couroupita guianensis* Aubl.

Ngo Quoc Luan[1*], Nguyen Phu Thach[1], Nguyen Van Minh Em[1], Nguyen Thi Ngoc Trinh[1], Phan Nhu Thao[2], Ngo Khac Khong Minh[3], Ngo Trong Nghia[4], Phan Nhat Minh[5,6], and Nguyen Tan Phat[4,5]

[1]*School of Education, Can Tho University, Viet Nam*

[2]*Nguyen Van Con High School, Tien Giang Province, Viet Nam*

[3]*Medical Faculty, Nam Can Tho University, Viet Nam*

[4]*Faculty of Environment and Natural Resource, Kien Giang University, Viet Nam*

[5]*Institute of Chemical Technology, Vietnam Academy of Science and Technology, Viet Nam*

[6]*University of Science and Technology, Vietnam Academy of Science and Technology, Viet Nam*

Correspondence: Ngo Quoc Luan (email: ngoquocluan@ctu.edu.vn)

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ABSTRACT

In this report, the extracts from the fruit and leaves of Couroupita guianensis were isolated using chromatographic methods and investigated for chemical composition. Four triterpenoid compounds were isolated and identified as betulinic acid, oleanolic acid, β-amyrin and friedelin. Their chemical structures were interpreted based on modern spectra such as MS, NMR and compared with previously published spectral data.

1. INTRODUCTION

1a. Stem

1b. Leaves

1c. Flowers

1d. Fruit

Figure 1. Some parts of *Couroupita guianensis* Aubl.

Couroupita guianensis Aubl. (Figure 1) was discovered and given scientific nomenclature in 1755 by the French botanist J. F. Aublet (Nelson & Wheeler, 1937). This plant originates from Guyana (South America). In Viet Nam and some countries with developed Buddhism, they have been typically grown within pagodas. Commonly they have Vietnamese names such as Dau lan, Ngoc ky lan, Ham Rong, Sala (Ho, 2003).

Worldwide, research projects have investigated the plant use for medicine, such as investigating the antibacterial and antioxidant activities of plant extracts. However, the number of publications on the chemical composition of this species remains minor.

In Vietnam, the plant use is mainly based on folk experience, to the best of our knowledge, up to now, there has not been any scientific study into the plant's chemical composition, as well as the biological activity of the active ingredients for safe use as medicinal herbs. Therefore, it is necessary to investigate the chemical composition of this species, especially the tree in the geographical conditions of Vietnam.

2. EXPERIMENT

2.1. Plant material

The fruit and leaves of *Couroupita guianensis* were harvested in September 2019 and December 2020, respectively, in Can Tho city, Vietnam. Voucher specimens were identified at the Department of Biology, School of Education, Can Tho University. After cleaning, the insect-infested parts were removed. Selected material was dried at 50°C to constant weight and then was grounded into fine powder.

2.2. General experimental procedures

2.2.1. Extraction and purification

Solid-liquid extracts were obtained with methanol, *n*-hexane, ethyl acetate and acetone. Solvent evaporating was accomplished by using RE-52A rotary evaporator system (China).

Thin layer chromatography (TLC) was carried out on pre-coated silica gel $60F_{254}$ (0.25 mm) aluminum sheet (Merck). Traces of compounds were detected by illuminating under UV light (254/365 nm) or spraying 10% H_2SO_4 solution in ethanol and then heating at 105°C for 1–2 min on an electric hot plate.

For normal phase column chromatography (NP-CC), silica gel 60 (0.040–0.063 mm, Merck), increasing polarity solvent systems including *n*-hexane (H), chloroform (C), ethyl acetate (E) and methanol (M) were used. Substances were cleaned by re-crystallization in pure solvents.

2.2.2. Structural elucidation and identification

The melting point (mp.) was read by a melting point device (Electrothermal 9100, UK), using capillary at Can Tho University. ^{1}H-NMR, ^{13}C-NMR, DEPT, HSQC, COSY, and HMBC spectra were recorded on a Bruker AM500, 600 FT-NMR spectrometer; Mass spectrum (MS) was recorded on mass spectrometer (HP 1100 series, LC/MSD Trap, Agilent) at the Vietnam Academy of Science and Technology.

2.3. Extraction and isolation

The dried fruit powder (3.0 kg) was exhaustedly extracted with methanol 70%vol (> 20 L) and then evaporated under reduced pressure to remove the

solvent to give the dry dark-green residue of crude methanol extract (FCGMe, 400 g).

The FCGMe extract (400 g) was distributed with *n*-hexane (10 L), ethyl acetate (15 L), and acetone (10 L), respectively, and the solvents were evaporated under poor pressure to obtain subextracts FCGHe (77.4 g), FCGEt (94.2 g), FCGAc (41.6 g) and the remainder was insoluble in distributed solvents (FCGW, 186.8 g).

The FCGEt subextract (94.2 g) was subjected to NP-CC with H:E (gradient, 0 to 100% E) and then with E:M (gradient, 0 to 100% E) solvent mixtures as eluent to give 11 fractions (FCGE1-11).

The fraction FCGE2 (H:E 8:2; 5.69 g) was treated with NP-CC (H:E, gradient, 0 to 100% E) to afford 5 subfractions (FCGE2.1-2.5). The subfraction FCGE2.3 (H:E 95:5, 1.32 g) was continued to take NP-CC (E:M 9:1) to get 4 subfractions (FCGE2.3.1-2.3.4). The subfraction FCGE2.3.3 (E:M 9:1, 80 mg) was re-crystallized in methanol to produce compound **3** (10.5 mg).

The fraction FCGE3 (H:E 7:3, 7.36 g) was performed NP-CC with C:M solvent systems (gradient, 0 to 100% M) to afford 6 subfractions (FCGE5.1-5.6). The subfraction FCGE3.3 (C:M 95:5, 1.84 g) was continued to take NP-CC (C:M 9:1) to produce 4 subfractions (FCGE3.3.1-3.3.4). The fraction FCGE33.2 (C:M 9:1, 120 mg) was re-crystallized two times in methanol to yield compound **1** (19.1 mg).

The dried powder of leaves was soaked in MeOH for three days and then filtered. The filtrates were concentrated under vacuum to produce the crude extracts of leaves (LCGMe, 743 g).

The LCGMe extract (700 g) was distributed with *n*-hexane (15 L) and acetone (15 L), respectively, and the solvents were evaporated under poor pressure to obtain subextracts LCGHe (221 g), LCGAc (275 g) and the remainder was insoluble in distributed solvents (LCGW, 160 g).

The LCGHe subextract (200 g) was taken NP-CC with eluent of H:E (gradient, 0 to 100% E) to give 6 subfractions (LCGH1-6). The fraction LCGH3 (H:E 9:1, 11.5 g) was continued to take NP-CC (H:E from 95:5 to 8:2) to get 5 subfractions (LCGH3.1-3.5). The fraction LCGH3.3 (H:E 85:15, 2.75 g) was re-crystallized several times in $CHCl_3$ to obtain compound **4** (12.3 mg).

The LCGAc subextract (250 g) was taken NP-CC with eluent of H:E (gradient, 0 to 100% E) to give 7 subfractions (LCGA1-7). The fraction LCGA4 (H:E 5:5, 14.3 g) was continued to take NP-CC (H:E from 7:3 to 6:4 and ending with E) to get 5 subfractions (LCGA4.1-4.5). The fraction LCGA4.3 (H:E 5:5, 2.45 g) was continued to take NP-CC (C:M from 95:5 to 85:1 and ending with M) to get 4 subfractions (LCGA4.3.1-4.3.4). The fraction LCGA4.3.2 re-crystallized several times in $CHCl_3$ to afford compound **2** (15.2 mg).

3. RESULTS AND DISCUSSIONS

All four isolated substances appeared to belong to the same group of natural compounds with some similar characteristics such as white solids, did not fluoresce under UV light and did not react positively with $FeCl_3$ reagent. Therefore, it can be inferred that they did not have the properties of phenols. Typical signals of protons and carbons in their 1D-NMR suggested that they had the patterns of triterpene backbones.

3.1. Compound 1

Compound **1** was isolated as white needle-shaped crystals, its mp. was about 316–318°C. It had violet-red TLC stain and no luminescence under UV lamp, which proved the absence of a conjugated system in its chemical structure.

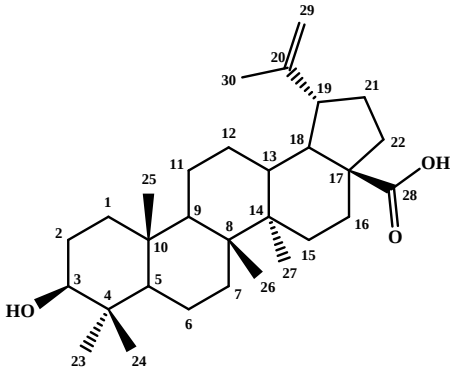
The molecular formula of compound **1** was established as $C_{30}H_{48}O_3$ (456 amu, seven degrees of unsaturation) by ESI-MS (*m/z* 455 $[M-H]^-$).

^{1}H-NMR spectrum of compound **1** unlocked typical proton signals of 6 tertiary methyl groups at δ_H [0.76 (3H, *s*), 0.83 (3H, *s*), 0.94 (3H, *s*), 0.97 (3H, *s*), 0.98 (3H, *s*), and 1.69 (3H, *s*)]; 1 oxygenated methine proton at δ_H [3.17-3.20 (1H, *dd*, 5.0, 11.5)]; two double-bonded methylene protons at δ_H [4.74 (1H, *d*, 2.0), 4.61 (1H, *t*, 1.5)] and about 27 other protons of methine, methylene, hydroxyl groups (Table 1).

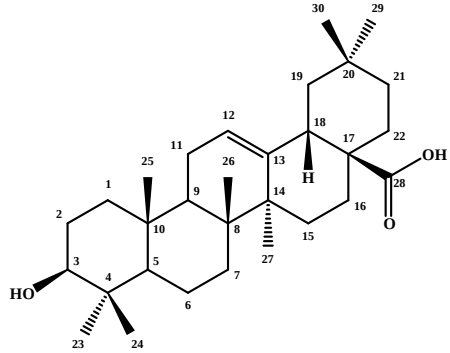
^{13}C-NMR and DEPT spectra appeared signals of total of 30 carbons containing 6 methyl, 11 methylene, 6 methine and 7 quaternary carbons. There were typical signals as one carbonyl group at δ_C 179.9; one oxygenated methine at δ_C 79.0; one exo-methylene at δ_C 109.7; one vinylic quaternary carbon at δ_C 150.4; 6 methyl carbons at δ_C [14.7, 15.3, 16.0, 16.1, 19.4, 28.0]. The rest were other aliphatic methyl, methylene and quaternary carbons (Table 1).

With the above-analyzed spectral characteristics, it is possible to confirm that compound **1** was a lupeol-

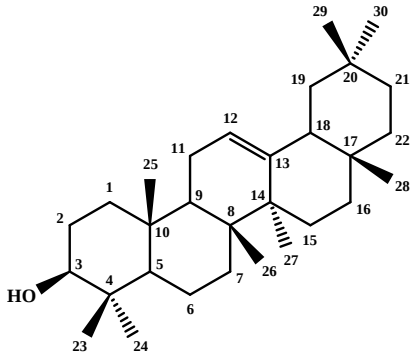
type triterpene derivative. The 1D-NMR spectral data of compound **1** were similar to those of betulinic acid (Figure 2) given in the previously reported (Chichir et al., 2018). Moreover, all correlation signals in HSQC and HMBC spectra of compound **1** conformed with the mentioned chemical structure, so compound **1** was determined to be betulinic acid.



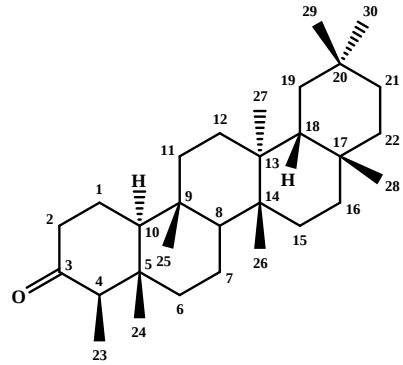
Betulinic acid (1)



Oleanolic acid (2)



***β*-Amyrin (3)**



Friedelin (4)

Figure 2. Chemical structures of the isolated compounds

Betulinic acid is apentacyclic triterpene which was commonly found in plants; however, this was first isolated from *Couroupita guianensis.* Its bioactivity has been verified toward cell lines of lung cancer (A549), colorectal carcinoma (DLD-1), breast cancer (MCF-7) and prostate cancer (PC-3), but no activity for cutaneous fibroblasts (WS1-1) (Chudzik et al., 2015).

3.2. Compound 2

Compound **2** was obtained as a white amorphous powder, its mp. was about 306–308°C. It had lotus purple chromatographic spot and no luminescence under UV lamp and negative reaction of phenolic reagent.

The molecular formula of compound **2** was speculated to be $C_{30}H_{48}O_3$ (456 amu, seven degrees of unsaturation) on the basis of ESI-MS (*m/z* 455 $[M-H]^-$).

The ^{1}H-NMR spectrum of compound **2** revealed 7 singlet signals of methyl protons at δ_H [0.75 (3H, *s*), 0.77 (3H, *s*), 0.90 (3H, *s*), 0.91 (3H, *s*), 0.93 (3H, *s*), 0.98 (3H, *s*) and 1.13 (3H, *s*)]; one oxygenated methine proton at δ_H 3.23 (1H, *dd*, 11.0, 4.0); one double-bonded methine proton at δ_H 5.28 (1H, *s*) and about 25 other protons of methine, methylene, hydroxyl groups (Table 1).

The ^{13}C-NMR and DEPT spectra of compound **2** exhibited signals of total 30 carbons including 7 methyl, 10 methylene, 5 methine and 8 quaternary carbons. In which, 2 carbons at δ_C 122.6 and 144.8 proved the presence of a double bond; carbon at δ_C 183.4 allowed to predict having a carboxylic group; and carbon at δ_C 79.1 was oxygenated methine group (Table 1).

From its typical 1D-NMR data, compound **2** gave the characteristic spectra pattern of a oleanane-type triterpene. Based on the above spectral data analysis and the parallel of those given in the literature (Güvenalp et al., 2009) and checking with its 2D-NMR spectra, compound **2** was identified as oleanolic acid (Figure 2).

Oleanolic acid is a common pentacyclic triterpene in plants, however, this was also first time it has been isolated from *Couroupita guianensis.* Oleanolic acid could be used to prevent the majority of the most common diseases of civilization *i.e.* cancer, cardiovascular diseases, atherosclerosis or diabetes (Paszel-Jaworska et al., 2014).

3.3. Compound 3

Compound **3** was also received as white amorphous powder, its mp. was 197–198°C. It had red purple TCL spot, no luminescence under UV light and no positive reaction of phenolics with Folin-Ciocalteu's reagent.

HRMS of compound **3** corresponded to the molecular formula of $C_{30}H_{50}O$ (426.39 amu, six degrees of unsaturation) by a peak at *m/z* 427.39397 $[M+H]^+$.

Most of 1D-NMR spectral signals of compound **3** were similar to those of compound **2** (Table 1) except for the difference which was compound **3** had only one more methyl carbon than compound **2** and instead of the disappearance of a carbonyl group. This showed that compound **3** was also the oleanane-type triterpene backbone.

Spectral data of compound **3** were compared with those given in the literature (Begum et al., 2009), 2D-NMR spectra of **3** were also used to check the fit of the predicted structure. As a result, compound **3** was identified as β-amyrin (Figure 2).

β-Amyrin had been founded from *Couroupita guianensis* before (Begum et al., 2009). It is believed to have good antioxidant, anti-inflammatory, and anti-cancer activities (Cardoso et al., 2018; Thirupathi et al., 2017).

3.4. Compound 4

Compound **4** was also received as white needle-shaped crystals, its mp. was 262–264°C. It had purple pink TCL trace, did not light up under UV lamp and was negative for phenolic reagent.

The molecular formula of compound **4** was the same as compound **3**, $C_{30}H_{50}O$ (426.39 amu, six degrees of unsaturation), based on its HRMS fragment at *m/z* 427.3881 $[M+H]^+$.

In general, 1D-NMR spectral signals of compound **4** and those of compound **3** were alike. However, compound **4** had a lack of a carbon-carbon double-bond, but was offset by a ketone group at δ_C 213.2 (Table 1). All of the above information allowed compound **4** to be identified as friedelane skeleton derivative.

Spectral data of compound **4** were compared with those of the published report (Ragasa et al., 2015). The elucidated structure was also tested by 2D-NMR spectra of compound **4** and then determined as friedelin (Figure 2).

Friedelin was found in *Couroupita guianensis* for the first time. It is reported to contain potent antimycobacterial, anti-inflammatory, analgesic, antipyretic, pathogenic fungi, and anti-Hela cancer cell activities (Ragasa et al., 2015).

4. CONCLUSIONS

This study reports part of the survey results on the chemical composition of the fruit and leaves of Couroupita guianensis collected in Can Tho city, Vietnam.

Except β-amyrin, three further isolated triterpenes, betulinic acid, oleanolic acid, and friedelin were seen for the first time from this plant. All these compounds have quite good biological activities according to previous reports. Further results will be published in the next paper.

Table 1. Assigned ^{1}D-NMR spectral data of isolated compounds

No.	Compound 1		Compound 2		Compound 3		Compound 4	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1	1.65-1.71 (1H, *m*), 0.87-0.91 (1H, *m*)	38.7	1.60 (1H, *m*), 0.95-0.96 (1H, *m*)	38.4	0.96-0.98 (1H, *m*), 1.63-1.65 (1H, *m*)	38.6	1.97 (1H, *m*), 1.69 (1H, *m*)	22.3
2	1.55-1.61 (1H, *m*)	27.4	1.74-1.76 (1H, *m*), 1.56 (1H, *m*)	27.2	1.59-1.63 (1H, *m*), 1.54-1.59 (1H, *m*)	27.3	2.39 (1H, *m*), 2.30 (1H, *m*)	41.5
3	3.17-3.20 (1H, *dd*, 5.0, 11.5)	79.0	3.23 (1H, *dd*, 11.0, 4.0)	79.1	3.20-3.24 (1H, *dd*, 4.5, 11.0)	79.1		213.2
4		38.9		38.8		38.8	2.25 (1H, *q*, 7.0)	58.3
5	0.67-0.69 (1H; *m*)	55.4	0.72-0.75 (1H, *overlap*)	55.3	0.73-0.76 (1H; *m*)	55.2		42.2
6	1.47-1.57 (1H, *m*), 1.37-1.47 (1H, *m*)	18.3	1.52-1.53 (1H, *m*), 1.32-1.35 (1H, *m*)	18.3	1.39-1.46 (1H, *m*), 1.54-1.59 (1H, *m*)	18.4	1.76 (1H, *m*), 1.29 (1H, *m*)	41.3
7	1.37-1.47 (1H, *m*)	34.4	1.43-1.44 (1H, *m*), 1.28-1.31 (1H, *m*)	32.6	1.49-1.54 (1H, *m*), 1.27-1.39 (1H, *m*)	32.7	1.49 (1H, *m*), 1.39 (1H, *m*)	18.3
8		40.7		39.3		39.8	1.40 (1H, *m*)	53.1
9	1.25-1.37 (1H, *m*)	50.6	1.54 (1H, *m*)	47.7	1.93-1.95 (1H, *dd*, 3.0, 12.0)	47.3		37.5
10		37.2		37.1		37.0	1.55 (1H, *m*)	59.5
11	1.37-1.47 (1H, *m*), 1.21-1.30 (1H, *m*)	20.9	1.89-1.90 (1H, *m*), 1.87-1.88 (1H, *m*)	23.4	1.89-1.93 (1H, *m*), 1.84-1.89 (1H, *m*)	23.6	1.45 (1H, *m*) 1.26 (1H, *m*)	35.7
12	1.65-1.71 (1H, *m*), 1.01-1.05 (1H, *m*)	25.5	5.29 (1H, *t*, 3.5)	122.6	5.18 (1H, *t*, 4.0)	121.6	1.34 (2H, *m*)	30.5
13	2.10-2.22 (1H, *m*)	38.4		144.8		145.2		39.7
14		42.5		41.6		41.8		38.3
15	1.47-1.57 (1H, *m*), 1.18-1.21 (1H, *m*)	29.7	1.13 (1H, *m*), 1.06 (1H, *m*)	27.7	1.71-1.84 (1H, *m*), 0.91-0.96 (1H, *m*)	26.2	1.51 (1H, *m*), 1.30 (1H, *m*)	32.5
16	2.26-2.29 (1H, *m*), 1.37-1.47 (1H, *m*)	32.2	1.99-2.00 (1H, *m*), 1.95 (1H, *m*)	22.9	1.98-2.04 (1H, *m*), 0.78-0.85 (1H, *m*)	27.0	1.58 (1H, *m*), 1.35 (1H, *m*)	36.0
17		56.3		46.5		32.5		30.0
18	1.55-1.61 (1H, *m*)	49.3	2.83 (1H, *dd*, 13.5, 4.0)	41.0	1.54-1.59 (1H, *m*)	47.7	1.56 (1H, *m*)	42.8
19	2.97-3.02 (1H, *m*)	46.9	1.64 (1H, *m*), 1.16-1.20 (1H, *m*)	45.9	1.65-1.71 (1H, *m*), 0.98-1.10 (1H, *m*)	46.9	1.37 (1H, *m*), 1.21 (1H, *m*)	35.4
20		150.4		30.7		31.1		28.2
21	1.95-1.99 (1H, *m*), 1.37-1.47 (1H, *m*)	30.6	1.37-1.41 (1H, *m*), 1.23-1.25 (1H, *m*)	33.8	1.39-1.46 (1H, *m*), 1.06-1.12 (1H, *m*)	34.8	1.47 (1H, *m*), 1.28 (1H, *m*)	32.8
22	1.95-1.99 (1H, *m*), 1.37-1.47 (1H, *m*)	37.0	1.77-1.80 (1H, *m*), 1.55 (1H, *m*)	32.4	1.39-1.46 (1H, *m*), 1.18-1.24 (1H, *m*)	37.2	1.53 (1H, *m*), 0.95 (1H, *m*)	39.3
23	0.98 (3H, *s*)	28.0	0.98 (3H, *s*)	28.1	0.79 (3H, *s*)	15.5	0.88 (3H, *d*, 6.5)	6.8
24	0.76 (3H, *s*)	15.3	0.77 (3H, *s*)	15.6	1.00 (3H, *s*)	28.1	0.73 (3H, *s*)	14.7
25	0.83 (3H, *s*)	16.1	0.91 (3H, *s*)	15.4	0.94 (3H, *s*)	15.6	0.87 (3H, *s*)	18.0
26	0.94 (3H, *s*)	16.0	0.75 (3H, *s*)	17.2	0.97 (3H, *s*)	16.8	1.01 (3H, *s*)	20.3
27	0.97 (3H, *s*)	14.7	1.13 (3H, *s*)	26.0	1.14 (3H, *s*)	26.0	1.05 (3H, *s*)	18.7
28		179.9		183.4	0.82 (3H, *s*)	28.4	1,18 (3H, *s*)	32.1
29	4.74 (1H, *d*, 2.0), 4.61 (1H, *t*, 1.5) 4.60 (1H, *s*)	109.7	0.90 (3H, *s*)	33.1	0.87 (3H, *s*)	33.3	0.95 (3H, *s*)	35.0
30	1.69 (3H, *s*)	19.4	0.93 (3H, *s*)	23.6	0.87 (3H, *s*)	23.7	1.00 (3H, *s*)	31.8

Note: All compounds were recorded in $CDCl_3$, 500/125 MHz.

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