

Chemical constituents of *Euphorbia tirucalli* L.

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Abstract—*Euphorbia tirucalli* has not been chemically studied much in Vietnam. This research described the isolation and elucidation of compounds isolated from the plant collected in Binh Thuan. Multiple chromatographic methods were applied, including normal phase silica gel column chromatography and thin-layer chromatography. Seven compounds were isolated and their chemical structures were elucidated by spectroscopic analysis as well as comparing their data with the ones in the literature. They are arjunolic acid (1), eriodictyol (2), quercitrin (3), afzelin (4), scopoletin (5), 3,3',4-trimethylellagic acid (6), and gallic acid (7). Among them, compound 1 a major component was isolated for the first time in *Euphorbia* genus, while three compounds 2, 4, and 5 were isolated from this species for the first time.

Keywords—arjunolic acid, phenolic compounds, flavonoid, *Euphorbia tirucalli*

1. INTRODUCTION

Euphorbia tirucalli L. belongs to the Euphorbiaceae family and is a very popular herb in traditional herbal medicine [1]. There are approximately 1600 species in the *Euphorbia* genus. Some species of this genus have long been used as herbal drugs in China, India, Brazil and Southeast Asia. *Euphorbia tirucalli* L. is traditionally used in Vietnam. The extract and pure compounds from *Euphorbia tirucalli* were evaluated some biological activity, including antioxidant and antimicrobial [3], antifungal, antiviral [4], anti-inflammatory [5], cytotoxicity [6], and enzyme inhibitory activities [7].

Previous phytochemical investigation on the plant *Euphorbia tirucalli* reported the presence of phytosterols, triterpenes, diterpenes, polyphenols,

tannins, [2, 8-10]. The latex from *Euphorbia tirucalli* showed the presence of ingenane- and tiglane-diterpenoids [2, 11, 12]. Up to 2017, no chemical studies on *Euphorbia tirucalli* were reported in Vietnam. Herein the isolation and structure elucidation of seven compounds (1–7) were presented (Fig. 1).

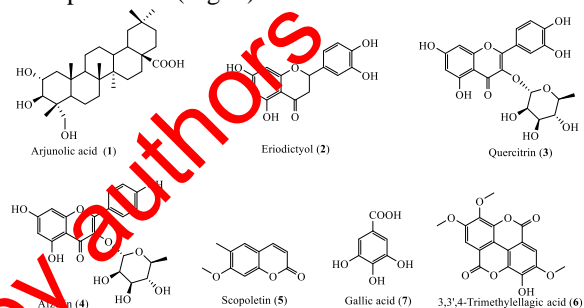


Fig.1. Chemical structures of 1–7

2. MATERIALS AND METHODS

General experimental procedures

NMR spectra were measured on Bruker Avance III (500 MHz for ^1H NMR and 125 MHz for ^{13}C NMR) spectrometer. Proton chemical shifts were referenced to the solvent residual signal of CD_3COCD_3 at δ_{H} 2.05. The ^{13}C NMR spectra were referenced to the central peak of CD_3COCD_3 at δ_{C} 29.4. HR-ESI-MS were recorded on a Bruker microTOF Q-II. TLC analyses were carried out on pre-coated silica gel 60 F₂₅₄ or silica gel 60 RP-18 F₂₅₄S (Merck) and spots were visualized by spraying with 10% H_2SO_4 solution followed by heating. Column chromatography (CC) was performed using silica gel 60 (0.040–0.063 mm, Himedia).

Plant material

The whole fresh plant of *Euphorbia tirucalli* (Euphorbiaceae) was collected from Hong Son village, Ham Thuan Bac, in Binh Thuan province in July 2014. The botanical sample was authenticated by Dr. Pham Van Ngot,

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Extraction and isolation

The dried sample was milled to obtain 3.5 kg of powder. The powder was extracted with EtOH (2 x 10 L) at 70 °C, to obtain the EtOH-soluble extract. While this solution was being evaporated, a precipitant (**P**, 250.4 g) occurred and was filtered off. The remaining solution was evaporated until dryness to obtain crude ethanolic extract (290.3 g).

The resultant ethanolic extract was sequentially partitioned with *n*-hexane, EtOAc, and *n*-BuOH to afford the extracts **H** (94.2 g), **EA** (61.8 g), and **Bu** (27.0 g), respectively. The **EA** extract was applied to a silica gel CC and eluted with a solvent system of *n*-hexane:EtOAc with the ratio 8:2, 5:5, and 0:10, to afford 3 fractions, **EA1** (10.32 g), **EA2** (2.5 g), and **EA3** (2.19 g), respectively. The fraction **EA2** was fractionated by CC with the solvent system *n*-hexane:EtOAc (1:4), to afford three fractions **EA2.1–3**. Fraction **EA2.1.3** (548.0 mg) was further applied to a silica gel CC eluted with *n*-hexane:EtOAc:EtOH:AcOH (5:1:0.2:0.1), to afford three sub-fractions **EA2.1.3.1–3**. Sub-fraction **EA2.1.3.1** (150.1 mg) was rechromatographed to obtain two compounds **5** (4.3 mg) and **7** (20 mg). Sub-fraction **EA2.1.3.2** (356 mg) was purified by preparative TLC using chloroform:MeOH:H₂O (4:0.38:0.02) to obtain three compounds **2** (6.5 mg), **3** (10.0 mg), and **4** (4.3 mg).

The precipitant **P** (250.4 g) was dissolved in hot solution of acetone:EtOH (1:1) then sequentially partitioned with *n*-hexane, EtOAc, and *n*-BuOH, to afford the extracts **PH** (100.4 g), **PA** (64.0 g), **PB** (21.0 g), respectively. The extract **PA** was applied to silica gel CC, and eluted with a solvent system of *n*-hexane:EtOAc (8:2, 5:5, and 0:10), to afford 3 fractions, **PA1** (11.26 g), **PA2** (8.42 g), and **PA3** (17.14 g), respectively. A part of fraction **PA1** (1.0 g) was concentrated to dryness and washed three times by acetone, to obtain compound **6** (120.0 mg). A part of fraction **PA2** (150.0 mg) was carried out in the same manner as fraction **PA1** to obtain compound **2** (10.3 mg).

Arjunolic acid (**1**). White amorphous powder; the ¹H and ¹³C NMR (acetone-*d*₆) spectroscopic data, see Table 1.

Eriodictyol (**2**). White amorphous powder; the ¹H and ¹³C NMR (acetone-*d*₆) spectroscopic data, see Table 2.

Quercitrin (**3**). Light-yellow amorphous powder; the ¹H and ¹³C NMR (acetone-*d*₆) spectroscopic data, see Table 2.

Afzelin (**4**). Light-yellow amorphous powder; the ¹H and ¹³C NMR (acetone-*d*₆) spectroscopic data, see Table 2.

Scopoletin (**5**). Light-yellow amorphous powder; ¹H NMR (acetone-*d*₆), δ: 3.90 (6-OCH₃), 6.17 (d, *J*=9.5 Hz, H-3), 6.80 (s, H-8), 7.19 (s, H-5), 7.84 (d, *J*=9.5 Hz, H-4). ¹³C NMR (acetone-*d*₆), δ: 160.4 (C-2), 112.4 (C-3), 143.7 (C-4), 109.1 (C-5), 141.9 (C-6), 154.1 (C-7), 102.8 (C-8), 150.9 (C-9), 111.2 (C-10), 55.7 (6-OCH₃).

3,3',4'-Trimethyllellagic acid (**6**). White amorphous powder; the ¹H and ¹³C NMR (acetone-*d*₆) spectroscopic data was suitable to those in the literature [9].

Galllic acid (**7**). White amorphous powder; the ¹H and ¹³C NMR (acetone-*d*₆) spectroscopic data was suitable to those in the literature [8].

3. RESULTS AND DISCUSSION

Three successive extractions were performed on the crude ethanol extract of the whole plant *Euphorbia tirucalli*. Further purification and isolation of compounds were carried out using silica gel chromatography, as described in the experimental section. Seven compounds were isolated and their structures were elucidated as arjunolic acid (**1**), eriodictyol (**2**), quercitrin (**3**), afzelin (**4**), scopoletin (**5**), 3,3',4'-trimethyllellagic acid (**6**), and gallic acid (**7**).

Compound **1** was obtained as a white amorphous powder. The ¹H NMR and HSQC spectra of **1** showed the presence of six quaternary methyls at δ_H 0.73 (3H, s), 0.80 (3H, s), 0.92 (3H, s), 0.94 (3H, s), 1.02 (3H, s), 1.17 (3H, s), two oxymethine protons at δ_H 3.39 (1H, d, *J* = 9.5 Hz) and 3.67 (1H, ddd, *J* = 11.5, 9.5, 4.5 Hz), one oxymethylene group at δ_H 3.28 (1H, d, *J* = 10.5 Hz) and 3.56 (1H, d, *J* = 10.5 Hz), and one olefinic proton at δ_H 5.25 (1H, dd, *J* = 7.0, 3.5 Hz). The ¹³C NMR spectrum, in accordance with HSQC spectrum, confirmed the presence of thirty carbons comprising two olefinic carbons (δ_C 122.9 and 145.0), one hydroxycarbonyl carbon (δ_C 178.9), two

oxymethines (δ_C 68.9 and 78.5), one oxymethylene (δ_C 67.1), and six methyls (δ_C 13.8, 17.5, 17.7, 23.7, 26.4, 33.4). The comparison of the ^{13}C NMR data of **1** and those of oleanolic acid [14] indicated the same structures of B, C, D, and E rings of **1** and those of oleanolic acid, except for the ^{13}C NMR signals in the A-ring. This finding led to the identification of the position of the oxygenated methine and methylene groups in the A-ring. In HMBC spectrum, proton H-3 (δ_H 3.39, d, 9.5 Hz) showed cross-peaks to signals at δ_C 47.4 (C-1), 68.9 (C-2), 43.4 (C-4), 13.8 (C-24) and proton H-2 (δ_H 3.67, ddd, 11.5, 9.5, 4.5 Hz) correlated to C-1, C-4, and C-3 (δ_C 78.5), which indicated their vicinal positions (Fig. 2). Moreover, the coupling constant ($J = 9.5$ Hz) between protons H-3 and H-2

indicated their *axial* positions [24]. On the other hand, while axial proton H-3 was at the β orientation, found in many oleanane triterpenes [14], proton H-2 was defined at the α one. The oxymethylene protons at δ_H 3.28 (H-23a, d, $J = 10.5$ Hz), and 3.56 (H-23b, d, $J = 10.5$ Hz) showed HMBC correlations to C-3, C-4, and C-24, to determine their positions. The comparison of NMR data of **1** and those of arjunolic acid [15,16] showed that they were identical, accordingly, **1** was elucidated as arjunolic acid (Table 1). Arjunolic acid was found in *Terminalia arjuna* tree and other plants [17] and isolated as a major component from the apolar fraction **H2.4** of *Euphorbia tirucalli*.

Table 1. NMR spectral data of compounds **1** and arjunolic acid

1 ^a			Arjunolic acid ^b	1 ^a			Arjunolic acid ^b
N	δ_C	δ_H , m J (Hz)	δ_C	N	δ_C	δ_H , m J (Hz)	δ_C
1	47.4		47.1	16	23.9		23.9
2	68.9	3.67 (ddd, 11.5, 9.5, 4.5)	68.9	17	46.9		47.0
3	78.5	3.39 (d, 9.5)	78.7	18	42.2		43.5
4	43.4		43.5	19	46.8		46.3
5	48.2		48.4	20	31.3		30.7
6	18.7		18.6	21	34.5		34.2
7	33.1		33.1	22	33.4		33.0
8	40.2		40.1	23	67.1	3.56 (d, 10.5) 3.28 (d, 10.5)	67.2
9	48.5		48.5	24	13.8	0.73 (s)	14.0
10	38.7		38.5	25	17.5	1.02 (s)	17.6
11	24.2		23.8	26	17.7	0.80 (s)	17.2
12	122.9	5.25 (dd, 7.0, 3.5)	122.5	27	26.4	1.17 (s)	26.1
13	145.0		144.1	28	178.9		178.6
14	42.6		42.4	29	33.4	0.92 (s)	32.9
15	28.4		28.3	30	23.7	0.94 (s)	23.7

^a recorded in acetone- d_6 ; ^b in pyridine- d_5

1 ^a			Arjunolic acid ^b	1 ^a			Arjunolic acid ^b
N	δ_C	δ_H , m J (Hz)	δ_C	N	δ_C	δ_H , m J (Hz)	δ_C
1	47.4		47.1	16	23.9		23.9
2	68.9	3.67 (ddd, 11.5, 9.5, 4.5)	68.9	17	46.9		47.0
3	78.5	3.39 (d, 9.5)	78.7	18	42.2		43.5
4	43.4		43.5	19	46.8		46.3
5	48.2		48.4	20	31.3		30.7
6	18.7		18.6	21	34.5		34.2
7	33.1		33.1	22	33.4		33.0
8	40.2		40.1	23	67.1	3.56 (d, 10.5) 3.28 (d, 10.5)	67.2
9	48.5		48.5	24	13.8	0.73 (s)	14.0
10	38.7		38.5	25	17.5	1.02 (s)	17.6
11	24.2		23.8	26	17.7	0.80 (s)	17.2
12	122.9	5.25 (dd, 7.0, 3.5)	122.5	27	26.4	1.17 (s)	26.1
13	145.0		144.1	28	178.9		178.6
14	42.6		42.4	29	33.4	0.92 (s)	32.9
15	28.4		28.3	30	23.7	0.94 (s)	23.7

^a recorded in acetone- d_6 ; ^b in pyridine- d_5

Nevertheless, it has not yet been found in the *Euphorbia* genus. Arjunolic acid possessed various biological activities such as antidiabetic, antifungal, antibacterial, anticholinesterase, antitumor, antiasthmatic, wound healing and insect growth inhibitory activity and its potential use was taken into account as a novel promising therapeutic strategy [17, 18].

Compound **2** was obtained as a white amorphous powder. The ^1H NMR data of **2** showed the presence of two *meta* coupled aromatic protons at δ_{H} 5.96 and 5.94 (each 1H, d, $J = 2.0$ Hz), three aromatic protons of a 1, 2, 4 trisubstitutedbenzene moiety at δ_{H} 7.03 (1H, d, $J = 1.5$), 6.87 (1H, dd, $J = 8.5, 1.5$ Hz), and 6.86 (1H, d, $J = 8.5$ Hz), one methylene group at δ_{H} 3.14 (1H, dd, $J = 17.0, 12.5$, Hz) and 2.72 (1H, dd, $J = 17.0, 3.0$, Hz), one oxymethine moiety at δ_{H} 5.40 (1H, dd, $J = 12.5, 3.0$, Hz), and one chelated hydroxy group at δ_{H} 12.17 (Table 2). The ^{13}C NMR spectrum in accordance with HSQC spectrum showed fifteen carbons comprising five aromatic methine carbons, one oxymethine at δ_{C} 80.0, one methylene δ_{C} 43.6, one carbonyl δ_{C} 197.3 and seven aromatic quaternary carbons (including five oxygenated ones). These findings led to the identification of the flavanone skeleton of **2**. In the A-ring, proton H-6 (δ_{H} 5.94) and H-8 (δ_{H} 5.96) showed HMBC correlations to signals at δ_{C} 167.4 (C-7) and δ_{C} 103.2 (C-10), confirming their positions. In the B-ring, protons H-2' (δ_{H} 7.03), H-5' (δ_{H} 6.87), and H-6' (δ_{H} 6.86) showed HMBC cross-peaks to signals at δ_{C} 146.1 (C-3') and 146.5 (C-4'), determining the two oxygenated carbons C-3' and C-4' (Fig. 3). Moreover, the HMBC correlation between proton H-2 and signals at δ_{C} 197.3 (C-4), 131.6 (C-1'), 114.8 (C-2'), and 119.2 (C-6'), indicating the connectivity between the B- and C- rings at C-2. The comparison of NMR data of **2** and those of eriodictyol showed that they were identical, thus **2** was elucidated as eriodictyol [19]. Eriodictyol was isolated in *E. acanthothamnus* [20] and many plants but this is the first time found in *Euphorbia tirucalli*. This compound possessed antiinflammatory effect [21].

Compound **3** was obtained as a light-yellow amorphous powder. Analysis of 1D NMR data of **3** indicated that **3** was a flavonoid glycoside with

the presence of L-rhamnopyranosyl moiety, comprising an anomeric proton signal at δ_{H} 5.19 (1H, d, $J = 1.5$ Hz, H-1"), four oxygenated proton signals in the ^1H zone of 3.1–4.0 ppm, and a characteristic methyl signal at δ_{H} 0.91 (3H, d, $J = 6.0$ Hz, H-6"). The NMR data of the aglycone moiety of **3** were similar with those of **2** (Table 2), except for the absence of the methine CH-2 and the methylene CH₂-3 groups in **2** and the presence of a new double bond between C-2 and C-3. This finding was confirmed by the HMBC correlation of H-2' and H-6' and C-2 (δ_{C} 158.4) (Fig. 3). The rhamnose moiety was linked to the aglycone moiety at its C-3, which was proved by the HMBC correlation of proton signal at δ_{H} 5.52 (H-1") and carbon C-3 (δ_{C} 135.9). All mentioned spectroscopic data well matched with those of quercitrin reported in literature [22]. Quercitrin was already reported from this species [10].

Compound **4** was obtained as a light-yellow amorphous powder. The comparison of 1D NMR data of **4** and **3** (both recorded in acetone-*d*₆) indicated that **4** was also a flavonoid glycoside with the presence of L-rhamnopyranosyl moiety, except for the absence of the hydroxy group in B-ring of the aglycone moiety (Table 2). This finding was confirmed by the presence of the 1,4-disubstituted benzene moiety in **4** instead of the 1,2,4 trisubstituted benzene in B-ring in **3**. The HMBC correlations between H-2'/6' and C-2 (δ_{C} 158.4), C-3' (δ_{C} 116.1), and C-4' (δ_{C} 161.0) supported this finding (Fig. 3). The comparison of NMR data of **4** and those of afzelin showed that they were identical, thus **4** was elucidated as afzelin [23]. Afzelin had been isolated in other *Euphorbia* plants such as *E. hirta* but this is the first time isolated in *Euphorbia tirucalli* [24].

Compound **5** was obtained as a light-yellow amorphous powder. Its ^1H NMR spectrum revealed the presence of seven resonances comprising two *cis* olefinic protons at δ_{H} 7.84 (1H, d, $J = 9.5$ Hz, H-4) and 6.17 (1H, d, $J = 9.5$ Hz, H-3), two *singlet* aromatic protons δ_{H} 6.80 (H-8) and 7.19 (H-5), one methoxy group at δ_{H} 3.90 (3H, s). The ^{13}C NMR of **5** showed ten carbon signals including one carbonyl ester group at δ_{C} 160.4 (C-2), four methine moieties at δ_{C} 143.7 (C-4), 112.4 (C-3), 109.1 (C-5), and 102.8 (C-8), one methoxy group at δ_{C} 55.7, and four aromatic quaternary carbons at δ_{C} 111.2 (C-10),

144.9 (C-6), 150.9 (C-9), and 154.1 (C-7) (three latter oxygenated). These NMR data of **5** were similar to those of scopoletin, accordingly, **5** was elucidated as scopoletin [25]. The HMBC correlations of **5** supported its structure as

described in Fig. 2. Scopoletin was found in *E. hirta* or *E. heteradena* [26] but this is isolated from *Euphorbia tirucalli* for the first time.

Table 2. NMR spectral data of compounds 2-4

Position	2			3			4		
	δ_C	δ_H , m	J (Hz)	δ_C	δ_H , m	J (Hz)	δ_C	δ_H , m	J (Hz)
2	80.0	5.40	dd, 12.5, 3.0	158.4			158.4		
3	43.6	3.14 2.72	dd, 17.0, 12.5 dd, 17.0, 12.5	135.9			135.6		
4	197.3			179.4			nd		
5	165.3			163.2			163.2		
6	96.8	5.94	d, 2.0	99.3	6.26	d, 2.0	98.8	6.26	d, 2.0
7	167.4			164.9			164.9		
8	95.9	5.96	d, 2.0	94.7	6.46	d, 2.0	93.5	6.46	d, 2.0
9	164.4			158.0			157.5		
10	103.2			105.7			104.5		
1'	131.6			122.9			121.0		
2'	114.8	7.03	d, 1.5	116.1	7.51	d, 2.0	128.5	7.86	d, 8.5
3'	146.1			149.0			116.1	7.01	d, 8.5
4'	146.5			145.8			161.0		
5'	116.1	6.86	d, 8.5	116.8	6.98	d, 8.5	116.8	7.01	d, 8.5
6'	119.2	6.87	dd, 8.5, 1.5	122.6	7.40	dd, 8.5, 2.0	128.5	7.86	d, 8.5
1''				102.8	5.52	m	102.8	5.54	d, 1.5
2''				71.3	4.22	m	71.2	4.21	dd, 3.0, 1.5
3''				71.5	3.74	dd, 9.0, 3.0	71.4	3.74	dd, 8.5, 3.0
4''				73.0	3.33	m	73.2	3.33	m
5''				72.2	3.33	m	72.2	3.30	m
6''				17.6	0.91	d, 6.0	17.9	0.90	d, 5.5
5-OH		12.17	s		12.72	s		12.71	S

nd: not determined

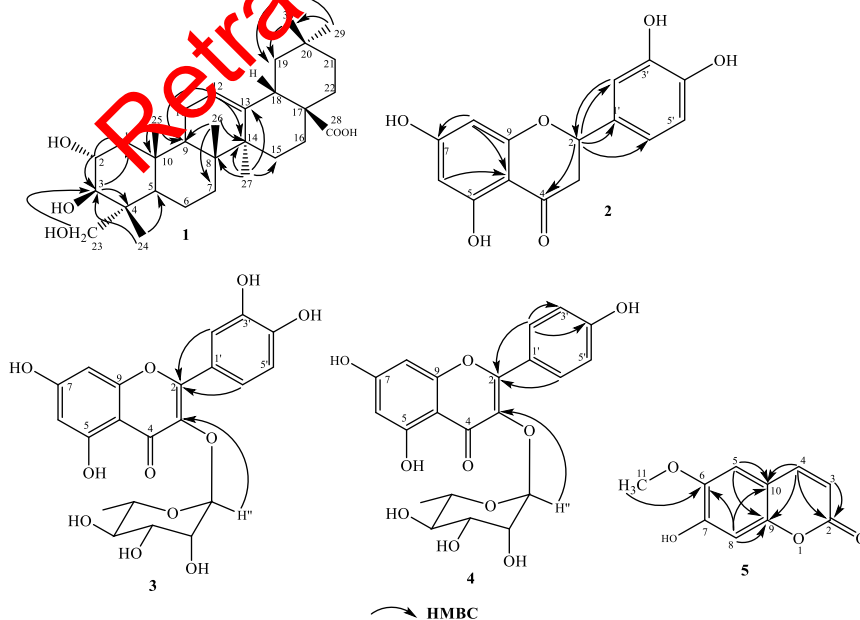


Fig. 2. Selected HMBC correlations of **1**, **2**, **3**, **4** and **5**

4. CONCLUSION

From *Euphorbia tirucalli* collected in Binh Thuan province, seven compounds were isolated and elucidated, including arjunolic acid (1), eriodictyol (2), quercitrin (3), afzelin (4), scopoletin (5), 3,3',4-trimethylellagic acid (6), and gallic acid (7). Among them, compound 1 was found for the first time in *Euphorbia* genus while three compounds 2, 4, and 5 were firstly isolated from this species. Furthermore, compounds 1 and 6 were confirmed as major components of this species.

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Thành phần hóa học của cây sanh giao (*Euphorbia tirucalli* L.)

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Tóm tắt— Cây Cành giao *Euphorbia tirucalli* chưa được nghiên cứu nhiều ở Việt Nam. Nghiên cứu này mô tả sự phân lập và xác định cấu trúc hóa học của một số hợp chất từ cây Cành giao sinh trưởng ở Bình Thuận. Các phương pháp sắc ký cột silica gel pha thuận và sắc ký lớp mỏng đã được sử dụng. Bảy hợp chất được cô lập và cấu trúc của chúng được xác định bằng các phương pháp phổ nghiệm cũng như so sánh với tài liệu tham khảo. Chúng là arjunolic acid (1), eriodictyol (2),

quercitrin (3), afzelin (4), scopoletin (5), 3,3',4'-trimethylellagic acid (6), và gallic acid (7) được cô lập. Trong số chúng, arjunolic acid được biết là thành phần chính trong cây thuộc chi *Euphorbia*. Các hợp chất 2, 4, 5 lần đầu tiên được cô lập từ loài *Euphorbia tirucalli*.

Từ khóa—arjunolic acid, các hợp chất phenolic, flavonoid, *Euphorbia tirucalli*