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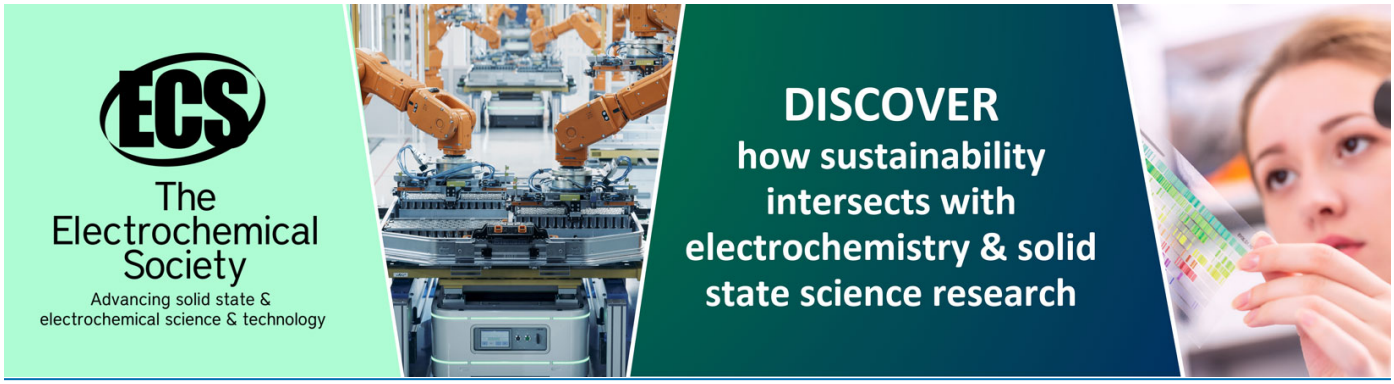

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Chemical constituents from *n*-hexane and ethyl acetate extracts of *Euphorbia hirta* L. grown in Vietnam

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Abstract –The medicinal plant *Euphorbia hirta* L. has been widely acknowledged for use in traditional medicine of Vietnam. The whole plants of *E. hirta* are commonly applied to cure a wide range of diseases, especially gastrointestinal disorders, skin problems, and respiratory system. This study focused on chemical composition of the *E. hirta* L. whole plant. The chemical investigation of *n*-hexane and ethyl acetate extracts of this species led to the isolation and structural determination of taraxerol (**1**), campesterol (**2**), astragalin (**3**), hymenoxin (**4**), luteolin-7-*O*-*β*-D-glucopyranoside (**5**) and quercetin 3-*O*-*α*-L arabinofuranoside (**6**) by spectroscopic methods including 1D-NMR, 2D-NMR, IR, HR-MS in combination with published literature.

1. Introduction

Euphorbia hirta L. is popular in most of tropical countries, especially in Vietnam and it is known to possess several medicinal functions.[1,2] According to plant classification, plant *Euphorbia hirta* belonging to *Euphorbia* genus, Euphorbiaceae family, and is frequently seen occupying open waste land and grasslands, road sides, and pathways all over of Vietnam. The whole plants of *E. hirta* L. are known to contain polyphenols compound including flavonoids, tannins and other natural products such as sterols, alkaloids, glycosides and triterpenoides.[3,4] Recently, modern pharmacological studies proved that *E. hirta* L. and its phytocomponents possessed many biological activities such as anti-inflammatory, antifungal, antibacterial, antidiarrheal, and antioxidant effects.[1,5,6] As parts of our continuing studies[7], this study reports the separation and characterization of six compounds from *n*-hexane and ethyl acetate extracts of *Euphorbia hirta* L. grown in Vietnam.

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2. Experimental

2.1.General procedures – NMR spectra were measured on a Bruker FTNMR spectrometer with model AM500 (Bruker, Karlsruhe, Germany) using TMS as an internal standard, Institute of Chemistry - Vietnam Academy of Science and Technology (VAST), Hanoi, Vietnam. ESI-MS spectra were recorded on a MicroOTOF-Q mass spectrometer (Bruker, Karlsruhe, Germany), Institute of Chemistry (VAST). IR spectra were recorded on Jasco-V730 (Kyoto, Japan), Can Tho University. Silicagel gel 60 (0.063–0.200mm, Merck) was used for column chromatograph. TLC F_{254} plate (Merck) was used for thin layer chromatography. The detection of compounds on TLC plates were done using UV lamp at 254 or 365 nm or a solution of $FeCl_3$/EtOH or H_2SO_4/EtOH.

2.2. Plant materials – The whole plants of *Euphorbia hirta* L. were collected at Can Tho city - Vietnam, in February 2017. The sample was authenticated by Dr. Dang Minh Quan, Department of Biology Education, Can Tho University and a voucher specimen (number EUPH1032017) was deposited in our department at Can Tho University.

2.3. Extraction and isolation – The plant materials were dried at room temperature, powdered, and extracted with 96% ethanol for four times (4 × 20 L). The extractive solutions were filtered and solvent was removed through rotary evaporator to obtain crude ethanol extract (700 g). The crude extract was then partitioned by a flash silica gel column chromatography and eluted with increasing polarity solvents, yielding the corresponding of *n*-hexane (160 g), ethyl acetate (95 g), *n*-butanol (185 g), and methanol (172 g) extracts.

The *n*-hexane extract (100g) was first subjected to a flash silica gel column chromatography (CC), eluted in a gradient solvent system consisting of *n*-hexane and ethyl acetate (100:1 – 0:100, gradient) to obtain eight fractions (HE1-8). Fraction HE3 was re-subjected to CC on silica gel, eluted with *n*-hexane: EtOAc (50:1-0:100) and 14 subfractions (HE3.1-14) were collected. Similarly, subfraction HE3.3 was further separated on a silica gel CC, with elution of *n*-hexane: EtOAc (50:1-0:100) to obtain four subfractions (HE3.3.1-4). Then, subfraction HE3.3.3 was subjected to a silica gel CC, eluted with *n*-hexane: EtOAc (80:1-50:1) to obtain four subfractions (HE3.3.3.1-4) and at last compound **1** (120 mg) was obtained from subfraction HE3.3.3.2 on a silica gel column, using mixture of *n*-hexane: EtOAc (80:1) as eluent. Fraction HE5 was also chromatographed on silica gel using *n*-hexane: EtOAc (20:1 - 0:100) to yield eight subfractions (HE6.1-8). Subfraction HE5.5 was repeatedly subjected to silica gel column using *n*-hexane: EtOAc (50:1, v/v) to obtain compound **2** (5 mg).

The ethyl acetate extract was partitioned through a flash silica gel column chromatography and eluted with *n*-hexane and ethyl acetate (100:0 – 0:100) to get eight fractions (EE1-8). Fraction EE9 was further passed over a silica gel column using $CHCl_3$: MeOH (30:1-1:1) as eluent to give seven subfractions (EE9.1-7). Subfraction EE9.1 was repeatedly subjected to silica gel column, eluted with $CHCl_3$: MeOH (2:1-0:100) to obtain four subfractions (EE9.1.1-4). Finally, compound **3** (4 mg) was obtained by eluting the column with $CHCl_3$: MeOH (5:95) from subfraction EE9.1.3. Similarly, fraction EE2 was also separated on a silica gel column using $CHCl_3$: MeOH (100:0-90:1) and compound **4** (8 mg) was obtained from subfraction EE2.3. In addition, subfraction EE8 was passed over a silica gel column eluting with *n*-hexane: EtOAc (7:3-3:7) to give five subfractions (EE8.1-5). Then, subfraction EE8.1 was recrystallized by ethyl acetate to obtain compound **5** (8.0 mg) and compound **6** was obtained from subfraction EE8.4 (5mg).

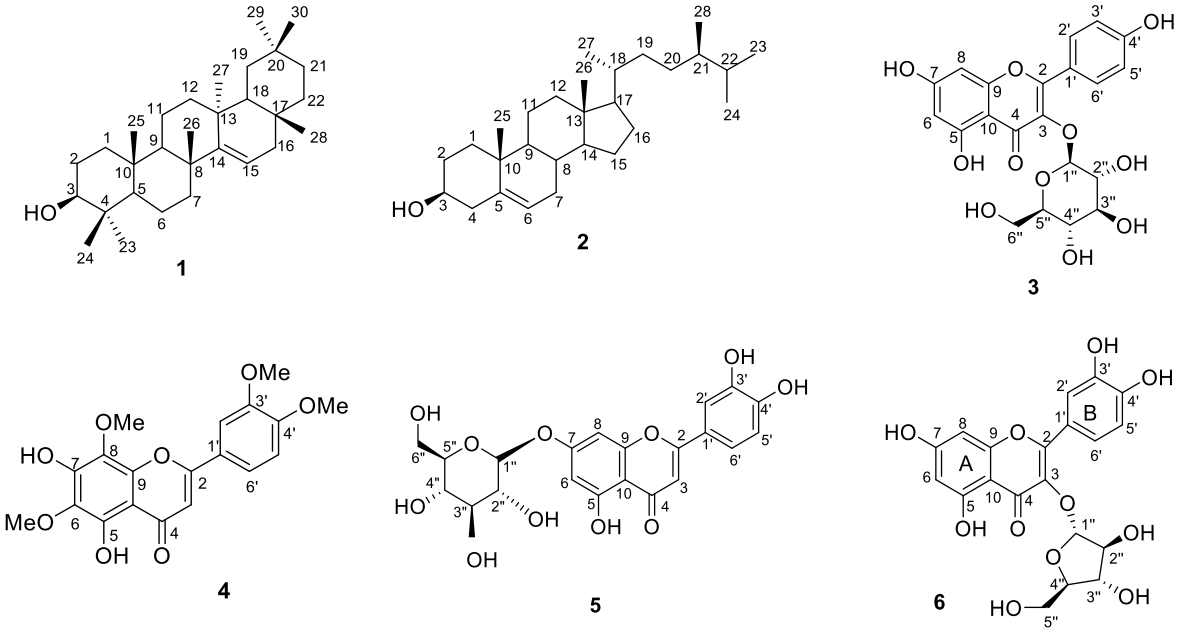

Figure 1. Chemical structures of isolated compounds (**1-6**)

3. Results and discussion

Compound **1** was obtained from *n*-hexane extract as white powder, m.p. 282-284ºC (MeOH). IR spectrum (KBr, ν_{max}, cm^{-1}): 3435 (-OH), 2941, 2869, 1642, 1457, 1381, 1030. The ^{13}C-NMR and DEPT spectra of **1** displayed 30 carbon signals, including 1 carbinol carbon >CH-OH at δ_C 79.1 (C-3), one tertiary olefinic carbon =CH- [δ_C 116.9 (C-15)], one quaternary olefinic carbon =C< [δ_C 158.1 (C-14)], 8 methyl carbons -CH_3, 10 methylene carbons, 3 methine carbons >CH- [δ_C 55.6 (C-5); 48.8 (C-9) and 49.3 (C-18)], and 6 quaternary carbons. The ^{1}H-NMR signals suggested the presence of triterpene skeleton – taraxerane, consisting of one hydroxyl group and one double bond. From HMBC, 2 methyl groups at C-29 and C-30 correlated with each other and also correlated with C-20, C-19 and C-21. Both methyl groups H_3-26 and H_3-27 correlated with the olefinic quaternary carbon at C-14. In addition, protons H_3-26 also correlated with C-8, C-9 and C-7. Protons H_3-27 also correlated with carbons at C-13, C-18 and C-12. Besides, olefinic proton at δ_H 5.53 corresponed with three quaternary carbons at C-8, C-13 and C-17; so the double bond must be at C-14 and C-15. Proton H_3-28 corresponded with four carbons at C-17, C-18, C-16 and C-22. Methyl proton H_3-25 correlated with three carbons at C-9, C-5 and C-1. Two methyl groups C-23 and C-24 correlated with each other and also correlated with carbon at C-4, C-5 and C-3. The double of doublet signal (J = 3.0 Hz, 8.0 Hz) appeared at δ_H 5.53 is assigned to the olefinic proton H-15. The broad doublet signal (J = 11.0 Hz and 4.5 Hz) was found at δ_H 3.19 could be assigned to proton H-3. This allow to identify compound **1** as *β*-taraxerol through the comparison of physical and spectral data with the published data.[8]

Compound **2** was obtained as white solid. The ^{1}H-NMR spectrum of **2** showed signals of δ_H (ppm) 5.35 (1H, *dd*, 2.0/3.0 Hz, H-6), 3.54 (1H, *tt*, 11.0/5.0 Hz, H-3), 2.28 (1H, *d*, 7.0 Hz, H-4), 0.86 - 2.31 (*m*, 20H 10 x CH_2 protons), 0.68-1.27 (*m*, 6 x CH_3, H-18, 19, 21, 26, 27 and H-28), 1.01 (3H, *s*, H-19); 0.96 (3H, *d*, 6.5 Hz, H-21); 0.84 (3H, *d*, 6.5 Hz, H-27); 0.86 (3H, *d*, 7.5 Hz, H-26); 0.82 (3H, *d*, 7.0 Hz, H-28); 0.96 (3H, *s*, H-18). The existence of proton carbinol >CH-OH at [δ_H 3.54, 11.0 Hz, H-3] indicated that -OH of C-3 was *β*-hydroxyl. The ^{13}C-NMR together with DEPT spectra of **2** appeared 28 carbon

signals which were classified into 6 methyl, 10 methylene, 9 methine and 3 quaternary carbons. Thus, compound **2** was established as campesterol.[9]

The ^{13}C-NMR spectral data of **3** displayed 21 carbon signals suggested the molecular formula as $C_{21}H_{20}O_{11}$. The ^{1}H-NMR spectrum of **3** indicated the characteristic signals of a flavonol glycoside including two singlet proton signals at δ_H 6.24 (*d*, 2.0 Hz, H-6) and δ_H 6.43 (*d,* 2.0 Hz, H-8) and two doublet proton signals at δ_H 6.91 (*d*, 9.0 Hz, H-2′, 6′) and δ_H 8.08 (*dd*, 1.8/6.8 Hz, H-3′,5′) that revealed the kaempferol-like nature of the aglycon part of the compound. ^{1}H-NMR spectrum of **3** also exhibited the existence of an anomeric proton at δ_H 5.27 (*d*, 7.5 Hz, H-1″) and was further supported by the presence of carbon signals of sugar moiety at δ_C 104.1 (C-1″), 75.7 (C-2″), 78.0 (C-3″), 71.4 (C-4″), 78.4 (C-5″), and 62.6 (C-6″). Furthermore, the attachment of glucose to C-3 of the aglycone was confirmed by crosspeak between H-1″ with C-3 in HMBC experiment. According to these data and by comparing them with the NMR data of different flavonol glycosides reported in the literature[10], the chemical structure of **3** was defined as astragalin.

The ^{13}C-NMR together with DEPT spectra of compound **4** displayed 15 carbon signals belong to a flavon skeleton. The ^{1}H-NMR spectra of compound **4** showed signals at δ_H 7.68 (*dd*, 8.5 and 2.2 Hz), 7.56 (*d*, 2.2 Hz), and 7.18 (*d*, 8.5 Hz) could be assigned to the proton H-6′, H-2′, and H-5′, respectively. In addition, the ^{1}H-NMR spectrum exhibited a singlet at δ_H 12.74 - a signal typical for a C-5 hydrogen-bonded hydroxyl group. Therefore, the structure of compound **4** was elucidated as hymenoxin.[11]

Compounds **5** and **6** were determined as luteolin-7-*O*-*β*-D-glucopyranoside (**5**) and quercetin 3-*O*-α-L arabinofuranoside (**6**). The ESI-MS spectrum of **5** showed a peak *m/z* 462.4 $[M+H]^+$ corresponding to the molecular formula $C_{22}H_{22}O_{11}$. The structure of **5** was identified by ^{1}H, ^{13}C, HSQC, and HMBC- NMR analyses. The structure to the aglycone was assigned as 5,7,3',4' - tetrahydroxy flavone. The NMR data revealed that the *β*-glucopyranose moiety is attached to C-7 of the flavone skeleton, in agreement with literature.[12] Analysis of ^{1}H and ^{13}C-NMR data of **6** showed that the aromatic signals are close to those reported for quercetin moiety. The sugar signals in ^{13}C-NMR, δ_C 109.6, 83.3, 78.7, 88.0 and 62.6 are comparable with those reported for *α*-L-arabinofuranose. ^{1}H-NMR spetrum appeared anomeric proton signal at δ_H 5.49 (1H, *s*) which confirmed the presence of *α*-linked sugar. In addition, the HMBC crosspeak between the anomeric proton with the C-3 proved that sugar moiety was linked to C-3 of the aglycone. Therefore, compound **6** could be quercetin-3-*O*-α-L-arabinofuranoside.[13] (**Figure 1**)

4. Conclusion

From *n*-hexane and methanol extracts of *Euphorbia hirta* L. grown in Vietnam, taraxerol (**1**), campesterol (**2**), astragalin (**3**), hymenoxin (**4**), luteolin-7-*O*-*β*-D-glucopyranoside (**5**) and quercetin 3-*O*-α-L arabinofuranoside (**6**) were isolated and determined. In which, methoxyl flavonoid (**4**) and two flavone glycoside (**5, 6**) were isolated from this species for the first times.

Acknowledgments

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