A TRITERPENOID AND TWO STEROIDS ISOLATED FROM THE STEM BARK OF *Calophyllum polyanthum*

PHÂN LẬP CÁC HỢP CHẤT TRITERPENOID VÀ STEROID TỪ THÂN VỎ CỦA CÂY CÒNG NHIỀU HOA *Calophyllum polyanthum*

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ABSTRACT

A triterpenoid, friedelin (**1**) and two steroids, *β*-sitosterol (**2**), stigmasterol (**3**) were isolated from dichloromethane extract of *Calophyllum polyanthum* stem bark. The chemical structures of the isolated compounds were determined by analysis of their NMR spectral data and comparison with those reported in the literature. As far as our knowledge, this is the first report about chemical constituents of *Calophyllum polyanthum* growing in Vietnam.

Keywords: *Calophyllum polyanthum*, triterpenoid, steroid, friedelin, *β*-sitosterol, stigmasterol.

TÓM TẮT

Từ cặn chiết dichloromethane của thân vỏ cây Còng nhiều hoa *Calophyllum polyanthum* chúng tôi đã phân lập được một triterpenoid, friedelin (**1**) và hai steroid gồm *β*-sitosterol (**2**) và stigmasterol (**3**). Cấu trúc hóa học của các hợp chất **1**-**3** được xác định dựa vào việc phân tích dữ kiện phổ NMR của các hợp chất kết hợp so sánh với dữ kiện phổ công bố trong các tài liệu tham khảo. Theo tìm hiểu của chúng tôi, đây là công trình đầu tiên công bố về thành phần hóa học của cây Còng nhiều hoa *Calophyllum polyanthum* ở Việt Nam.

Từ khóa: *Calophyllum polyanthum*, triterpenoid, steroid, friedelin, *β*-sitosterol, stigmasterol.

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1. INTRODUCTION

The genus *Calophyllum* is one of the five largest and most important genera of the Clusisaceae family, mainly distributed in the tropical regions such as India, Malaysia, Indonesia, and Madagascar, in which about 130 species have been reported [1-3]. Many plants of the *Calophyllum* are used in traditional medicine for treating peptic ulcer, malaria, high blood pressure, inflammation, trauma infection,… [4-6]. Previous phytochemical studies of *Calophyllum* species revealed rich sources of active natural compounds, namely coumarins, xanthones, chromanones, flavonoids, terpenoids [4-8]. In Vietnam, more than 30 known *Calophyllum* species have been found, including *Calophyllum polyanthum* (*C. polyanthum*) which is an evergreen tree that can grow up to 15 m tall with straight trunk, quadrangulate glabrous branches and white flowers [9]. Courmarines isolated from *C. polyanthum* exhibited significant cell protective activities against H_2O_2-induced human umbilical vein endothelial cell damage [10], xanthones isolated from *C. polyanthum* exhibited higher CYP1A2 enzyme inhibitory effects than that of the positive control *α*-naphthoflavone [11]. However, in Vietnam there has not been any research about this plant.

As part of our continuing investigations for bioactive compounds from Clusisaceae family, including *Garcinia* and *Calophyllum* genus, we described herein the chemical constituent research of *C. polyanthum* collected in Dam Rong district, Lam Dong province. As a result, a triterpenoid, friedelin (**1**), and two steroids, namely *β*-sitosterol (**2**) and stigmasterol (**3**), have been isolated from the dichloromethane (DCM) extract of *C. polyanthum* stem bark.

2. EXPERIMENTAL SECTION

2.1. General

Column chromatography (CC) was carried out on silica gel 60 (Merck, 40 - 63µm), silica gel 60 (Merck, 63 - 200µm)

eluted with gradient solvent. Thin layer chromatography plates were visualized using UV light (254 and 365nm), staining with vanillin in H_2SO_4 10% solution. Commercial solvents were purified and dried, when necessary, by standard methods just prior to use.

NMR spectra were recorded on a Bruker Advance 600 spectrometer (Institute of Chemistry - Vietnam Academy of Science and Technology), operating at 600 and 125MHz for ^1H and ^{13}C, respectively. Chemical shifts were shown in δ (ppm) in $CDCl_3$ using tetramethylsilane (TMS) as an internal reference. Melting points were measured on Buchi B545 apparatus (no correction).

2.2. Plant materials

Stem bark of *C. Polyanthum* was collected in Dam Rong district, Lam Dong province, in September 2021. The plant materials were identified by Dr. Nguyen Quoc Binh, Vietnam National Museum of Nature. A voucher specimen (CP2021006) has been deposited at Faculty of Chemical Technology - Hanoi University of Industry.

2.3. Extraction and isolation

The stem bark of *C. polyanthum* (6.0kg) was crushed into small pieces and was dried in the oven at the temperature of 45°C in three days to achieve 5.1 kg dried stem bark. Then the material was extracted with methanol (MeOH) (10L × 3 times) at room temperature using conventional ultrasound-assisted technique. The solvent was evaporated under reduced pressure to give a dark red residue (821.0g). The residue was further extracted with DCM (1L × 3 times) to yield DCM extract (207.3g). The left residue was then extracted respectively with ethylacetate (EtOAc) (500mL × 3 times) and acetone (500mL × 3 times) to afford EtOAc extract (150.6g), acetone extract (235.8g) and methanol residue (212.8g).

The DCM extract was subjected to column chromatography (CC) over silica gel, eluted with *n*-hexane-acetone in a polarity gradient manner (v/v, 100:0 to 0:100) to afford 28 fractions (Frs. CPT1–CPT28). Fraction GPT3 (3.8 g) was chromatographed over silica gel using *n*-hexane-DCM (v/v, 100:0 to 0:100) as an eluent to give eight subfractions CPT3.1-3.8. Subfraction CPT3.3 (1.4g) was isolated by CC eluting with 50% DCM in *n*-hexane to afford five subfractions CPT3.3.1-3.3.5. Crystallization of subfraction CPT3.3.4 in *n*-hexane-DCM (v/v, 1:1) yielded compound **1** as white needle crystal (0.26g).

Fraction CPT7 (4.1g) was fractioned by CC using eluent of 20% DCM in *n*-hexane as the mobile phase to give six subfractions CPT7.1-7.6. Repeated chromatography of subfraction CPT7.2 (0.6g) over silica gel using *n*-hexane-acetone (v/v, 60:1) as eluent providing compound **2** as white needles (0.08g).

Fraction CPT8 (7.5g) was separated on CC eluted with 30 % DCM in *n*-hexane as the mobile phase to give four subfractions CPT8.1-8.4. Subfraction CPT8.1 (2.4g) was isolated by CC using eluent of 10% acetone in *n*-hexane followed by crystalization in *n*-hexane-DCM (v/v, 1:1) to give compound **3** as white needle crystal (0.12g).

Friedelin (**1**): white needle crystal, m.p. 263 - 265°C. ^1H NMR ($CDCl_3$) δ_H 2.39 (1H; ddd; J = 13.8, 4.8, 1.8Hz; H-2); 2.30 (1H; ddt; J = 13.8, 7.2, 0.6Hz; H-2), 2.25 (1H; q; J = 7.2, 6.6, 6.0Hz; H-4), 1.96 (1H; tq; J = 7.2; 6.0, 2.4Hz; H-1), 1.75 (1H; td; J = 12.6, 3.0Hz; H-1), 1.18 (3H, s, H-28), 1.05 (3H, s, H-27), 1.01 (3H, s, H-26), 1.00 (3H, s, H-30), 0.95 (3H, s, H-29), 0.88 (3H; dd; J = 6.6Hz, H-23), 0.87 (3H, s, H-25), 0.73 (3H, s, H-24); ^{13}C NMR ($CDCl_3$) δ_C 213.2 (C-3), 59.5 (C-10), 58.3 (C-4), 53.1 (C-8), 42.8 (C-18), 42.2 (C-5), 41.6 (C-2), 41.3 (C-6), 39.7 (C-13), 39.3 (C-22), 38.3 (C-14), 37.5 (C-9), 36.0 (C-16), 35.6 (C-11), 35.4 (C-19), 35.0 (C-29), 32.8 (C-21), 32.4 (C-28), 32.1 (C-15), 31.8 (C-30), 30.5 (C-12), 30.0 (C-17), 28.2 (C-20), 22.3 (C-1), 20.3 (C-26), 18.7 (C-27), 18.3 (C-7), 18.0 (C-24), 14.7 (C-25), 6.8 (C-23).

***β*-Sitosterol** (**2**): white needle crystal, m.p. 137 - 140°C. ^1H NMR ($CDCl_3$) δ_H 5.35 (1H; t; J = 6.0Hz, H-6); 3.53 (1H; dt; J = 10.8, 4.8Hz; H-3); 1.01 (3H; s; H-18); 0. 95 (3H; d; J = 7.2Hz; H-21), 0.85 (3H; d; J = 7.2Hz; H-29), 0.83 (6H; d; J = 6.6Hz; H-26, 27), 0.68 (3H; s; H-19). ^{13}C NMR ($CDCl_3$) δ_C 140.8 (C-5), 121.7 (C-6), 71.8 (C-3), 56.8 (C-14), 56.1 (C-17), 50.2 (C-9), 45.9 (C-24), 42.4 (C-13), 42.3 (C-4), 39.8 (C-12), 37.3 (C-1), 36.5 (C-10), 36.3 (C-20), 33.9 (C-22), 31.9 (C-8), 32.0 (C-7), 31.8 (C-2), 29.1 (C-25), 28.3 (C-16), 26.1 (C-23), 24.3 (C-15), 23.1 (C-28), 21.2 (C-11), 19.8 (C-26), 19.5 (C-19), 19.0 (C-27), 18.8 (C-21), 11.8 (C-18).

Stigmasterol (**3**): white needle crystal, m.p. 161 - 162°C. ^1H NMR ($CDCl_3$) δ_H 5.35 (1H; ts; J = 3.6, 1.8Hz; H-6), 5.15 (1H; dd; J = 15.6, 9.0Hz; H-22), 5.01 (1H; dd; J = 15.0, 8.4Hz; H-21), 3.53 (1H; dt; J = 10.8, 4.8Hz; H-3), 1.02 (3H; d; J = 6.6Hz; H-19), 1.01 (3H; s; H-29), 0.85 (3H; d; J = 6.6Hz; H-27), 0.81 (3H; t; J = 6.6Hz; H-24), 0.80 (3H, d, J = 6.6Hz, H-26), 0.70 (3H; s; H-28). ^{13}C NMR ($CDCl_3$) δ_C 140.8 (C-5), 138.3 (C-22), 129.3 (C-23), 121.7 (C-6), 71.8 (C-3), 56.9 (C-14), 56.0 (C-17), 51.3 (C-24), 50.2 (C-9), 42.3 (C-13), 42.2 (C-4), 40.5 (C-20), 39.7 (C-12), 37.3 (C-1), 36.5 (C-10), 31.9 (C-8), 31.88 (C-7), 31.88 (C-25), 31.7 (C-2), 28.9 (C-16), 25.4 (C-28), 24.4 (C-15), 21.2 (C-11), 21.1 (C-26), 21.1 (C-21), 19.4 (C-19), 19.0 (C-27), 12.2 (C-29), 12.1 (C-18).

3. RESULTS AND DISCUSSION

Repeatedly chromatography over silica gel of the DCM extract of *C. polyanthum* stem bark collected in Dam Rong district, Lam Dong province resulted in a triterpenoid and two steroids. The ^{1}H and ^{13}C NMR spectral data of these compounds established compound **1** as a pentacyclic triterpenoid while compounds **2** and **3** as sterol derivatives. The structures of the isolated compounds are shown in Figure 1.

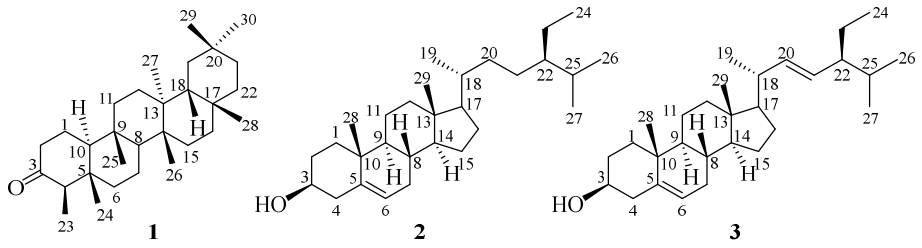

Figure 1. Chemical structures of compounds **1**-**3**

Compound **1** was isolated as white needle crystal. The ^{1}H NMR spectrum revealed the presence of seven tertiary methyl singlets at δ_H 1.18, 1.05, 1.01, 1.00, 0.95, 0.87, 0.73 and a secondary methyl doublet at δ_H 0.88 (3H; dd; J = 6.6Hz; H-23). Two protons appeared at δ_H 2.39 (1H; ddd; J = 13.8, 4.8, 1.8Hz; H-2) and 2.30 (1H; ddt; J = 13.8, 7.2, 0.6Hz; H-2) representing the two nonequivalent methylene protons H-2 due to negative inductive effect of the carbonyl group. For the same reason, a proton appeared as a quartet at δ_H 2.25 (1H; q; J = 7.2, 6.6, 6.0Hz; H-4) suggested resonance of H-4 and two nonequivalent methylene protons at δ_H 1.96 (1H; tq; J = 7.2, 6.0, 2.4Hz; H-1) and 1.75 (1H; td; J = 12.6, 3.0Hz; H-1) were determined as signals of H-1. The ^{13}C NMR spectrum showed signals of a carbonyl group at δ_C 213.2 and 29 saturated carbons all appeared at strong field with δ_C < 60ppm. The NMR spectra of compound **1** suggested the structure of a friedelane-type triterpenoid. In fact, the NMR data of compound **1** were identical to those of friedelin illustrated in the literature [12, 13], thus compound **1** was elucidated as friedelin.

Compound **2** was isolated as white needles. The ^{1}H NMR spectra of compound **2** presented characteristic resonance of a hydroxymethine proton appeared as a doublet triplet at δ_H 3.53 (1H; dt; J = 10.8, 4.8Hz; H-3) together with signal of an olefinic proton as a doublet at δ_H 5.35 (1H; d; J = 6.0Hz; H-6). Signals of six methyls including four doublet signals appeared at δ_H 0.95, 0.85, 0.83, 0.83 and two singlet protons at δ_H 1.01, 0.67. The ^{13}C NMR showed 29 carbon signals with two olefin carbons appeared at δ_C 140.8, 121.7 and a carbinol carbon at δ_C 71.8. The ^{1}H and ^{13}C NMR data of compound **2** suggested the existence of a steroid frame. By analysis and comparison NMR data of **2** with those of *β*-sitosterol [12, 14], we concluded that compound **2** was *β*-sitosterol.

Compound **3** was isolated as white needles. The ^{1}H NMR and ^{13}C NMR spectra of compound **3** was similar to those of **2** with 29 carbon signals, characteristic resonances of a hydroxymethine group at δ_H 3.53 (1H; dt; J = 10.8, 4.8Hz; H-3)/ δ_C 71.8, four methyls appeared as doublets at δ_H 1.02, 1.01, 0.85, 0.81, 0.80 and two singlet methyls at 1.01, 0.70. However, the ^{1}H NMR spectrum of **3** showed signals of three olefinic protons at δ_H 5.35 (1H; ts; J = 3.6, 1.8Hz; H-6), 5.15 (1H; dd; J = 15.6, 9.0Hz; H-22) and 5.01 (1H; dd; J = 15.0, 8.4Hz; H-21) and the ^{13}C NMR revealed resonances of four alkene carbons appeared at δ_C 140.8, 138.3, 129.3, 121.7. Thus, the ^{1}H and ^{13}C NMR data of compound **3** suggested the structure of stigmasterol. By comparison the NMR data of 3 with previously reported data [12, 15], the structure of compound **3** was determined as stigmasterol.

4. CONCLUSION

Phytochemical investigation of a DCM extract from *C. polyanthum* stem bark led to the isolation of three compounds including a triterpenoid and two steroids. The structures of friedelin (**1**), *β*-sitosterol (**2**) and stigmasterol (**3**) were identified using NMR spectroscopy together with comparison with previously reported data. These compounds are proved to exhibit precious biological activities such as anti-inflammatory, anti-cancer, anti-oxidant, anti-diabetes,… [16-18] making them potential sources in pharmaceutical applications or synthetic precursors for new biologically active compounds. This is the first time the chemical constituents of *C. polyanthum* growing in Vietnam have been revealed. In additon, the high content of friedelin suggested *C. polyanthum* for a rich source of this biologically active compound.

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THÔNG TIN TÁC GIẢ

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