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CYTOTOXIC ACTIVITY OF XANTHONES FROM THE TWIGS AND LEAVES OF *CRATOXYLUM COCHINCHINENSIS* (LOUR.) BLUME

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Summary

This study aimed to isolate and identify bioactive compounds from the ethyl acetate extract of *Cratoxylum cochinchinensis* twigs and leaves. As a result, five xanthones were obtained and structurally characterized as 1,8-dihydroxy-3-methoxy-6-methylxanthone (**1**), cochinchinone A (**2**), 1,3,7-trihydroxy-2,4-diisoprenylxanthone (**3**), α-mangostin (**4**), and 1,3,5,6-tetrahydroxy-7-isoprenylxanthone (**5**). Notably, compounds **1** and **5** were isolated for the first time from *C. cochinchinensis*. Furthermore, the cytotoxic activity of these compounds was evaluated *in vitro* against three cancer cell lines (A549, MCF-7, and HepG2). Among them, compounds **2–4** exhibited cytotoxic activities against all tested cell lines, with IC_{50} values ranging from 10.24 to 16.86 µM. Meanwhile, compound **5** showed selective activity against MCF-7 and HepG2, with IC_{50} values of 13.55 ± 0.74 and 16.69 ± 0.81 µM, respectively.

Keywords: *Cratoxylum cochinchinensis; Xanthones; Cytotoxic activity.*

1. Introduction

Cratoxylum cochinchinensis (Lour.) Blume, also known as Thành ngạnh nam, belongs to the Hypericaceae family. In Vietnam, this plant is mainly found in Son La, Quang Ninh, Tuyen Quang, Thai Nguyen, Bac Giang, Phu Tho, Hoa Binh, and Hanoi. In Vietnamese traditional medicine, it has been used to treat urinary retention, gonorrhea, diarrhea, dizziness, and stomach pain, and to improve blood circulation, especially for postpartum women [1]. Chemical studies have identified about 161 compounds from this plant, including xanthones (127 compounds), terpenoids (13 compounds), flavonoids (11 compounds), anthranoids (5 compounds), essential oils, and other compounds [2]. Biological studies showed that its extracts and isolated compounds have significant effects against cancer and diabetes, along with anti-inflammatory, antioxidant, antibacterial, and antimicrobial properties [2].

As part of the National Scientific Program "Study on anticancer and immune-modulation of some Vietnamese medicinal plants" (2020-2024) under the Vietnam-Korea Science and Technology Cooperation Agreement, the 70% ethanol (EtOH) extract from the twigs and leaves of *C. cochinchinensis* has shown potential *in vitro* anticancer activity [3]. However, research on its anticancer effects is still limited. To further explore its potential, this study presents the isolation of five compounds from this plant and evaluates their cytotoxic activity against three human cancer cell lines (HepG2, A549, and MCF-7).

2. Material and methods

2.1. Plant materials

Twigs and leaves of *Cratoxylum cochinchinensis* (Lour.) Blume (Hypericaceae) were collected in March 2022, at Son Dong, Bac Giang Province. The scientific name was identified by MSc. Nguyen Quynh Nga and MSc. Phan Van Truong from the Medicinal Material Resources Center, NIMM. A voucher specimen (MT.03.06) has been deposited in the Herbarium of NIMM.

2.2. General experiment procedures

The melting point was recorded using the Melting Point M-560 instrument from BuChi Labortechnik AG (Flawil, Switzerland). Nuclear magnetic resonance (NMR) spectra were obtained using a Bruker AM 600 FT-NMR spectrometer (Karlsruhe, Germany) with TMS as the internal standard, and coupling constants (*J*) in Hz. Mass spectrometry (MS) data were analyzed using an LC-MS/MS system equipped with a DAD detector (Shimadzu, Japan). Column chromatography (CC) was performed with *silica gel* (40-63 µm, Merck), YMC gel (ODS-A 12 nm, S-75 µm, Japan), and MCI gel (75-150 µm, Japan). Thin-layer chromatography (TLC) was conducted on *silica gel* $60F_{254S}$ (0.25 mm, Merck) and RP-$18F_{254}$ (0.25 mm, Merck) plates. Spots were visualized under UV light at 254 and 366 nm or by spraying with 10% sulfuric acid in 96% EtOH followed by heating.

2.3. Extraction and isolation

Dried powdered *C. cochinchinense* twigs and

leaves (16.3 kg) were extracted with 70% EtOH at 65°C (three times, 3 hours each, 1:8 material/solvent ratio). After filtration, the combined extracts were concentrated under reduced pressure to obtain the 70% EtOH extract (1703 g). This extract was suspended in hot water and successively partitioned with *n*-hexane and ethyl acetate (EtOAc) (each three times, 1:1 ratio). The organic layers were evaporated under reduced pressure, yielding *n*-hexane extract (136.2 g), EtOAc extract (141.1 g), and aqueous residue (1202 g).

The EtOAc extract (141.2 g) was fractionated using *silica gel* CC and sequentially eluted with 100% *n*-hexane, *n*-hexane-EtOAc (9:1 - 1:2, v/v), EtOAc-MeOH (9:1, v/v), and 100% MeOH, yielding 15 fractions (E1-E15). Fraction E4 (3.0 g) was separated using *silica gel* CC with an *n*-hexane-acetone solvent system (15:1 - 6:1, v/v), affording 11 fractions (E4.1-E4.11). Fraction E4.2 (160 mg) was applied to RP-C_{18} CC with an acetone-water system (4:1, v/v), leading to 3 fractions (E4.2.1-E4.2.3). Fraction E4.2.1 (15 mg) was subjected to liquid-liquid extraction with EtOAc, affording compound **1** (7.0 mg). Fraction E4.9 (470 mg) was further processed using RP-C_{18} CC with an acetone-water system (3:1 - 9:1, v/v), obtaining 11 fractions (E4.9.1-E4.9.11). Purification of fraction E4.9.10 (29.3 mg) using *silica gel* CC with a DCM-MeOH system (70:1 - 20:1, v/v) gave compound **2** (12.1 mg). Fraction E4.10 (50 mg) was subjected to MCI CC with an acetone-water system (3:2, 2:1, 3:1, v/v), producing 2 fractions (E4.10.1-E4.10.2), and subsequent purification of fraction E4.10.1 (12 mg) via *silica gel* CC with a DCM-MeOH system (40:1, v/v) obtained compound **3** (5.2 mg). Fractionation of E7 (4.3 g) on *silica gel* CC with an *n*-hexane-acetone system (15:1 - 5:1, v/v) resulted in nine fractions (E7.1-E7.9). Fraction E7.4 (1.0 g) was isolated using an MCI CC with an acetone-water system (2:1 – 3:1, v/v), affording compound **4** (5.8 mg). A similar purification method was applied to fraction E9 (4.0 g), which was separated using MCI CC with an acetone-water system (1:3 - 5:4, v/v), affording 15 fractions (E9.1-E9.15). Fraction E9.11 (61.8 mg) was purified using *silica gel* CC with a DCM-MeOH system (50:1, v/v), leading to the isolation of compound **5** (8.0 mg).

Compound **1**: Yellow powder; ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H (ppm): 6.69 (1H, d, J = 2.4 Hz, H-2), 7.37 (1H, d, J = 2.4 Hz, H-4), 7.63 (1H, d, J = 1.2 Hz, H-5), 7.09 (1H, d, J = 0.6 Hz, H-7), 3.94 (3H, s, 3-OCH_3), 2.45 (3H, s, 6-CH_3), 12.31 (1H, s, 1-OH), 12.11 (1H, s, 8-OH); ^{13}C-NMR (150 MHz, $CDCl_3$) δ_C (ppm): 165.5 (C-1), 107.0 (C-2), 166.8 (C-3), 108.5 (C-4), 121.5 (C-5), 148.7 (C-6), 124.8 (C-7), 162.5 (C-8), 114.0 (C-8a), 182.3 (C-9), 110.6 (C-9a), 56.3 (3-OCH_3), 22.4 (6-CH_3).

Compound **2**: Amorphous yellow powder; ESI-MS: m/z = 379.30 $[M-H]^-$; ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H (ppm): 7.36 (1H, d, J = 9.0 Hz, H-5), 7.25 (1H, dd, J = 3.0, 9.0 Hz, H-6), 7.61 (1H, d, J = 3.0 Hz, H-8), 3.47 (2H, d, J = 6.6 Hz, H-1′), 5.27 (1H, m, H-2′), 1.77 (3H, s, H-4′), 1.85 (3H, s, H-5′), 3.54 (2H, d, J = 6.6 Hz, H-1″), 5.27 (1H, m, H-2″), 1.74 (3H, s, H-4″), 1.88 (3H, s, H-5″), 13.12 (1H, s, 1-OH), 6.45 (1H, s, 7-OH); ^{13}C-NMR (150 MHz, $CDCl_3$) δ_C (ppm): 158.3 (C-1), 108.9 (C-2), 161.0 (C-3), 105.3 (C-4), 153.1 (C-4a), 150.6 (C-4b), 119.0 (C-5), 123.9 (C-6), 152.1 (C-7), 109.2 (C-8), 120.8 (C-8a), 180.9 (C-9), 103.3 (C-9a), 21.6 (C-1′), 121.8 (C-2′), 135.4 (C-3′), 25.8 (C-4′), 18.0 (C-5′), 21.9 (C-1″), 121.5 (C-2″), 133.8 (C-3″), 25.9 (C-4″), 18.0 (C-5″).

Compound **3**: Yellow powder; ESI-MS: m/z = 447.35 $[M-H]^-$; ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H (ppm): 7.27 (1H, d, J = 8.4 Hz, H-5), 7.22 (1H, dd, J = 1.8, 8.4 Hz, H-6), 7.60 (1H, s, H-8), 3.44 (2H, d, J = 6.0 Hz, H-1′), 5.27 (1H, m, H-2′), 1.84 (3H, s, H-4′), 1.76 (3H, s, H-5′), 3.53 (1H, d, J = 6.0 Hz, H-1″), 5.27 (1H, m, H-2″), 2.05 (2H, m, H-4″), 2.09 (2H, m, H-5″), 5.05 (1H, br t, J = 6.6 Hz, H-6″), 1.57 (3H, s, H-8″), 1.87 (3H, s, H-9″), 1.63 (3H, s, H-10″), 13.01 (1H, s, 1-OH); ^{13}C-NMR (150 MHz, $CDCl_3$) δ_C (ppm): 158.3 (C-1), 109.1 (C-2), 161.1 (C-3), 105.1 (C-4), 153.0 (C-4a), 150.4 (C-4b), 118.9 (C-5), 124.1 (C-6), 152.4 (C-7), 109.0 (C-8), 120.6 (C-8a), 181.0 (C-9), 103.2 (C-9a), 21.7 (C-1′), 121.6 (C-2′), 134.9 (C-3′), 17.9 (C-4′), 25.9 (C-5′), 21.8 (C-1″), 121.6 (C-2″), 137.9 (C-3″), 39.7 (C-4″), 26.5 (C-5″), 123.9 (C-6″), 131.8 (C-7″), 17.7 (C-8″), 16.3 (C-9″), 25.7 (C-10″).

Compound **4**: Yellow needle-shaped crystals; ESI-MS: m/z = 409.25 $[M-H]^-$; ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H (ppm): 6.27 (1H, s, H-4), 6.78 (1H, s, H-5), 3.43 (2H, d, J = 6.6 Hz, H-1′), 5.27 (1H, m, H-2′), 1.83 (3H, s, H-4′), 1.83 (3H, s, H-5′), 4.08 (2H, d, J = 6.6 Hz, H-1″), 5.27 (1H, m, H-2″), 1.75 (3H, s, H-4″), 1.69 (3H, s, H-5″), 3.80 (3H, s, 7-OCH_3), 13.75 (1H, s, 1-OH); ^{13}C-NMR (150 MHz, $CDCl_3$) δ_C (ppm): 160.6 (C-1), 108.8 (C-2), 161.6 (C-3), 93.2 (C-4), 154.6 (C-4a), 155.0 (C-4b), 101.6 (C-5), 155.7 (C-6), 142.6 (C-7), 137.1 (C-8), 112.1 (C-8a), 182.0 (C-9), 103.6 (C-9a), 21.5 (C-1′), 121.6 (C-2′), 135.2 (C-3′), 18.2 (C-4′), 25.8 (C-5′), 26.5 (C-1″), 123.2 (C-2″), 132.1 (C-3″), 17.9 (C-4″), 25.8 (C-5″), 62.0 (7-OCH_3).

Compound **5**: Yellow powder; ESI-MS: m/z = 327.15 $[M-H]^-$; ^{1}H-NMR (600 MHz, CD_3OD) δ_H (ppm): 6.41 (1H, s, H-2), 6.14 (1H, s, H-4), 7.43 (1H, s, H-8), 3.39 (2H, d, J = 7.2 Hz, H-1′), 5.39 (1H, t, J = 7.2 Hz, H-2′), 1.78 (3H, s, H-4′), 1.75 (3H, s, H-5′); ^{13}C-NMR (150 MHz, CD_3OD) δ_C (ppm): 164.6 (C-1), 95.4 (C-2), 167.7 (C-3), 99.2 (C-4), 159.3 (C-4a), 146.2 (C-4b), 159.3 (C-5), 152.8 (C-6), 127.3 (C-7), 116.2 (C-8), 133.3 (C-8a), 181.6 (C-9), 103.0 (C-9a), 29.1 (C-1′), 123.2 (C-2′), 133.8 (C-3′), 26.0

(C-4′), 17.8 (C-5′).

2.4. In vitro cytotoxic activity assay

Three human cancer cell lines, including HepG2 (human hepatocellular carcinoma), A549 (human lung carcinoma), and MCF-7 (human breast carcinoma), were used to evaluate cytotoxic activity. These cell lines were cultured following the standard guidelines of the American Type Culture Collection. The cytotoxic activities of the compounds on the cancer cells were assessed using the sulforhodamine B (SRB) assay, following a previously established protocol [4]. This assay measures cell density by quantifying total cellular protein stained with SRB. Briefly, trypsinized cells were seeded in 96-well plates and treated with the test samples for 72 hours. Negative control wells contained cells exposed to a diluted solution. After incubation, cells were fixed with trichloroacetic acid (TCA) for 1 hour and then stained with SRB for 30 minutes at 37°C. Excess dye was removed by washing the plates three times with acetic acid before air drying at room temperature. The bound SRB-stained protein was dissolved in 10 mM unbuffered Tris base, and the plate was gently shaken for 10 minutes at room temperature. Optical density (OD) at 540 nm was recorded using an ELISA Plate Reader (BioTek, Winooski, VT, USA). Ellipticine served as a positive control. The percentage of cell growth inhibition was calculated using the following formula: (%) inhibition = 100% - $[(OD_{sample} - OD_{day\ 0})/ (OD_{DMSO} - OD_{day\ 0})] \times 100$.

2.5. Data analysis

The experimental data were processed and recorded. Each experiment was performed three times, and the results were reported as the mean ± standard deviation (SD) using Microsoft Excel 2023. The IC_{50} values were calculated with TableCurve 2Dv4 software.

3. Results and discussion

3.1. Structural determination of isolated compounds

From the EtOAc extract, five compounds (**1**–**5**) were isolated and structurally determined (**Fig. 1**).

Compound **1** was isolated as a yellow powder. The ^{1}H-NMR spectrum of **1** showed signals for two pairs of *meta*-substituted aromatic protons [δ_H 7.63 (1H, d, J = 1.2 Hz, H-5), 7.09 (1H, d, J = 0.6 Hz, H-7) and 7.37 (1H, d, J = 2.4 Hz, H-4), 6.69 (1H, d, J = 2.4 Hz, H-2)], two hydroxyl protons [δ_H 12.31 (1H, s, 1-OH) and 12.11 (1H, s, 8-OH)], one methoxy group [δ_H 3.94 (3H, s, 3-OCH_3)], and one methyl group [δ_H 2.45 (3H, s, 6-CH_3)]. The ^{13}C NMR spectrum revealed signals for 15 carbons, including one carbonyl carbon [δ_C 182.3 (C-9)], one methoxy carbon (δ_C 56.3), one methyl carbon (δ_C 22.4), and 12 aromatic carbons. These NMR data suggested that **1** belonged to the xanthone skeleton. The HMBC spectrum confirmed the positions of key functional groups. The hydroxyl protons at δ_H 12.31 and 12.11 showed correlations with carbons at δ_C 107.0 (C-2), 110.6 (C-9a), 165.5 (C-1) and δ_C 124.8 (C-7), 114.0 (C-8a), 162.5 (C-8), respectively, confirming their placement at C-1 and C-8. The methoxy proton at δ_H 3.94 correlated with δ_C 166.8 (C-3), and the methyl proton at δ_H 2.45 correlated with δ_C 124.8 (C-7), 148.7 (C-6), and 121.5 (C-5), confirming their positions at C-3 and C-6, respectively (**Fig. 2**). Based on these spectral data and comparison with the literature [5], compound **1** was identified as 1,8-dihydroxy-3-methoxy-6-methylxanthone.

Compound **2** was obtained as an amorphous yellow powder. The ESI-MS spectrum of **2** displayed a deprotonated molecular ion peak at *m/z* 379.30 $[M-H]^-$, consistent with the molecular formula $C_{23}H_{24}O_5$. The ^{1}H-NMR spectrum showed signals for three aromatic protons in an ABX spin system at δ_H 7.25 (1H, dd, J = 3.0, 9.0 Hz, H-6), 7.36 (1H, d, J = 9.0 Hz, H-5), and 7.61 (1H, d, J = 3.0 Hz, H-8). Characteristic signals of two isoprenyl groups were observed at δ_H 1.74 (3H, s), 1.77 (3H, s), 1.85 (3H, s), 1.88 (3H, s), 3.47 (2H, d, J = 6.6 Hz), 3.54 (2H, d, J = 6.6 Hz), and 5.27 (2H, m). The ^{13}C NMR spectrum revealed 23 carbon signals, including one carbonyl carbon (δ_C 180.9, C-9), four methyl groups [δ_C 18.0 (C-5′ and C-5″), 25.8 (C-4′), and 25.9 (C-4″)], four olefinic carbons [δ_C 121.5 (C-2″), 121.8 (C-2′), 135.4 (C-3′), and 133.8 (C-3″)], two methylene groups [δ_C 21.6 (C-1′), 21.9 (C-1″)], and 12 aromatic carbons. The HMBC correlations between H-1′ and C-1/C-2/C-3/C-2′/C-3′ and between H-4′/H-5′ and C-2′/C-3′ confirmed the presence and position of an isoprenyl group at C-2. Meanwhile, correlations between H-1″ and C-3/C-4/C-4a/C-2″/C-3″, as well as between H-4″/H-5″ and C-2″/C-3″, also indicated the presence and position of another isoprenyl group at C-4 (**Fig. 1**). Based on these spectral data and comparison with the literature [6], compound **2** was determined to be 1,3,7-trihydroxy-2,4-diisoprenylxanthone.

Compound **3** was obtained as a yellow powder. Its ESI-MS spectrum displayed a deprotonated molecular ion peak at *m/z* 447.35 $[M-H]^-$, consistent with the molecular formula $C_{28}H_{32}O_5$. The ^{1}H-NMR spectrum showed signals corresponding to three aromatic protons in an ABX system at δ_H 7.60 (1H, s, H-8), 7.27 (1H, dd, J = 8.4 Hz, H-5), and 7.22 (1H, dd, J = 8.4, 1.8 Hz, H-6). Characteristic signals of an isoprenyl group were observed at δ_H 5.27 (1H, m), 3.44 (2H, d, J = 6.0 Hz), 1.84 (3H, s), and 1.76 (3H, s). Additionally, the presence of a geranyl group was confirmed by signals at δ_H 5.27 (1H, m), 5.05 (1H, br t, J = 6.6 Hz), 3.53 (1H, d, J = 6.0 Hz), 2.05 (2H, m), 2.09 (2H, m), 1.87 (3H, s), 1.63 (3H, s), and 1.57 (3H, s). The ^{13}C-NMR spectrum revealed 28 carbon signals, including a carbonyl group at δ_C 181.0 (C-

9), five methyl groups [δ_C 17.9 (C-4′), 25.9 (C-5′), 17.7 (C-8″), 16.3 (C-9″), and 25.7 (C-10″)], six olefinic carbons [δ_C 121.6 (C-2′), 134.9 (C-3′), 121.6 (C-2″), 137.9 (C-3″), 123.9 (C-6″), and 131.8 (C-7″)], four methylene groups [δ_C 21.7 (C-1′), 21.8 (C-1″), 39.7 (C-4″), and 26.5 (C-5″)], and 12 aromatic carbons. The HMBC correlations between H-1′ and C-1/C-2/C-3/C-2′/C-3′ and between H-4′/H-5′ and C-2′/C-3′ confirmed the position of the isoprenyl group at C-2. Meanwhile, correlations between H-1″ and C-3/C-4/C-4a/C-2″/C-3″, H-9″ and C-3″, H-4″ and C-2″/C-6″, H-5″ and C-3″/C-6″, as well as H-8″/H-10″ and C-6″/C-7″ indicated the geranyl group at C-4 (**Fig. 1**). The chemical structure of **3** was similar to **2**, except that the isoprenyl group at C-4 in **2** was replaced by a geranyl group in **3**. Comparison with the literature [7] and analysis of the spectral data confirmed that compound **3** was cochinchinon A.

Compound **4** was purified as yellow needle-shaped crystals with melting point of 181.2°C. Its ESI-MS spectrum exhibited a deprotonated molecular ion peak at *m/z* 409.25 $[M-H]^-$, corresponding to the molecular formula $C_{24}H_{26}O_6$. The ^{1}H-NMR spectrum exhibited two singlet aromatic proton signals at δ_H 6.27 (1H, s, H-4) and 6.78 (1H, s, H-5), indicating the presence of two benzene rings. Additionally, characteristic signals of four methyl groups [δ_H 1.83 (6H, s, H-4′, H-5′), 1.75 (3H, s, H-4″), and 1.69 (3H, s, H-5″)], two methylene groups [δ_H 3.43 (2H, d, J = 6.6 Hz, H-1′) and 4.08 (2H, d, J = 6.6 Hz, H-1″)], and two olefinic protons [δ_H 5.27 (2H, m, H-2′, H-2″)] were observed. These signals confirmed the presence of two isoprenyl groups in the structure of compound **4**. The ^{13}C-NMR spectrum revealed 24 carbon signals, including a carbonyl group [δ_C 182.0 (C-9)], four methyl groups [δ_C 25.8 (C-5′ and C-5″), 18.2 (C-4′), and 17.9 (C-4″)], four olefinic carbons [δ_C 123.2 (C-2″), 121.6 (C-2′), 135.2 (C-3′), and 132.1 (C-3″)], a methoxy group [δ_C 62.0 (7-OCH_3)], two methylene groups [δ_C 21.5 (C-1′) and 26.5 (C-1″)], and 12 aromatic carbons. The HMBC correlations between H-1′ and C-1/C-2/C-3/C-2′/C-3′, as well as between H-4′/H-5′ and C-2′/C-3′, confirmed the position of an isoprenyl group at C-2. Meanwhile, correlations between H-1″ and C-7/C-8/C-8a/C-2″/C-3″, along with those between H-4″/H-5″ and C-2″/C-3″, established the second isoprenyl group at C-8. The methoxy group was assigned to C-7 based on the HMBC correlation between the proton at δ_H 3.80 (3H, s) and the carbon at δ_C 142.6 (C-7) (**Fig. 1**). Based on these spectral data and comparison with the literature [8], compound **4** was identified as *α*-mangostin.

Compound **5** was isolated as a yellow powder. Its ESI-MS spectrum revealed a deprotonated molecular ion peak at *m/z* 327.15 $[M-H]^-$, which corresponds to the molecular formula $C_{18}H_{16}O_6$. The ^{1}H-NMR spectrum displayed three aromatic proton signals at δ_H 6.41 (1H, s, H-2), 6.14 (1H, s, H-4), and 7.43 (1H, s, H-8). Characteristic signals of an isoprenyl group were observed at δ_H 1.75 (3H, s), 1.78 (3H, s), 3.39 (2H, d, J = 7.2 Hz), and 5.39 (1H, t, J = 7.2 Hz). The ^{13}C-NMR spectrum revealed 18 carbon signals, including a carbonyl group [δ_C 181.6 (C-9)], two methyl groups [δ_C 17.8 (C-5′) and 26.0 (C-4′)], two olefinic carbons [δ_C 123.2 (C-2′) and 133.8 (C-3′)], a methylene group [δ_C 29.1 (C-1′)], and 12 aromatic carbons. The HMBC correlations between H-1′ and C-6/C-7/C-8/C-2′/C-3′, along with those between H-4′/H-5′ and C-2′/C-3′, confirmed the location of the isoprenyl group at C-7. Additionally, the correlation between the proton at δ_H 7.43 (1H, s) and C-4b/C-6/C-8a/C-9/C-1′ confirmed its position at C-8. The HMBC correlation between the proton at δ_H 6.41 (1H, s) and C-1/C-3/C-4/C-9a established its location at C-2 (**Fig. 1**). Structurally, compound **5** closely resembled cochinchinone F, except for the position of the isoprenyl and hydroxyl groups. Specifically, **5** featured an isoprenyl group at C-7 and a hydroxyl group at C-5, whereas in cochinchinone F, these groups were interchanged. Compound **5** was identified for the first time in *Garcinia xipshuanbannaensis* using LC-MS analysis [9]. Based on these spectral data, **5** was elucidated as 1,3,5,6-tetrahydroxy-7-isoprenylxanthone.

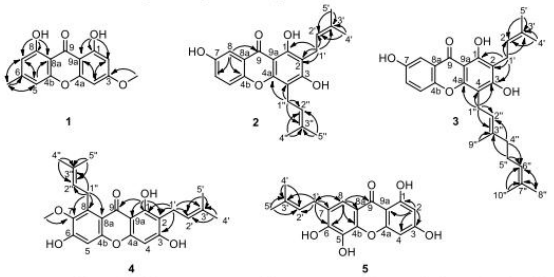

Fig. 1. Chemical structure and key HMBC correlations (→) of isolated compounds **1**–**5** from *C. cochinchinensis*

3.2. Cytotoxic activity of isolated compounds

Five xanthones isolated from *C. cochinchinensis* were evaluated for their *in vitro* cytotoxic activity against A549, MCF-7, and MDA-MB-231 cancer cell lines. The results are shown in Table 1.

Table 1. Cytotoxic activity of isolated compounds from *C. cochinchinensis*

Compounds	IC_{50} (μM)		
	HepG2	A549	MCF-7
1	>20	>20	>20
2	10.29 ± 0.38	13.54 ± 0.43	10.67 ± 0.43
3	12.14 ± 0.32	16.86 ± 0.47	10.24 ± 0.47
4	11.85 ± 0.39	13.71 ± 0.44	13.09 ± 0.49
5	16.69 ± 0.81	>20	13.55 ± 0.74
Ellipticine	1.46 ± 0.12	1.62 ± 0.12	1.38 ± 0.08

Among the tested compounds, compounds **2**–**4**

displayed moderate cytotoxic activity against the tested cell lines, with IC_{50} values ranging from 10.24 ± 0.47 to 16.86 ± 0.47 μM. Specifically, compound **2** exhibited the strongest activity against HepG2 and MCF-7 cells (IC_{50} = 10.29 ± 0.38 and 10.67 ± 0.43 μM, respectively), while its effect on A549 was slightly weaker (IC_{50} = 13.54 ± 0.43 μM). Compound **3** exhibited moderate cytotoxic activity across all tested cell lines, with its most potent effect observed against MCF-7 (IC_{50} = 10.24 ± 0.47 μM), followed by HepG2 and A549. Compound **4** displayed IC_{50} values of 11.85 ± 0.39 μM (HepG2), 13.71 ± 0.44 μM (A549), and 13.09 ± 0.49 μM (MCF-7), suggesting moderate and similar cytotoxic effects across all three cell lines. Besides, compound **5** also exhibited moderate activity, with an IC_{50} of 16.69 ± 0.81 μM against HepG2, 13.55 ± 0.74 μM against MCF-7, and >20 μM against A549. However, compound **1** did not exhibit significant cytotoxic activity against any of the three cancer cell lines (IC_{50} >20 μM). These findings suggest that compounds **2**–**4** may be promising candidates for further development as anticancer agents. However, since these compounds were only tested on cancer cell lines, further evaluation on normal cell lines is necessary to assess their selectivity and potential applicability in cancer treatment.

Previous studies on *C. cochinchinensis* have shown that xanthones are its main constituents. In this study, we also isolated five xanthone compounds. Among these compounds, 1,8-dihydroxy-3-methoxy-6-methylxanthone (**1**) and 1,3,5,6-tetrahydroxy-7-isoprenylxanthone (**5**) were identified for the first time in *C. cochinchinensis*. To date, investigations into their biological activities remain limited. Meanwhile, cochinchinon A (**2**), 1,3,7-trihydroxy-2,4-diisoprenylxanthone (**3**), and *α*-mangostin (**4**) have also been identified in this plant [10], [11]. Recent studies have shown that these compounds exhibited cytotoxic activity against various cancer cell lines, including NCI-H187 (small cell lung carcinoma) [10], HL-60 (promyelocytic leukemia), PC-3 (prostate adenocarcinoma), and MDA-MB-231 (human breast adenocarcinoma) [12], with varying levels of inhibition. These results showed that xanthones from *C. cochinchinensis* could be useful for further studies, especially on their anticancer effects.

4. Conclusion

Using combined chromatographic methods, five compounds were isolated from the EtOAc fraction. Their structures were identified based on NMR spectral data and comparison with references, including 1,8-dihydroxy-3-methoxy-6-methylxanthone (**1**), cochinchinone A (**2**), 1,3,7-trihydroxy-2,4-diisoprenylxanthone (**3**), *α*-mangostin (**4**), and 1,3,5,6-tetrahydroxy-7-isoprenylxanthone (**5**). Among them, compounds **1** and **5** were reported for the first time from this species. Additionally, compounds **2–4** exhibited cytotoxic activity against all three tested cancer cell lines, with IC_{50} values ranging from 10.24 to 16.86 μM. Meanwhile, compound **5** demonstrated cytotoxic effects on two cancer cell lines, MCF-7 and HepG2, with IC_{50} values of 13.55 ± 0.74 and 16.69 ± 0.81 μM, respectively.

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