

# A further investigation on the chemical constituents from *Euphorbia tirucalli* growing in Binh Thuan province

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## ABSTRACT

**Introduction:** *Euphorbia tirucalli* L. is a medicinal plant popularly distributed in Asian countries. In Vietnam, only one study on the polar extract the plant *Euphorbia tirucalli* growing in Binh Thuan province, Vietnam was reported, revealing several phenolic components. As of 2019, no chemical reports on the non-polar extract from the Vietnamese plant were found. This research described the isolation and elucidation of compounds isolated from the non-polar extract of *E. tirucalli* growing in Binh Thuan province. **Method:** The *n*-hexane extract of this plant was carried out by using normal phase silica gel column chromatography, thin-layer chromatography, and gel chromatography (Sephadex LH-20). Analysis of spectroscopic data and a comparison of the NMR data with that in the literature led to the structural elucidation of isolated compounds. **Results:** Three terpenoid compounds, euphol (1), lupenone (2), and vomifoliol (3), along with ergosterol peroxide (4), ferulic acid (5), and vanillic acid (6) were isolated and elucidated. **Conclusions:** Among them, compound 3 and 4 were reported in the first time from *E. tirucalli*.

**Key words:** Euphorbia tirucalli, terpenoid, euphol, vomifoliol

## INTRODUCTION

*Euphorbia tirucalli* L. is a shrub or small tree widely distributed in Africa, Asia, and Indochina and is a medicinal plant in various tropical countries<sup>1</sup>. In India, this plant is used for the treatment of cancer, asthma, and leucorrhoea. Pharmacological properties of *E. tirucalli* indicated diverse bioactivities, comprising antioxidant and antimicrobial, antifungal, antiviral, anti-inflammatory and cytotoxicity, as well as enzyme inhibitory activities. Chemical profile of this plant provided three common skeletons such as terpenoids, polyphenols, and tannins<sup>1-3</sup>. In Vietnam, phytochemical investigation on this plant was scarce. Our previous report focusing on the ethyl acetate extract revealed seven phenolic compounds with the ellagic acid being a major component (Le et al., 2018). As a continuation of our research focused on the diversity of bioactive metabolites from Vietnamese medicinal plants<sup>4,5</sup>, the phytochemical study was performed on the less polar extract of the title plant. Multiple chromatographic methods included normal phase silica gel column chromatography, thin-layer chromatography, and gel chromatography was applied to the *n*-hexane extract. As a result, six compounds have been obtained. Their structures were elucidated from analysis of 1D and 2D NMR along with a comparison with literature reports. Herein we report on the structure elucidation and isolation of six

compounds.

## MATERIALS AND METHODS

### General experimental procedures

Bruker Advance III (500 MHz for <sup>1</sup>H NMR and 125 MHz for <sup>13</sup>C NMR) spectrometer with TMS as internal standard recorded NMR spectra. Chemical shifts are expressed in ppm with reference of acetone-*d*<sub>6</sub> at  $\delta_H$  2.05,  $\delta_C$  206.26 and 29.84 and of chloroform-*d*<sub>1</sub> at  $\delta_H$  7.26 and  $\delta_C$  77.80. The HR-ESI-MS were recorded on a HR-ESI-MS Bruker microOTOF Q-II. TLC was carried out on pre-coated silica gel 60 F<sub>254</sub> or silica gel 60 RP-18 F<sub>254</sub>S (Merck Millipore, Billerica, Massachusetts, USA) and spots were visualized by spraying with 10% H<sub>2</sub>SO<sub>4</sub> solution followed by heating. Gravity column chromatography was performed with silica gel 60 (0.040–0.063 mm) (HiMedia, Mumbai, India).

### Plant material

Whole plants of *Euphorbia tirucalli* were collected from Hong Son village, Ham Thuan Bac, in Binh Thuan province in July 2014. The botanical sample was identified by Dr. Pham Van Ngot, Department of Botany, Faculty of Biology, Ho Chi Minh University of Education. A voucher specimen (No UP002) is

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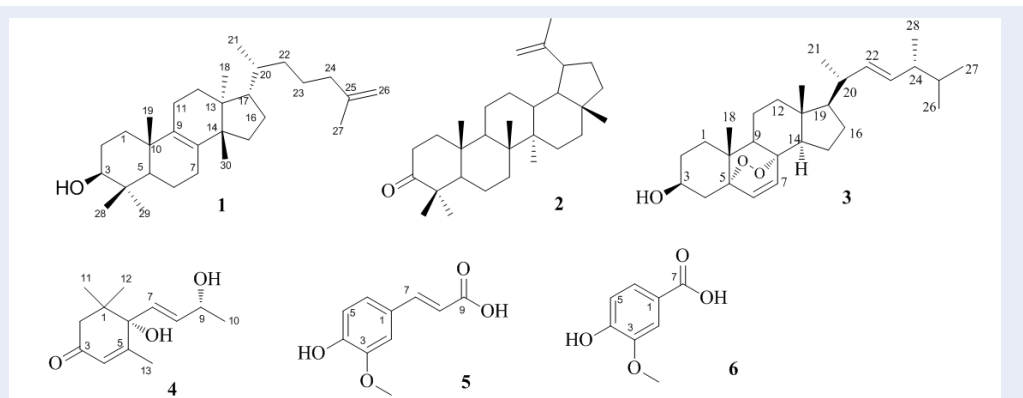


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**Figure 1:** Chemical structures of euphol (1), lupenone (2), vomifoliol (3), ergosterol peroxide (4), ferulic acid (5), and vanilic acid (6).

deposited in the herbarium of the Department of Organic Chemistry, Faculty of Chemistry, Ho Chi Minh University of Education.



**Figure 2:** *Euphorbia tirucalli* L.

### Extraction and isolation

The clean, air-dried and ground material (3.5 kg) was extracted by maceration with EtOH (10 L x 2) at 70°C. A precipitate occurred as the crude extract was evaporated under reduced pressure and was filtered off to give 250.4 g of precipitate **P**. The filtered solution was evaporated to dryness to obtain the crude ethanol extract (290.3 g). The dry residue of this latter extract was subsequently partitioned using liquid-liquid extraction with the solvents of increasing polarities: *n*-hexane (**H**, 94.2 g), EtOAc (**EA**, 61.8 g) and *n*-BuOH (**B**, 27.0 g). Extract **H** (94.2 g) was applied to silica gel CC, eluted with the solvent system *n*-hexane/EtOAc/Acetone (12:1:1 to 5:1:1; *v/v/v*)

to afford three fractions **H1-H3**. Fraction **H2** (15.7 g) was fractionated by Sephadex LH-20 CC using MeOH to yield three subfractions (**H2.1-H2.3**). Subfraction **H2.1** (4.1 g) was applied to normal phase silica gel CC and eluted isocratically with the solvent system *n*-hexane/EtOAc/EtOH/Acetic acid (9:2:1:0.2; *v/v/v/v*) to give eight subfractions **H2.1.1-H2.1.8**. Fraction **H.2.1.1** (1.8 g) was subjected to silica gel CC using *n*-hexane/EtOAc/acetone (12:1:1) to isolate compound **2** (21 mg). Fraction **H2.1.3** (489.0 mg) was further chromatographed by reverse phase C18 silica gel CC and isocratically eluted with a MeOH/Acetone/H<sub>2</sub>O (1:3:1) solvent system to obtain three subfractions **H2.1.3.1-H2.1.3.3**. Fraction **H2.1.3.3** was rechromatographed using the solvent system *n*-hexane/chloroform/EtOAc/Acetone (100:40:24:10) to yield **1** (21.0 mg), **4** (3.2 mg), **5** (1.8 mg), and **6** (4.7 mg). Fraction **H2.3** (3.7 g) was fractionated by normal phase silica gel CC using *n*-hexane/EtOAc/Acetone (7:1:1) as mobile phase to obtain three fractions **H2.3.1-H2.3.3**. Subfraction **H2.3.1** (241 mg) was further purified using the same chromatographic procedure to afford **3** (11 mg).

- **Euphol (1)**. White GUM; the <sup>1</sup>H and <sup>13</sup>C NMR (CDCl<sub>3</sub>) spectroscopic data, see Table 1.
- **Lupenone (2)**. White amorphous powder; the <sup>1</sup>H and <sup>13</sup>C NMR (CDCl<sub>3</sub>) spectroscopic data, see Table 1.
- **Vomifoliol (3)**. White amorphous powder; the <sup>1</sup>H and <sup>13</sup>C NMR (Acetone-*d*<sub>6</sub>) spectroscopic data, see Table 1.
- **Ergosterol peroxide (4)**. Colorless needle; the <sup>1</sup>H and <sup>13</sup>C NMR (CDCl<sub>3</sub>) spectroscopic data, see Table 2.

- **Ferulic acid (5).** Colorless needle; the  $^1\text{H}$  and  $^{13}\text{C}$  NMR (Acetone- $d_6$ ) spectroscopic data, see Table 2.
- **Vanillic acid (6).** Colorless needle; the  $^1\text{H}$  and  $^{13}\text{C}$  NMR (Acetone- $d_6$ ) spectroscopic data, see Table 2. The NMR data are consistent with those in the literature<sup>6</sup>.

## RESULTS AND DISCUSSION

Compound **1** was isolated as a white gum. The  $^1\text{H}$  NMR data exhibited resonances for an isobutenyl  $-\text{CH}=\text{C}(\text{CH}_3)_2$  group characterizing by one olefinic proton at  $\delta_{\text{H}}$  5.03 and two methyls at  $\delta_{\text{H}}$  1.68 and 1.61 and six upfield methyls ( $\delta_{\text{H}}$  0.76, 0.80, 0.86, 0.88, 0.95, 1.00), in which one was doublet ( $\delta_{\text{H}}$  0.86, d, 6.5 Hz). Moreover, the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra revealed the signal of one oxymethine at  $\delta_{\text{H}}$  3.25 (1H,  $J = 4.5, 11.5$  Hz) and  $\delta_{\text{C}}$  79.1. Analysis of the coupling pattern of this proton indicated that the hydroxy group was at  $\beta$  position. The  $^{13}\text{C}$  NMR spectrum showed the presence of 30 carbons including four  $\text{sp}^2$  carbons at  $\delta_{\text{C}}$  134.3, 133.7, 131.0, and 125.4 along with seven  $\text{sp}^3$  methylene carbons, three  $\text{sp}^3$  methine carbons, and five  $\text{sp}^3$  quaternary carbons. Spectroscopic features indicated the structure to be tetracyclic triterpenes such as euphanes or tirucallanes (Ghosh, 2017). The comparison of NMR data of **1** with those of euphol<sup>7</sup> showed that they were identical, thus **1** was elucidated as euphol.

Compound **2** was isolated as a white amorphous powder. The  $^1\text{H}$  NMR data exhibited seven *singlet* methyls ( $\delta_{\text{H}}$  0.80, 0.93, 0.96, 1.03, 1.07, 1.07, and 1.68), two *gem* olefinic protons at  $\delta_{\text{H}}$  4.57 and 4.69 with the coupling constant being  $J = 2.5$  Hz. The  $^{13}\text{C}$  NMR spectrum showed the presence of 30 carbons including one  $\text{sp}^2$  substituted carbon at  $\delta_{\text{C}}$  151.1 and one  $\text{sp}^2$  methylene at  $\delta_{\text{C}}$  109.9 which was assignable for one isopropenyl group  $-\text{C}(\text{CH}_3)=\text{CH}_2$ , one ketone carbon at  $\delta_{\text{C}}$  218.5, ten methylene carbons, five methine carbons, and five quaternary carbons. The comparison of NMR data of **2** with those of lupenone<sup>8</sup> showed that they were identical; thus **2** was elucidated as lupenone.

Compound **3** was isolated as a white amorphous powder. The  $^1\text{H}$  NMR spectrum exhibited signals for six methyl groups at  $\delta_{\text{H}}$  0.81 (s, H-19), 0.83 (d,  $J = 6.5$  Hz, H-27), 0.82 (d,  $J = 7.0$  Hz, H-26), 0.88 (s, H-18), 0.91 (d,  $J = 7.0$  Hz, H-28) and 1.00 (d,  $J = 6.5$  Hz, H-21), four olefinic protons containing two signals at  $\delta_{\text{H}}$  5.14 (dd,  $J = 15.5, 7.7$  Hz, H-22) and 5.22 (dd,  $J = 15.5, 7.7$  Hz, H-23) assignable for the double bond C-22-C-23 and two signals at  $\delta_{\text{H}}$  6.24 (d,  $J =$

8.5 Hz, H-6) and 6.50 (d,  $J = 8.5$  Hz, H-7) assignable for the double bond at C-6-C-7, one oxymethine at  $\delta_{\text{H}}$  3.97 (m, H-3) and twenty protons at  $\delta_{\text{H}}$  1.23–2.10. The  $^{13}\text{C}$  NMR spectrum showed the presence of 28 carbons, including six methyls, seven methylenes, eleven methines (one bearing oxygen and four olefinic carbons) and four quaternary carbons (two bearing oxygen). The NMR data of **3** were similar to those of (5 $\alpha$ ,8 $\alpha$ )-ergosterol peroxide<sup>9</sup>; thus it was assigned being (5 $\alpha$ ,8 $\alpha$ )-ergosterol peroxide.

Compound **4** was obtained as a white amorphous powder. The  $^1\text{H}$  NMR spectrum exhibited one trans double bond ( $\delta_{\text{H}}$  5.88, dd, 15.5, 4.5 and 5.84, d, 15.5, 10.5), one olefinic proton ( $\delta_{\text{H}}$  5.85, br), four methyls ( $\delta_{\text{H}}$  1.00, 1.04, 1.20, 1.88), one oxymethine ( $\delta_{\text{H}}$  4.33, m), one methylene ( $\delta_{\text{H}}$  2.42, d, 16.5 and 2.10, d, 16.5). The  $^{13}\text{C}$  NMR spectrum showed signals of 13 carbons including one ketone carbon, four olefinic carbons, four methyls, one oxymethine, one methylene and two quaternary carbons, one of which was oxygenated (Table 2). HMBC cross peaks of H-7, H-8, H<sub>3</sub>-11, H<sub>3</sub>-12, H<sub>3</sub>-13 to C-6 defined the attachment of the hydroxy group at this carbon while HMBC correlations of H<sub>3</sub>-11 to C-2, of H<sub>2</sub>-2 to C-3 and C-4, of H-4 to C-2 and C-3, of H<sub>3</sub>-13 to C-4 and C-5 defined the connectivity through C-1-C-6 (see Figure 3). Besides, H-9 gave HMBC cross peaks to C-9 and C-10 and *vice versa* H<sub>3</sub>-10 gave HMBC correlations to C-8 and C-9, indicating the structure of the side chain (see Figure 3). The comparison of NMR data of **4** with those of vomifoliol<sup>10</sup> showed that they were identical, thus **4** was elucidated as vomifoliol.

Compound **5** was obtained as a white amorphous powder. The  $^1\text{H}$  NMR spectrum of **5** revealed the presence of an ABX benzenoid system, a (*E*)-configured double bonds ( $\delta_{\text{H}}$  7.31 d, 15.5 and 6.97, d, 15.5), one hydroxy group ( $\delta_{\text{H}}$  7.95), and one methoxy group ( $\delta_{\text{H}}$  3.91). The  $^{13}\text{C}$  NMR spectrum of **5** showed signals of one carbonyl carbon ( $\delta_{\text{C}}$  168.1), three aromatic methines, two olefinic carbons, and three substituted aromatic carbons, two of which were oxygenated ( $\delta_{\text{C}}$  146.3 and 148.6). HMBC correlations of both H-7 and H-8 to C-1 and C-9 defined the connectivity of the side chain to C-1 of the benzene ring. In addition, HMBC cross peaks of all H-2, H-5, and OCH<sub>3</sub> to C-3 defined the position of the methoxy group while HMBC cross peaks of all H-2, H-6, and 5-OH to C-4 defined the attachment of a hydroxy group at C-4. NMR data of **5** closely resembled those of ferulic acid<sup>3</sup>; accordingly, **5** was elucidated as ferulic acid.

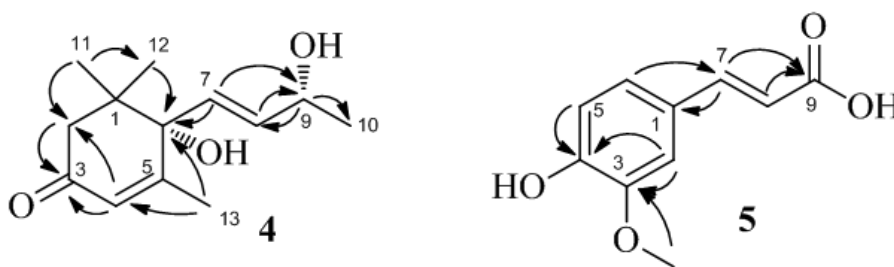
Euphol (**1**), a common component from *Euphorbia tirucalli* growing in the world had strong cytotoxicity toward various cancer cell lines, anti-inflammatory

**Table 1: Nuclear magnetic resonance of compounds 1-3 (in CDCl<sub>3</sub>)**

N	1 <sup>a</sup>		2 <sup>b</sup>		3	
	$\delta_H, J$ (Hz)	$\delta_C$	$\delta_H, J$ (Hz)	$\delta_C$		
1		35.6		39.8	34.9	
2		28.1	2.40, m ; 2.49, m	34.3	30.2	
3	3.20, dd, 4.5, 11.5	79.3		218.5	3.97, m	66.5
4		37.4		47.5		37.1
5		50.1		55.1		82.3
6		19.1		19.8		135.6
7		27.8		33.7		130.9
8		134.3		41.0		79.6
9		133.7		50.0		51.3
10		39.1		37.1		37.1
11		21.7		21.1		23.6
12		28.2		25.3		39.3
13		44.3	1.71, m	38.4		44.6
14		50.2		43.2		51.7
15		31.1		27.6		20.6
16		29.9		36.0		28.9
17		49.8		43.1		56.2
18	0.80, s	15.8		48.4	0.88, s	18.3
19	0.95, s	20.3	2.37, m	48.1	0.81, s	13.0
20		36.0		151.1		39.9
21	0.86, d, 6.5	19.1		30.1	1.00, d, 6.5	21.0
22		35.4		40.1	5.14, dd, 15.5, 7.7	135.4
23		24.9	1.07, s	26.6	5.22, dd, 15.5, 7.7	132.5
24	5.03, t, 7.0	125.4	1.03, s	21.2		42.9
25		131.0	0.93, s	16.1		33.2
26	1.68, s	25.9	1.07, s	16.0	0.82, d, 7.0	19.8
27	1.61, s	17.8	0.96, s	14.6	0.83, d, 7.0	20.1
28	0.88, s	24.6	0.80, s	18.2	0.91, d, 6.5	17.7
29	1.00, s	28.3	4.69, d, 2.5 4.57, dd, 2.5, 1.5	109.9		
30	0.76, s	15.7	1.68, s	19.5		

**Table 2: Nuclear magnetic resonance of compounds 4-6 (in acetone-d<sub>6</sub>)**

	4		5		6	
	$\delta_H$ , J(Hz)	$\delta_C$	$\delta_H$ , J(Hz)	$\delta_C$	$\delta_H$ , J(Hz)	$\delta_C$
1		41.8		127.7		122.3
2	2.42, d, 16.5 2.10, d, 16.5	50.5	7.16, d, 2.0	115.2	7.56, d, 1.0	112.6
3		197.6		146.3		148.0
4	5.85, br	129.4		148.6		152.1
5		161.0	6.87, d, 8.0	115.7	6.91, d, 8.0	115.5
6		79.5	7.04, dd, 8.0, 2.0	116.3	7.59, d, 8.0, 1.0	123.8
7	5.88, dd, 15.5, 4.5	137.1	6.97, d, 15.5	137.5		166.8
8	5.84, d, 15.5, 10.5	126.9	7.31, d, 15.5	122.4		
9	4.33, m	67.9		168.1		
10	1.20, d, 6.5	24.5				
11	1.00, s	24.2				
12	1.04, s	23.4				
13	1.88, br	19.2				
6-OH	4.11, s					
9-OH	3.82, br					

**Figure 3:** Key Heteronuclear Multiple Bond Correlations of **4** and **5**.

activity as well as diverse pharmacological properties<sup>1,11-13</sup>. Lupenone (**2**) was found in the first time from the plant *E. tirucalli* growing in China<sup>14</sup> since 2011 but it could be found in many higher plants belonging to the *Euphorbia* genus. Ferulic acid (**5**) was reported as a significant phenolic compound detected through HPLC-UV in all extracts of *E. tirucalli* from Brazil<sup>3</sup> and proposed to be responsible to the high antioxidant of this plant; nevertheless, this compound was isolated with the minute amount. Although compound **3** and **4** have been investigated from some *Euphorbia* plants, such as vomifoliol from *E. heteradena*<sup>15</sup>, *Euphorbia prostrata*<sup>16</sup>... or ergosterol per-

oxide from *E. lagascae*<sup>17,18</sup>, to the best of our knowledge, two compounds **3** and **4** were isolated from this species for the first time.

## CONCLUSION

From the plant *E. tirucalli* growing in Binh Thuan province, six compounds were isolated and elucidated as being euphol (**1**), lupenone (**2**), vomifoliol (**3**), ergosterol peroxide (**4**), ferulic acid (**5**), and vanillic acid (**6**). Two compounds **3** and **4** were isolated from this species for the first time.

## ABBREVIATIONS

<sup>1</sup>H NMR: Proton nuclear magnetic resonance, <sup>13</sup>C NMR: Carbon-13 nuclear magnetic resonance, CC: column chromatography, TLC: Thin layer chromatography, HSQC: Heteronuclear single quantum coherence, HMBC: Heteronuclear multiple bond correlation, s: singlet, d: doublet, m: multiplet

## CONFLICTS OF INTEREST

The authors declare no competing financial interest.

## AUTHOR CONTRIBUTION

Duong T. H. has contributed in conducting experiments, acquisition of data, interpretation of data, searching the bibliography and gave final approval of the manuscript to be submitted.

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