ISOLATION OF PHENOLIC COMPOUNDS FROM THE AERIAL PARTS OF *EXCOECARIA COCHINCHINENSIS*

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TÓM TẮT

PHÂN LẬP MỘT SỐ HỢP CHẤT PHENOLIC TỪ BỘ PHẬN TRÊN MẶT ĐẤT CỦA CÂY ĐƠN LÁ ĐỎ (*EXCOECARIA COCHINCHINENSIS*)

*Cây Đơn lá đỏ (Excoecaria cochinchinensis) thuộc họ Thầu dầu (*Euphorbiaceae*), từ lâu đã được sử dụng rộng rãi trong y học dân gian để điều trị các bệnh ngoài da như nhọt, phát ban, cũng như đau bụng, tiêu chảy, viêm khớp, rối loạn da và một số bệnh nhiễm trùng. Trong nghiên cứu này, từ dịch chiết methanol của E. cochinchinensis thu hái tại tỉnh Hòa Bình, sáu hợp chất đã biết đã được phân lập, bao gồm kaempferol (**1**), quercetin (**2**), kaempferol 3-O-β-D-xylopyranoside (**3**), scopoletin (**4**), methyl protocatechuate (**5**) và methyl gallate (**6**). Cấu trúc của các hợp chất này được xác định thông qua phổ NMR và so sánh với tài liệu tham khảo. Đặc biệt, hai hợp chất số **3** và **5** lần đầu tiên được phân lập từ cây này.*

Từ khóa: *Excoecaria cochinchinensis, flavonoid, coumarin, hợp chất phenolic.*

1. INTRODUCTION

Excoecaria cochinchinensis, a medicinal herb from the Euphorbiaceae family, is widely utilized in traditional medicine to treat various conditions, including skin diseases like boils and rashes, as well as abdominal pain, diarrhea, arthritis, dermatological disorders, and certain infections [1]. Previous studies have identified that *E. cochinchinensis* contains various bioactive compounds, including flavonoids [2], terpenoids [3-5], lignans [6], and phenolic compounds [7]. Additionally, this plant is a beneficial source for pharmaceutical research due to its diverse biological activities, such as anti-inflammatory [8], antioxidant [9], and anticancer activities [10]. Further studies on the isolation and identification of active compounds from methanol extract will help maximize the medicinal potential of this plant. Herein, we report the isolation of six known compounds (Figure 1) and their structural characterization through NMR spectroscopic analysis, with comparisons to existing literature.

2. EXPERIMENT

2.1. General experimental procedures

The NMR spectra were recorded using a Bruker Avance III 500 MHz spectrometer and a Bruker Avance 600 MHz spectrometer, with TMS as the internal standard. Column chromatography was

conducted using silica gel 60G (Merck), RP-C18 gel (40–63 μm, Merck, Darmstadt, Germany), and Sephadex LH-20 (GE Chemical Corporation). Thin-layer chromatography (TLC) was performed on silica gel 60 F254 plates (Merck) and silica gel 60 RP-18 F254S plates (Merck), with spot visualization achieved by spraying with a 10% H_2SO_4 solution followed by heating.

2.2. Plant materials

The aerial parts of *E. cochinchinensis* were collected in March 2023 from Luong Son District, Hoa Binh Province, Vietnam. The plant was authenticated by botanist Cao Ngoc Giang at the Research Center of Ginseng and Medicinal Materials, National Institute of Medicinal Materials. A voucher specimen (TBDD-0923) is preserved in the Department of Chemistry, Ho Chi Minh University of Education, Ho Chi Minh City, Vietnam.

2.3. Extraction and isolation

The air-dried aerial parts of *E. cochinchinensis* (20.0 kg) were powdered and extracted with methanol (50 L × 3) at room temperature. The solvent was then evaporated under reduced pressure, and the resulting residues were combined, suspended in water, and sequentially partitioned with *n*-hexane, *n*-hexane-ethyl acetate (1:1, v/v), and ethyl acetate. This procedure yielded three extracts: H (70.0 g), HE (255.0 g), and EA (217.0 g). The HE extract (255.0 g) was further purified using silica gel column chromatography with an *n*-hexane-ethyl acetate gradient (10:1 to 1:1, v/v), resulting in six fractions (HE1–HE6). Fraction HE6 (5.0 g) was fractionated on a silica gel column using *n*-hexane-ethyl acetate (4:1) to obtain three subfractions (HE6.1–HE6.3). Subfraction HE6.2 (3.0 g) was purified on a Sephadex LH-20 column using CH_2Cl_2-MeOH (3:7) to get three subfractions (HE6.2.1–HE6.2.3). Subfraction HE6.2.2 (550.5 mg) was separated on a silica gel column using CH_2Cl_2-MeOH (4:0.07) to afford compound **5** (26.4 mg). Compound **6** (9.2 mg) was obtained from subfraction HE6.2.3 (105.3 mg) by using a reversed-phase silica gel CC (RP-18) using MeOH-H_2O (1:2). The EA extract (217.0 g) was similarly purified using silica gel CC with a stepwise gradient of *n*-hexane–ethyl acetate (1:5 to 0:1, v/v), yielding seven fractions (EA1–EA7). Fraction EA3 (6.3 g) was chromatographed on a silica gel column using *n*-hexane-ethyl acetate (2:1) to get three subfractions EA3.1–EA3.3. Subfraction EA3.1 (1.5 g) was purified on a silica gel column using *n*-hexane-CH_2Cl_2-acetone (2:0.25:0.25) and then on an RP-18 CC with MeOH-H_2O (1:2) to afford **1** (9.8 mg). Compound **2** (19.0 mg) was yielded from subfraction EA3.2 (1.2 g) by using Sephadex LH-20 with CH_2Cl_2-MeOH (3:7). Subfraction EA3.3 (2.1 g) was isolated on a silica gel column using *n*-hexane-CH_2Cl_2-acetone (1.3:0.4:0.4) to get three subfractions EA3.3.1–EA3.3.3. Compounds **3** (10.4 mg) and **4** (19.3 mg) were isolated using an RP-18 CC with MeOH-H_2O (1:2 to 2:1) from subfraction EA3.3.2 (120.5 mg).

Kaempferol (**1**): Yellow powder. ^{1}H NMR (500 MHz, acetone-d_6) δ_H (ppm): 12.17 (1H, s, 5-OH), 8.15 (2H, d, J = 8.5 Hz, H-2′, H-6′), 7.01 (2H, d, J = 9.0 Hz, H-3′, H-5′), 6.53 (1H, d, J = 2.5 Hz, H-8), 6.27 (1H, d, J = 2.0 Hz, H-6). ^{13}C NMR (125 MHz, acetone-d_6) δ_C (ppm): 176.6 (C-4), 165.0 (C-7), 162.3 (C-9), 160.2 (C-4′), 157.8 (C-5), 147.0 (C-2), 136.6 (C-3), 130.5 (C-2′, C-6′), 123.3 (C-1′), 116.3 (C-3′, C-5′), 104.2 (C-10), 99.2 (C-6), 94.5 (C-8).

Quercetin (**2**): Yellow powder. ^{1}H NMR (500 MHz, acetone-d_6) δ_H (ppm): 12.17 (1H, s, 5-OH), 7.83 (1H, d, J = 2.5 Hz, H-2′), 7.70 (1H, dd, J = 8.5, 2.0 Hz, H-6′), 6.99 (1H, d, J = 8.5 Hz, H-5′), 6.52 (1H, d, J = 2.0 Hz, H-8), 6.26 (1H, d, J = 2.0 Hz, H-6). ^{13}C NMR (125 MHz, acetone-d_6) δ_C (ppm): 176.6 (C-4), 165.0 (C-7), 162.3 (C-5), 157.8 (C-9), 148.3 (C-4′), 146.9 (C-2), 145.8 (C-3′), 136.8 (C-3), 123.8 (C-1′), 121.5 (C-6′), 116.2 (C-5′), 115.8 (C-2′), 104.1 (C-10), 99.1 (C-6), 94.4 (C-8).

Kaempferol 3-*O*-*β*-D-xylopyranoside (**3**): Yellow powder. ^{1}H NMR (600 MHz, DMSO-d_6) δ_H (ppm): 12.58 (1H, s, 5-OH), 8.02 (2H, d, J = 8.4 Hz, H-2′, H-6′), 6.90 (2H, d, J = 8.4 Hz, H-3′, H-5′), 6.44 (1H, d, J = 1.8 Hz, H-8), 6.21 (1H, d, J = 1.8 Hz, H-6), 5.33 (1H, d, J = 7.2 Hz, H-1″), 3.62 (1H, dd, J = 11.4, 4.8 Hz, H-5″a), 3.29-3.19 (3H, m, H-2″, H-3″, H-4″), 2.96 (1H, dd, J = 11.4, 9.6 Hz, H-5″b). ^{13}C NMR (150 MHz, DMSO-d_6) δ_C (ppm): 177.4 (C-4), 164.2 (C-7), 161.2 (C-5), 160.1 (C-4′), 156.4 (C-9), 156.2 (C-2), 133.1 (C-3), 130.8 (C-2′, 6′), 120.7 (C-1′), 115.3 (C-3′, 5′), 103.9 (C-10), 101.7 (C-1″), 98.8 (C-6), 93.7 (C-8), 75.8 (C-3″), 73.7 (C-2″), 69.4 (C-4″), 65.9 (C-5″).

Scopoletin (**4**): Light-yellow powder. ^{1}H NMR (500 MHz, DMSO-d_6) δ_H (ppm): 10.33 (1H, br s, 7-OH), 7.91 (1H, d, J = 9.5 Hz, H-4), 7.22 (1H, s, H-5), 6.79 (1H, s, H-8), 6.21 (1H, d, J = 9.5 Hz, H-3), 3.81 (3H, s, 6-OCH$_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ_C (ppm): 160.7 (C-2), 151.2 (C-7), 149.5 (C-9), 145.3 (C-6), 144.5 (C-4), 111.7 (C-3), 110.5 (C-10), 109.6 (C-5), 102.8 (C-8), 56.0 (6-OCH$_3$).

Methyl protocatechuate (**5**): White amorphous powder. ^{1}H NMR (500 MHz, methanol-d_4) δ_H (ppm): 7.42 (1H, br s, H-2), 7.41 (1H, dd, J = 8.0, 2.0 Hz, H-6), 6.81 (1H, d, J = 8.0 Hz, H-5), 3.83 (3H, s, 7-OCH$_3$). ^{13}C NMR (125 MHz, methanol-d_4) δ_C (ppm): 168.9 (C-7), 151.7 (C-4), 146.2 (C-3), 123.6 (C-6), 122.5 (C-1), 117.4 (C-2), 115.9 (C-5), 52.3 (7-OCH$_3$).

Methyl gallate (**6**): White amorphous powder. ^{1}H NMR (500 MHz, DMSO-d_6) δ_H (ppm): 9.17 (3H, s, 3-OH, 4-OH, 5-OH), 6.93 (2H, s, H-2, H-6), 3.74 (3H, s, 7-OCH$_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ_C (ppm): 166.3 (C-7), 145.6 (C-3, C-5), 138.4 (C-4), 119.3 (C-1), 108.5 (C-2, C-6), 51.6 (7-OCH$_3$).

3. RESULTS AND DISCUSSION

Compound **1** was acquired as a yellow powder. The ^{1}H NMR spectroscopic data of **1** revealed the presence of an AA′BB′ spin system was assigned as two doublets at δ_H 8.15 (2H, d, J = 8.5 Hz, H-2′, H-6′), and 7.01 (2H, d, J = 9.0 Hz, H-3′, H-5′). Two *meta*-coupled doublets at δ_H 6.53 (1H, d, J = 2.5 Hz, H-8) and 6.27 (1H, d, J = 2.0 Hz, H-6) suggest the occurrence of a tetrasubstituted phenyl ring (ring A). Besides that, one hydrogen bonding at δ_H 12.17 (1H, s, 5-OH) was observed in **1**. The ^{13}C NMR spectrum showed 13 signals for 15 carbons, including a carbonyl carbon at δ_C 176.6 (C-4), eight quaternary carbons at δ_C [165.0 (C-7), 162.3 (C-9), 160.2 (C-4′), 157.8 (C-5), 147.0 (C-2), 136.6 (C-3), 123.3 (C-1′), and 104.2 (C-10)], six methines carbons at δ_C [130.5 (C-2′, C-6′), 116.3 (C-3′, C-5′), 99.2 (C-6), and 94.5 (C-8)]. The ^{1}H and ^{13}C NMR spectroscopic data of **1** were close to those of kaempferol [11]. Thus, the structure of **1** was determined.

Compound **2** was isolated in the form of a yellow powder. The ^{1}H NMR of **2** showed the presence of the ABX spin system at δ_H

7.83 (1H, d, J = 2.5 Hz, H-2′), 7.70 (1H, dd, J = 8.5, 2.0 Hz, H-6′), and 6.99 (1H, d, J = 8.5 Hz, H-5′) indicated a trisubstituted benzene ring (ring B). Two *meta*-coupled protons at δ_H 6.52 (1H, d, J = 2.0 Hz, H-8) and 6.26 (1H, d, J = 2.0 Hz, H-6) were also observed in the ^{1}H NMR. The ^{13}C NMR spectrum displayed 15 carbon signals, including a carbonyl carbon (δ_C 176.6), nine quarternary carbons [δ_C 165.0 (C-7), 162.3 (C-5), 157.8 (C-9), 148.3 (C-4′), 146.9 (C-2), 145.8 (C-3′), 136.8 (C-3), 123.8 (C-1′), 104.1 (C-10)], and five methines carbon [δ_C 121.5 (C-6′), 116.2 (C-5′), 115.8 (C-2′), 99.1 (C-6), and 94.4 (C-8)]. The NMR data of **2** were similar to those of quercetin [12]; thus, **2** was quercetin.

Compound **3** was separated as a yellow powder. The ^{1}H NMR spectral data of **3** exhibited six aromatic protons at δ_H [8.02 (2H, d, J = 8.4 Hz, H-2′, H-6′), 6.90 (2H, d, J = 8.4 Hz, H-3′, H-5′), 6.44 (1H, d, J = 1.8 Hz, H-8), 6.21 (1H, d, J = 1.8 Hz, H-6)] which are typical of kaempferol unit. An aromatic proton at δ_H 5.33 (1H, d, J = 7.2 Hz, H-1″) indicated a sugar moiety in **3**. The ^{13}C NMR displayed 22 carbon resonances, 15 carbons deduced to kaempferol [δ_C 177.4 (C-4), 164.2 (C-7), 161.2 (C-5), 160.1 (C-4′), 156.4 (C-9), 156.2 (C-2), 133.1 (C-3), 130.8 (C-2′, 6′), 120.7 (C-1′), 115.3 (C-3′, 5′), 103.9 (C-10), 98.8 (C-6), 93.7 (C-8)], and five for the xylose unit [δ_C 101.7 (C-1″), 75.8 (C-3″), 73.7 (C-2″), 69.4 (C-4″), 65.9 (C-5″)]. The xylose unit was β-xylopyranose due to the large coupling constant at δ_H 5.33 (1H, d, J = 7.2 Hz). Furthermore, the upfield carbon at C-3 (δ_C 133.1) suggested that the xylose was attached at this position. The spectroscopic data of **3** were fit with kaempferol 3-*O*-β-D-xylopyranoside [13]. Compound **3** was first isolated from this plant.

Compound **4** was purified as a light-yellow powder. The ^{1}H NMR spectrum exhibited two *ortho*-coupled protons with a coupling constant of 9.5 Hz, appearing at δ_H 7.91 (1H, d, J = 9.5 Hz) and 6.21 (1H, d, J = 9.5 Hz), corresponding to H-4 and H-3, respectively. In addition, two aromatic singlet protons at δ_H 7.22 (1H, s, H-5), 6.79 (1H, s, H-8), and one methoxy group at δ_H 3.81 (3H, s, 6-OCH$_3$) were found in the ^{1}H NMR spectrum. The ^{13}C NMR data showed 10 carbon resonances containing a coumarin skeleton at δ_C [160.7 (C-2), 151.2 (C-7), 149.5 (C-9), 145.3 (C-6), 144.5 (C-4), 111.7 (C-3), 110.5 (C-10), 109.6 (C-5), 102.8 (C-8)], and a methoxy group at δ_C 56.0. Analysis of NMR data showed that compound **4** has the same structure as scopoletin [14]. Compound **4** was compared to isoscopoletin to further confirm its structure, which was different at C-6 (δ_C 145.3 in **4** and δ_C 143.6 in isoscopoletin) [14].

Compound **5** was obtained in the form of a white amorphous powder. The ^{1}H NMR spectrum showed three aromatic protons at δ_H 7.41 (1H, dd, J = 8.0, 2.0 Hz, H-6), 6.81 (1H, d, J = 8.0 Hz, H-5), indicating the presence of trisubstituted phenyl ring. The ^{13}C NMR spectrum showed signals for seven carbons, including a carbonyl carbon at δ_C 168.9 (C-7), six aromatic carbons at δ_C [151.7 (C-4), 146.2 (C-3), 123.6 (C-6), 122.5 (C-1), 117.4 (C-2), 115.9 (C-5)], and a methoxy group at δ_C 52.3. The ^{1}H and ^{13}C NMR spectra of compound **5** matched those of methyl protocatechuate [15].

Compound **6**, isolated as a white amorphous powder. The ^{1}H NMR spectrum showed the existence of three

hydroxy groups at δ_H 9.17 (3H, s, 3-OH, 4-OH, 5-OH), two protons in the aromatic region at δ_H 6.93 (2H, s, H-2, H-6), and a methoxy group at δ_H 3.74 (3H, s, 7-OCH3). The ^{13}C NMR spectrum exhibited seven carbons [δ_C 166.3 (C-7), 145.6 (C-3, C-5), 138.4 (C-4), 119.3 (C-1), 108.5 (C-2, C-6), 51.6 (7-OCH_3)]. The structure of **6** closely resembled that of **5**, except for the presence of a hydroxy group at C-5. In addition, the NMR data of **6** were consistent with methyl gallate [16], thus determining its structure.

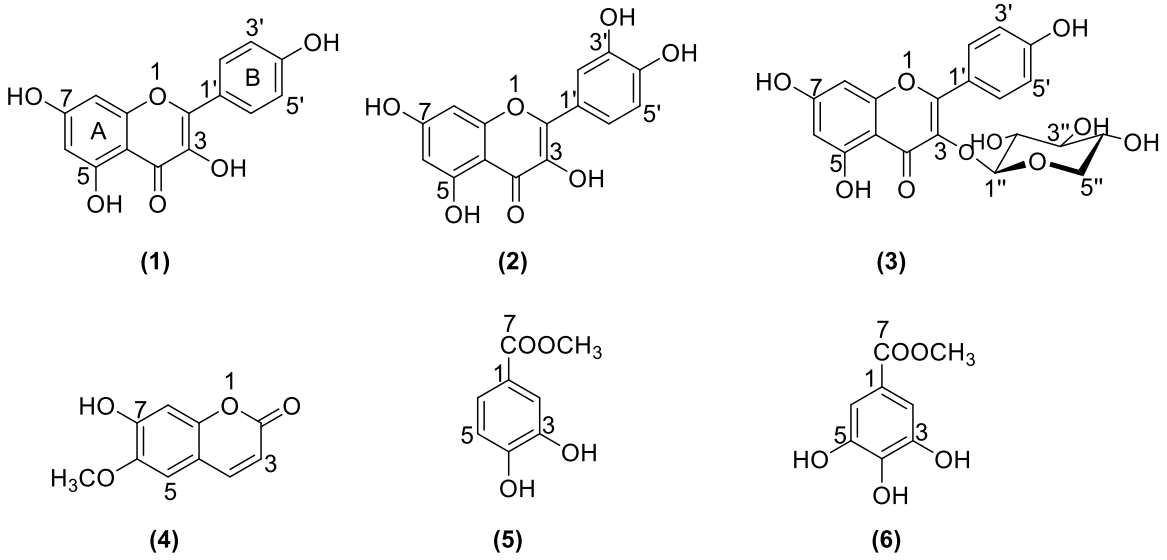

*Figure 1. Structures of compounds **1–6***

4. CONCLUSION

In conclusion, six previously described phenolic compounds were isolated from the methanol extract of the aerial parts of *E. cochinchinensis*, including three flavonols (**1**–**3**), a coumarin (**4**), and two derivatives of gallic acid (**5**–**6**). Their structures were elucidated by NMR spectroscopic analysis and compared with the literature reports. Notably, compounds **3** and **5** were isolated for the first time from this species.

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