Chemical constituents of leaves *Dialium cochinchinense* Pierre

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Abstract

The genus *Dialium* belongs to the Caesalpinioideae family, consisting of approximately 30 species distributed in the tropical regions. Secondary metabolites from the *Dialium* genus have been reported to exhibit various biological activities including antioxidant, cytotoxicity and antimicrobial activities. This work describes the isolation and characterization of five compounds from the leaves of *Dialium cochinchinense* Pierre. Their structures were established by spectroscopic analysis, including MS and NMR spectra. Accordingly, the isolated compounds were identified to be lupeone (1), β-sitostenone (2), β-sitosterol (3), daucosterol (4), and dihydrokaempferide (5). To the best of our knowledge, this is the first report of the isolation of compounds 1 and 5 from the genus *Dialium*.

Keywords

*Dialium cochinchinense*, Caesalpinioideae, Phytochemistry, Terpenoids, Flavonoids

Introduction

*Dialium* is a genus of legume in the Caesalpinioideae family. The genus consists of approximately 30 species, which most occur in Africa (Junior et al., 2016) with some species in Asia (Schmidt & Nguyen, 2005). Previous phytochemical studies of the *Dialium* genus revealed the presence of steroids, terpenoids, polyphenols, and saponin (Awantu et al., 2011; Adeleye et al., 2014; Ayessou et al., 2014; Tuo et al., 2015; Ijoma & Ajiwe, 2017; Moronkola et al., 2017). Some bioactivities studies of this genus have reported cytotoxic (Awantu et al., 2011), antimicrobial (Orji et al., 2012; Ajiboye et al., 2015; Ijoma et al., 2016), antioxidant (Ogu et al., 2013), and anticancer activities (Prakash et al., 2013).

*Dialium cochinchinense* is a significant and well-recognized tree in Vietnam. It is distributed in many regions, but is especially predominant in the Kon Tum and Gia Lai provinces. In Vietnam, several parts of the plant have been used in folk medicine for the treatment of various diseases. The *D.cochininense* fruits are also
used as a supplemental food to improve appetite and digestion. Traditional practitioners use the leaves and fruits of *D. cochinchinense* to treat waterborne parasitic diseases, fever, and malaria. Our previous screening program on plants belonging to the *Dialium* genus in Vietnam found that the ethyl acetate extract of the leaves of *D. cochinchinense* (at a concentration of 1 μg/mL) showed a potent cytotoxic effect against KB cancer cell line. In this study, the isolation and identification of five compounds from the leaves of *D. cochinchinense* were presented. This study aimed to investigate the chemical composition of the leaves of *D. cochinchinense*. This is the first report on the phytochemical of the leaves of this plant.

**Materials and Methods**

**General procedures**

All chromatographic solvents were purchased from Sigma-Aldrich (Merck) or redistilled before use. The silica gel plates (Merck silica gel 60F254) and RP-18 modified silica gel coated with fluorescent indicator F254S plates were used for thin-layer chromatography (TLC). The spots were visualized first under UV light (254nm and 365nm) and by heating silica gel plates sprayed with cerium (IV) sulfate reagent. Column chromatography (CC) was carried out on silica gel (40-63μm, Kieselgel 60, Merck) and on sephadex LH-20 (Merck). A Model Thermo Scientific Mel-Tem 3.0 instrument was used for determining the melting points of isolated compounds. NMR spectra were recorded on a Bruker Avance 500 MHz spectrometer. ESI-MS was performed on a LTQ Orbitrap XL™ instrument. The chemical formulas were drawn using ChemBioOffice Ultra Version 14.0 software.

**Plant materials**

The leaves of *D. cochinchinense* Pierre were collected in Kon Chu Rang (Gia Lai, Vietnam) in 2005 and were identified by Dr. Nguyen Quoc Binh (Vietnam Academy of Science and Technology). The samples were washed, removed damaged leaves, dried at 40°C, powdered and vacuum sealed in plastic bags, then, stored in the sample store room.

**Extraction and Isolation**

The phytochemical tests were conducted utilizing standard procedures with some modifications as described by Ijoma et al. (Ijoma & Ajiwe, 2017). The leaves of *D. cochinchinense* are taken for phytochemical analysis to study the presence of steroids, terpenoids, anthraquinones, phenolic glycosides, saponins and polyphenols. Dried and grounded leaves of *D. cochinchinense* (1.0kg) were successively extracted with n-hexane, ethyl acetate, and methanol by maceration (4 times x 4L) for one day at 30°C and then ultrasonically for 30min. The n-hexane, ethyl acetate, and methanol solution were concentrated under reduced pressure to dry and to obtain the n-hexane (3.7g, 0.37%), ethyl acetate (16.4g, 1.64%) and MeOH (17.5g, 1.75%) crude extracts.

The ethyl acetate crude extract from the leaves (16 g) was chromatographed on a silica gel column (70 x 80cm) by gradient elution with n-hexane in the EtOAc system (1:0 to 0:1, each 500 mL) to obtain four fractions (EA1+EA4). Fraction EA1 (610mg) was subjected to column chromatographic separation over silica gel (2.5 x 60 cm) and eluted with n-hexane-EtOAc (1:0 to 0:1, 100mL) as the mobile phase to give ten subfractions (EA1.1+EA1.10). Fraction EA1.3 (35mg) was further purified on a silica gel column chromatography (1.0 x 40cm) using n-hexane-CH₂Cl₂ (100:1, 120mL) to concentrate compound 1 (5.3mg). Fraction EA2 (504mg) was chromatographed on a silica gel column using n-hexane-EtOAc (99:1 to 0:1, each 100mL) to provide four subfractions (EA2.1+EA2.4). Fraction EA2.2 (65mg) was then purified by a silica gel column using n-hexane-EtOAc (9/1, 150mL) to yield compounds 2 (3.0mg). Fraction EA4 (1.81g) was chromatographed on a silica gel column using a gradient solvent of n-hexane-EtOAc (10:1 to 0:10, each 150mL) as eluent, to yield seven fractions (EA4.1-EA4.7). Fraction EA4.3 (377mg) was further subjected to column chromatography (2.0 x 60cm) with n-hexane-EtOAc (9:1, 280mL) to give compound 3 (103mg).
The methanol crude extract (17.0 g) was chromatographed on a silica gel column and eluted with a gradient solvent system of n-hexane-CH₂Cl₂ and MeOH. A total of 150 fractions (20 mL each) were collected and then combined based upon similar TLC profiles to yield 10 main fractions (Me1-Me10). Fraction Me6 (553 mg) was further subjected to column chromatography (2.0 x 60 cm) and eluted with a CH₂Cl₂-EtOAc-MeOH (85:15:5, 550 mL) to give compound 4 (92.2 mg). Fraction Me9 (1.3 g) was subjected to column chromatographic separation (2.5 x 60 cm) over silica gel eluting with a CHCl₃-MeOH-formic acid system (5:1:0.1, 750 mL) to obtain eight subfractions (MeF9.1-MeF9.8). Fraction MeF9.8 was purified by column chromatographic separation over Sephadex LH-20 with MeOH/CH₂Cl₂ (9/1) as the mobile phase to yield compound 5 (2.0 mg).

The structures of compounds 1-5 were identified by comparison of their physical and spectra with the literature values, and also with standards by TLC.

**Lupeone (1):** C₃₀H₄₄O, colourless needles from n-hexan-CH₂Cl₂, mp 168-170°C. ¹H-NMR (500 MHz, CDCl₃, δ, ppm, J/Hz): 0.80 (3H, s, H-28), 0.93 (3H, s, H-25), 0.96 (3H, s, H-26), 1.23 (3H, s, H-27), 1.78 (6H, s, H-23 and H-24), 1.68 (3H, s, H-30), 4.57 (1H, d, J = 2.5, H-29b), 4.69 (1H, d, J = 2.5, H-29a). ¹³C-NMR (125 MHz, CDCl₃, δ, ppm): 14.5 (C-27), 15.1 (C-26), 15.9 (C-25), 18.0 (C-28), 19.3 (C-30), 19.7 (C-6), 21.0 (C-24), 21.5 (C-11), 25.2 (C-12), 26.8 (C-23), 27.5 (C-15), 29.9 (C-21), 33.6 (C-7), 34.2 (C-2), 35.5 (C-16), 36.9 (C-10), 38.2 (C-13), 39.6 (C-1), 39.9 (C-22), 40.8 (C-8), 42.9 (C-14), 43.0 (C-17), 47.3 (C-4), 47.9 (C-19), 48.3 (C-18), 49.8 (C-9), 54.9 (C-5), 109.4 (C-29), 150.9 (C-20), 218.2 (C-3).

**β-Sitostenone (2):** C₂₉H₄₄O, white solid, mp 93-94°C. ¹H-NMR (CDCl₃, 500 MHz, δH, ppm, J/Hz): 0.70 (3H, s, H-18), 0.81 (3H, d, J = 6.8, H-27), 0.83 (3H, d, J = 6.8, H-26), 0.84 (3H, t, J = 7.4, H-29), 0.91 (3H, d, J = 6.5, H-21), 1.17 (3H, s, H-19), 5.71 (1H, s, H-4). ¹³C-NMR (125 MHz, CDCl₃): δC 11.9 (C-29), 12.0 (C-18), 17.4 (C-19), 18.7 (C-21), 19.0 (C-27), 19.8 (C-26), 21.0 (C-11), 23.1 (C-28), 24.2 (C-15), 26.1 (C-23), 28.2 (C-16), 29.2 (C-25), 32.1 (C-7), 32.9 (C-6), 33.9 (C-2), 34.0 (C-22), 35.6 (C-1), 35.7 (C-8), 36.1 (C-20), 38.6 (C-10), 39.6 (C-12), 42.4 (C-13), 45.8 (C-24), 53.8 (C-9), 55.9 (C-14), 56.0 (C-17), 123.7 (C-4), 171.6 (C-5), 199.6 (C-3).

**β-sitosterol (3):** C₂₀H₃₆O₅ white powder, mp 140-142°C. ¹H-NMR (500 MHz, CDCl₃, δ, ppm, J/Hz): 0.68 (3H, s, H-18), 0.82 (3H, d, J = 7.0, H-26), 0.83 (3H, d, J = 7.0, H-27), 0.85 (3H, t, J = 7.0, H-29), 0.92 (3H, d, J = 7.0, H-21), 1.01 (3H, s, H-19), 3.52 (1H, m, H-3), 5.35 (1H, t, J = 3.0, H-6). ¹³C-NMR (125 MHz, CDCl₃, δ, ppm): 12.0 (C-29), 12.0 (C-18), 18.8 (C-21), 19.1 (C-26), 19.4 (C-27), 19.8 (C-19), 21.1 (C-11), 23.1 (C-28), 24.3 (C-15), 26.1 (C-23), 28.3 (C-16), 29.2 (C-25), 31.7 (C-2), 31.9 (C-7), 31.9 (C-8), 34.0 (C-22), 36.2 (C-20), 36.5 (C-10), 37.3 (C-1), 39.8 (C-12), 42.3 (C-4), 42.4 (C-13), 45.9 (C-24), 50.2 (C-9), 56.1 (C-17), 56.8 (C-14), 71.8 (C-3), 121.7 (C-6), 140.8 (C-5).

**Daucosterol (4):** C₃₅H₆₆O₆, white solid, mp 272-273°C. ¹H-NMR (500 MHz, DMSO-d₆, δH, J/Hz): 0.65 (3H, s, H-18), 0.79 (3H, t, J = 7.0, H-29), 0.82 (3H, s, H-26), 0.83 (3H, s, H-27), 0.90 (3H, d, J = 6.5, H-21), 0.95 (3H, s, H-19), 3.50 (1H, m, H-3), 5.35 (1H, brs H-6). ¹³C-NMR (125 MHz, DMSO-d₆): δC 11.7 (C-29), 11.8 (C-18), 18.6 (C-21), 19.1 (C-27), 19.7 (C-19), 19.8 (C-26), 20.6 (C-11), 22.6 (C-28), 23.9 (C-15), 25.5 (C-23), 27.8 (C-16), 28.7 (C-25), 29.3 (C-2), 31.4 (C-7), 31.4 (C-8), 33.4 (C-22), 35.5 (C-20), 36.2 (C-10), 36.8 (C-1), 38.2 (C-12), 41.9 (C-4), 39.9 (C-12), 41.9 (C-13), 45.1 (C-24), 49.6 (C-9), 55.4 (C-17), 56.2 (C-14), 61.1 (C-6′) 70.1-77.0 (C-2′-C-5′), 73.4 (C-3), 100.8 (C-1′), 121.2 (C-6), 140.5 (C-5).

**Dihydrokaempferide (5):** C₁₆H₁₂O₆, yellow amorphous powder. ¹H-NMR (500 MHz, CDCl₃, δ, ppm, J/Hz): 3.82 (3H, s, OCH₃-4′), 4.56 (1H, d, J = 11.5, H-3), 5.02 (1H, d, J = 12.0, H-2), 6.06 (1H, d, J = 2.0, H-6), 6.12 (1H, d, J = 2.0, H-8), 6.92 (2H, d, J = 9.0, H-3′ and H-5′), 7.42 (2H, d, J = 8.5, H-2′ and H-6′), 11.18 (1H, s, OH-5). This compound was compared to the standard by TLC.

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**Results and Discussion**

From the leaves of *D. cochinchinense*, five compounds (1-5) (Figure 1) were isolated and their structures were elucidated.

Compound 1 appeared as colorless needles from n-hexan-CH₂Cl₂. Its ESI-MS showed an ion peak at m/z 425 [M+H]⁺ corresponding to the molecular formula of C₃₀H₄₈O. The ¹H-NMR spectrum of 1 exhibited two doublet protons for two olefinic methylene protons at δ_H 4.69 (H-29a) and 4.57 (H-29b), representing the exocyclic double bond protons, and seven methyl groups at δ_H 1.68 (H-29), 1.08 (H-23 and H-24), 1.02 (H-27), 0.96 (H-26), 0.93 (H-25), and 0.80 (H-28). The ¹³C- and DEPT-NMR spectrum of 1 showed thirty carbon signals including seven methyl groups (δ_C 14.5, 15.1, 15.9, 18.0, 19.3, 21.0), five methine groups (δ_C 38.2, 47.9, 48.3, 49.8, 54.9), eleven methylene groups (δ_C 19.7, 21.5, 25.2, 27.5, 29.9, 33.6, 34.2, 35.5, 39.6, 39.9, 109.4), five high-field quaternary carbons (δ_C 36.9, 40.8, 42.9, 43.0, 47.3), one olefinic quaternary carbon (δ_C 150.9), and one carbonyl carbon (δ_C 218.2). The ¹³C-NMR spectrum of compound 1 showed a saturated carbonyl group at δ_C 218.2 and the alkene carbons at δ_C 150.4 and 108.8; suggesting the presence of a lupane triterpene having a carbonyl group in its structure. Our spectroscopic data was consistent with published ones (Wang et al., 2011). Therefore, compound 1 was determined to be lupeone.

Compound 2 appeared as a white solid. The ¹H-NMR spectrum of 2 showed a singlet olefinic proton at δ_H 5.74 (H-4). The ¹H-NMR spectrum also revealed singlet protons at δ_H 1.18 and 0.70 (each 3H, s) corresponding to two methyl groups at C-10 (H-19) and C-13 (H-18), respectively. Nine doublet protons at δ_H 0.91 (J = 6.5), 0.83 (J = 6.8) and 0.81 (J = 6.8) were demonstrated for methyl groups at C-20 (H₂-21), and C-25 (H₂-26 and H₂-27). Three triplet protons (J = 7.4) at δ_H 0.84 were observed for another methyl group at C-28 (H₃-29). The ¹³C-NMR spectrum of 2 showed the resonances of 29 carbons, including one carbonyl group at δ_C 199.6 (C-3) and one olefinic methine carbon at δ_C 123.7 (C-4). In addition, its DEPT-NMR showed signals of six methyl carbons (δ_C 11.9, 12.0, 17.4, 18.7, 19.0, 19.8), eleven methylene groups (δ_C 21.0, 23.1, 24.2, 26.1, 28.2, 32.1, 32.9, 33.9, 34.0, 35.6, 39.6), five methine groups (δ_C 29.2, 35.7, 36.1, 45.8, 53.8, 55.9, 56.0), and three quaternary carbons (δ_C 38.6, 42.4, 171.6). On this basis, the structure of compound 2 was determined to be β-sitostenone.

The identification of compound 2 was confirmed by the comparison of its ¹H- and ¹³C-NMR spectral data with the published values for β-sitostenone (Prachayasittikul et al., 2009).

Compound 3 was isolated as a white powder. The ¹H-NMR spectrum of 3 revealed one multiplet proton at δ_H 3.52 (H-3). The typical H-6 of the steroidal skeleton appeared as a multiplet at δ_H 5.35 for one proton. The ¹H-NMR spectrum also showed six methyl groups which were similar in the ¹H-NMR spectrum of compound 2. Moreover, the ¹³C- and DEPT-NMR spectra displayed twenty-nine carbon signals including: 6 sp³ methyls at δ_C 12.0, 12.0, 17.4, 18.7, 19.0, 19.8), 11 sp² methylens at δ_C 21.1, 23.1, 24.3, 26.1, 28.3, 31.7, 31.9, 34.0, 37.3, 39.8, 42.4), 9 sp² methines at δ_C 29.2, 31.9, 36.2, 45.9, 50.9, 56.1, 56.8, 71.3, 121.7), and 3 quaternary carbons (δ_C 36.5, 42.2, 140.8). All these properties confirmed that the structure of 3 was β-sitosterol (Mahato & Kundu, 1994).

Compound 4 was determined by comparing its spectral data with the spectral data of compound 3, and with previously reported values. The ¹H-NMR spectrum of compound 4 showed six signals at δ 0.65 (H-18), 0.79 (H-29), 0.82 (H-26), 0.83 (H-27), 0.90 (H-21), and 0.95 (H-19) for methyl groups (-CH₃). One proton at C-3 appeared as a multiplet at δ_H 3.50 ppm. A doublet proton at δ_H 5.35 ppm was the characteristics of a trisubstituted double bond between quaternary carbon (C-5) and methine carbon (C-6). The proton signals at δ_H 3.0-4.0 ppm indicated the presence of protons for the sugar moiety. ¹³C-NMR indicated that compound 4 consisted of 35 carbons. From DEPT spectra, the carbon signals of 4 additional six carbons in comparison to that of β-sitosterol (3) including
five oxygenated methine carbons and one oxygenated methylene carbon. These carbinol signals were assigned for the carbons of glucopyranosyl group. These data indicate the structure of β-sitosterol glucoside, in which a O-glucosyl group binding to C-3 of β-sitosterol. Above 1H-NMR and 13C-NMR data of 4 revealed it to be β-sitosterol-D-glucoside (daucosterol) which was confirmed by Rahmana et al. (2009).

Compound 5 was isolated as a yellow amorphous powder. Its 1H-NMR spectrum presented signals of six aromatic protons, including an A2B2 system [δH 6.92 (2H, d, J = 9.0, H-3’ and 5’), 7.42 (2H, d, J = 8.5, H-2’ and 6’)] and two doublets with meta-coupling at δH 6.06 (J = 2.0, H-6) and 6.12 (J = 2.0, H-8). The 1H-NMR spectrum also exhibited three protons for two doublet signals at δH 4.56 (J = 11.5, H-3), 5.02 (J = 12.0, H-2), one methoxy group at δH 3.82 (OCH3-4’) and one intramolecular chelated hydroxyl group at δH 11.84 (OH-5). The NMR data of 5 was characteristic of a flavonoid which is similar to dihydrokaempferide (Hattori et al., 2011).

Conclusions

The results demonstrated that the five compounds isolated belonged to three structure classifications, including one terpenoid, three steroids and one flavonoid. They were determined to be lupeone (1), β-sitostenone (2), β-sitosterol (3), daucosterol (4), and dihydrokaempferide (5). Other compounds are currently under identification. It is important to continue chemical research on these isolated compounds to evaluate their cytotoxic effects in an attempt to discover a new source of bioactive compounds from the flora in Vietnam.

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![Figure 1. Structures of the compounds isolated from the leaves of D. cochinchinense](https://vjas.vnua.edu.vn/1135)
Chemical constituents of leaves *Dialium cochinchinense* Pierre

References


