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Xanthones and Coumarins from the Twigs of *Mesua ferrea* L.

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Abstract

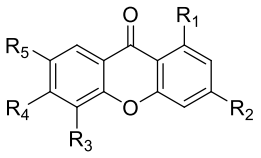
Five xanthones **1–5** and four phenylcoumarins **6–9** were isolated from acetone extracts of twigs of *Mesua ferrea* L. The structures of these compounds were elucidated by spectroscopic analysis and comparison with reported data such as 1D and 2D NMR, UV and IR spectroscopy. Compounds **4–7** were isolated from this plant for the first time. Compound **3**, 1,5,6-trihydroxyxanthone, showed significant antioxidant activities with IC_{50} values of free radical scavenging in DPPH and ABTS assays of 45.0 and 197.0 µg/mL, respectively, as compared to the control, trolox, (77.6±1.0 µg/mL and 282.9±1.2 µg/mL for DPPH and ABTS assay, respectively). Besides, mammea A/AA cyclo F, compound **9**, formed in a single crystal and was reported.

Keywords: *Mesua ferrea* L., Calophyllaceae, xanthones, phenylcoumarins

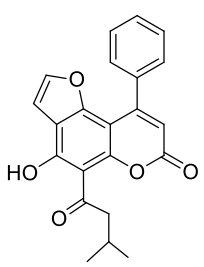
1. Introduction

Mesua ferrea L., is known locally as "bunnak", belongs to the Calophyllaceae family and is distributed throughout Southeast Asia, India and Sri Lanka. In India, aerial parts of this plant have been used in traditional medicine to treat various diseases. A decoction of the flowers with sugar candy is used to stop bloody stool (1). The essential oil of fruits and flowers was used to treat rheumatism and cure skin diseases (2). The dried flowers exhibit stomachic properties and anti-inflammatory (3). The seed oil is a safe pharmaceutical excipient and could be used in medicinal formulations (4). *M. ferrea* is used as an anticancer, an antimicrobial, an antipyretic, a cardiotonic, a carminative, a diuretic and an expectorant agent (5, 6). In Thailand, the bark is traditionally used for the treatment of cough, dysentery, and vomiting (7), while the seeds are used as an aromatic, an expectorant, and a wound healer (8).

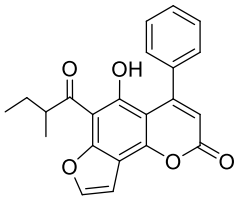
We previously reported the isolation of several xanthones from roots of *M. ferrea* and some of those compounds had cytotoxic properties (9). In this study nine known compounds, including five xanthones **1–5** and four phenylcoumarins **6–9**, are isolated and characterised from twigs of *M. ferrea* (Figure 1), identified as 5-hydroxy-1,6-dimethoxyxanthone **1** (9), 5-hydroxy-1,3,6,7-tetramethoxyxanthone **2** (10), 1,5,6-trihydroxyxanthone **3** (11), 1,3,7-trihydroxyxanthone **4**. 1,7-dihydroxy-3-methoxy xanthone **5** (12), isodisparfuran A **6**, disparfuran B **7** (13), mammea A/AA cyclo D **8** (14) and mammea A/AA cyclo F **9** (15, 16). Although these compounds are known compounds, to the best of our knowledge no NMR data have been published for compounds **1** and **2**. Therefore, the present report presents the complete assignment of ^{1}H and ^{13}C-NMR spectral data of compounds **1** and **2** along with the single-crystal X-ray diffraction analysis of compound **9**. Also, compounds **4–7** are isolated from this plant and reported for the first time. Compounds **3–5** and **9** are evaluated for their antioxidant properties based on the DPPH and ABTS radical scavenging activities and antibacterial activity.



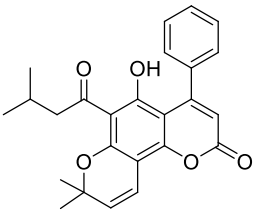
1: 5-hydroxy-1,6-dimethoxyxanthone, R_1 = OCH_3, R_2 = H, R_3 = OH, R_4 = OCH_3, R_5 = H
2: 5-hydroxy-1,3,6,7-tetramethoxyxanthone, R_1 = OCH_3, R_2 = OCH_3, R_3 = OH, R_4 = OCH_3, R_5 = OCH_3
3: 1,5,6-trihydroxyxanthone, R_1 = OH, R_2 = H, R_3 = OH, R_4 = OH, R_5 = H
4: 1,3,7-trihydroxyxanthone, R_1 = OH, R_2 = OH, R_3 = H, R_4 = H, R_5 = OH
5: 1,7-dihydroxy-3-methoxyxanthone, R_1 = OH, R_2 = OCH_3, R_3 = H, R_4 = H, R_5 = OH



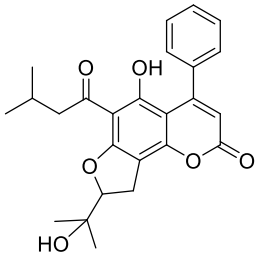
6: isodisparfuran A



7: disparfuran B



8: mammea A/AA cyclo D



9: mammea A/AA cyclo F

Figure 1 Structures of compounds **1–9**

2. Materials and Experiment

2.1 Plant material

The twigs of *Mesua ferrea* L. were collected from Trang Province, Thailand, in April 2017 and the identification was made by Mr. Sukid Ruangrua, the Forest Herbarium (BKF). A voucher specimen (BKF NO. 194350) has been deposited at the Forest Herbarium (BKF), Chatuchak, Bangkok.

2.2 General experimental procedures

The infrared (IR) spectra were recorded on a PerkinElmer 783 FTS 165 FT-IR spectrometer. Ultraviolet (UV) absorption spectra were measured in MeOH on a UV-160A spectrophotometer (Shimadzu). The ^{1}H and ^{13}C NMR spectra were recorded on 300 MHz and 500 MHz Bruker FTNMR Ultra Shield spectrometer with tetramethylsilane (TMS) as an internal standard. Quick column chromatography (QCC) was carried out on silica gel 60 GF_{254} (Merck). Column chromatography (CC) was performed using silica gel (Merck) type 100 (70—230 mesh ASTM). Thin–layer chromatography (TLC) and precoated TLC (PTLC) were carried out on silica gel 60 F_{254} (Merck).

2.3 Extraction and isolation of compounds

The acetone extract was extracted for one week from 8.3 kg of the twigs with acetone at room temperature. A brownish crude acetone extract was furnished at a mass of 43.0 g which was subjected to QCC using a hexane eluent. Polarity was increased with acetone and MeOH .The eluates were combined based on TLC characteristics to afford 13 fractions (F1–F13). Fraction F5 was purified by CC with acetone–hexane (1.5:4.0, v/v) to yield **4** (12.5 mg) and **7** (2.1 mg). Fraction F6 gave **5** (3.1 mg). Compounds **8** (11.5 mg) and **9** (10.5 mg) were isolated from fraction F10 (615.0 mg). Fractions F11 (6.4 g) was purified by QCC with a gradient of hexane-CH_2Cl_2-EtOAc to yield **3** (2.1 mg) and **6** (1.3 mg). Compounds **1** (4.3 mg) and **2** (1.5 mg) were isolated from fractions F13 (5.6 mg) and F12 (145.5 mg), respectively.

2.3.1 5-hydroxy-1,6-dimethoxyxanthone (1)

Pale yellow solid, UV λ_{max} (MeOH) (log ε): 240 (3.95), 263 (3.42) and 344 (4.17); IR (neat) υ_{max}: 3280, 1650 and 1575 cm^{-1}. ^{1}H NMR (acetone-d_6, 300 MHz) δ: 3.94 (3H, s, 1-OCH_3), 3.98 (3H, s, 6-OCH_3), 6.94 (1H, d, J = 8.0 Hz, H-2), 7.10 (1H, d, J = 8.7 Hz, H-7), 7.11 (1H, d, J = 8.0 Hz, H-4), 7.67 (1H, d, J = 8.7 Hz, H-8), 7.67 (1H, t, J = 8.0 Hz, H-3). ^{13}C NMR (acetone-d_6, 75 MHz) δ: 55.6 (1-OCH_3), 55.9 (6-OCH_3), 106.0 (C-2), 108.2 (C-7), 109.7 (C-4), 112.0 (C-9a), 116.4 (C-8), 117.9 (C-8a), 134.0 (C-5), 134.6 (C-3), 144.3 (C-10a), 151.6 (C-6), 158.2 (C-4a), 160.9 (C-1), 174.3 (C-9).

2.3.2 5-hydroxy-1,3,6,7-tetramethoxyxanthone (2)

Pale yellow solid; UV λ_{max} (MeOH) (log ε): 215 (3.61), 263(3.03) 317 (3.17) and 382 (3.82); IR (neat) υ_{max}: 3125, 1623 and 1578cm^{-1}. ^{1}H NMR (CD_3OD, 500 MHz) δ: 3.91 (3H, s, 3-OCH_3), 3.92 (3H, s, 1-OCH_3), 3.93 (3H, s, 6-OCH_3), 3.94 (3H, s, 7-OCH_3), 6.48 (1H, d, J = 2.0 Hz, H-2), 6.74 (1H, d, J = 2.0 Hz, H-4), 7.13 (1H, s, H-8). ^{13}C NMR (CD_3OD, 125 MHz) δ: 55.0 (7-OCH_3), 55.1 (6-OCH_3), 55.2 (1-OCH_3), 59.8 (3-OCH_3), 92.7 (C-4), 94.7 (C-8), 94.8 (C-2), 106.0 (C-9a), 118.0 (C-8a),

137.3 (C-5), 142.3 (C-6), 150.2 (C-7), 150.4 (C-10a), 160.1 (C-4a), 161.7 (C-1), 165.3 (C-3), 178.8 (C-9).

2.4 X-ray crystallographic of 9

The crystal data of **9** were collected on CCD SMART APEX diffractometer with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å) at 296(2) K with 13086 reflections. The *SMART* program was utilized for data collection and *SAINT* Program was used for cell refinement and data reductions, respectively (17). Absorption correction was applied by using the multi-scan program *SADABS* (17). The structure was solved by direct methods refined by a full-matrix least-squares procedure based on F^2 with the *SHELXT* program packages (18). The C-bound H atoms were positioned geometrically with the distance of C—H = 0.93 Å, U_{iso} = 1.2U_{eq} for C-sp^2 (for aromatic H atoms) and distances of C—H = 0.96-0.98 Å, U_{iso} = 1.5U_{eq} for C-sp^3, respectively. All H atoms of carbon atoms are constrained to ride on their parent atoms. The hydrogen atoms of hydroxyl groups and water molecules were located in a difference map and the coordinates were refined isotropically and the O–H distances are in the range of 0.79(5)–0.90(4) Å. The *WinGX*v2014.1 and *Mercury* programs were used to prepare the materials and molecular graphic for publication (18, 19, 20). Crystallographic data for **9** were deposited at the Cambridge Crystallographic Data Center (CCDC Number: 1998635). Copies of the data can be obtained, free of charge, through application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, (fax: +44-(0)1223-336033, or by e-mail at http://www.ccdc.cam.ac.uk/deposit@ccdc.cam.ac.uk).

2.4.1 Crystallographic data of Mammea A/AA cyclo F (9)

Colourless crystals, $C_{25}H_{26}O_6$, H_2O, M = 440.47, W = 0.71073 Å, 0.202 x 0.156 x 0.104 mm^3, monoclinic, $P2_1/c$, a = 9.7967(5) Å, b = 21.7164(10) Å, c = 11.3173(6) Å, α = 90.00°, β = 110.0020(10)°, γ = 90.00°, V = 2262.5(2) $Å^3$, Z = 4, D_x = 1.293 Mg/m^3, crystal size 0.202 x 0.156 x 0.104 mm^3, μ(Mo Kα) = 0.094 mm^{-1}, F(000) = 936, Reflections collected = 13086, Independent reflections = 3984, R(reflections) = 0.0566 (2306), $wR2$(reflections) = 0.1566(3984), S = 0.988, $Npar$ = 307

2.5 Antioxidant assay

To measure antioxidant assays, the concentration of 1,000 mg/L of each plant-derived compound was firstly prepared by dissolving in 95% methanol. A derivative vitamin E with potent antioxidant properties, Trolox, was added as a positive control. The free radical scavenging capacities of the compound was tested *in vitro* by 2,2′- diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethyl benzthiazo line-6-sulphonic acid) (ABTS), assay according to the previous method (21). Briefly, an aliquot (100 µL) of each sample was carefully mixed with 80 µM DPPH ethanol solution (100 µL) in a 96-well plate. After incubation in the dark for 30 min at ambient temperature, the absorbance of the solution was measured at 520 nm comparing to an appropriate blank to obtain the DPPH radical (DPPH·) bleaching. ABTS radical cation ($ABTS^{\cdot+}$) was generated by mixing 2 mM ABTS and 2.45 mM potassium persulfate at a volume ratio of 1:1. The mixture was incubated in the dark at room temperature for 16 h. Then, the absorbance of the solution was adjusted to 0.70 ± 0.05 at 734 nm with ethanol. An aliquot (100 µL) of each sample was mixed with 100 µL of $ABTS^+$ solution. After 6 min of incubation, the absorbance was then taken at 734 nm.

2.6 Antibacterial activity assay

The antibacterial activity of isolated compounds against *S. aureus* ATCC23235 was evaluated using a modified broth microdilution method from the Clinical and Laboratory Standards Institute (CLSI) (22). Briefly, the bacterial suspension of tested bacteria in Mueller-Hinton broth was mixed with the 2-fold serial dilutions of isolated compounds in 96-well plates. The plates were then incubated at 37 °C for 24 hours. After incubation, the minimal inhibitory concentration (MIC) of each compound is defined as the lowest concentration of the compounds that completely inhibits the bacterial growth.

3. Results and Discussion

Compound **1** was obtained as a pale yellow powder which was previously reported from the hydrolysis of 1,6-dimethoxy-5-toluene-*p*-sulphonyloxyxanthone (23). The UV spectrum presented absorption bands characteristic of xanthone at 240, 263 and 344 nm. The IR spectrum also presented the main peaks of xanthone such as the hydroxyl group at 3280 cm^{-1}, the conjugated carbonyl group at 1650 cm^{-1} and an aromatic at 1575 cm^{-1} (9). The ^{1}H NMR spectrum of **1** showed AMX pattern signals of aromatic protons at δ_H 6.94 (d, J = 8.0 Hz), 7.67 (t, J = 8.0 Hz) and 7.11 (d, J = 8.0 Hz), signals of two *ortho*-coupled aromatic protons at δ_H 7.10 (d, J = 8.7 Hz) and 7.67 (d, J = 8.7 Hz) and singlet signals of methoxyl protons at δ_H 3.94 (s, 1-OCH_3) and δ_H 3.98 (s, 6-OCH_3). The ^{13}C NMR data of **1** presented 15 carbon signals, including a conjugated ketone carbonyl carbon (δ_C 174.3), seven quaternary aromatic carbons (δ_C 160.9, 158.2, 151.6, 144.3, 134.0, 117.9 and 112.0), five methine aromatic carbons (δ_C 134.6, 116.4, 109.7, 108.2, 106.0) and two methoxy carbons (δ_C 55.9 and 55.6). In the HMBC spectrum of compound **1**, the correlations between H-3 and C-1 (δ_C 160.9) and H-8 and C-6 (δ_C 151.6) and the NOESY correlations) H-2↔1-OCH_3 and H-7↔6-OCH_3 (indicated the location of the two methoxy groups at C-1 and C-6,

respectively (Figure 2). The significantly high field-shifted signal at δ_C 134.0 as well as the HMBC correlation from H-7 to C-5 (δ_C 134.0) indicated that the hydroxyl group was attached to C-5 (Figure 2). Thus, compound **1** was assigned as 5-hydroxy-1,6-dimethoxyxanthone.

Compound **2** was previously isolated from *Swertia mileensis* (24) and *Swertia franchetiana* (25). In this study, it was isolated as a pale yellow solid. Compound **2** presented similar UV and IR data to **1**, indicating that it was also a xanthone derivative. The ^{1}H and ^{13}C NMR spectra of **2** were also similar to those of **1**. The differences were that, instead of the signals of *ortho*-coupled and AMX aromatic protons produced by **1**, compound **2** presented one singlet signal of an aromatic proton at δ_H 7.13 (H-8, δ_C 94.7) and signals of *meta*-coupled aromatic protons at δ_H 6.48 (d, J = 2 Hz, H-2, δ_C 94.8) and δ_H 6.74 (d, J = 2 Hz, H-4, δ_C 92.7). Furthermore, the ^{1}H NMR spectrum of **2** exhibited an additional two methoxyl proton signals at δ_H 3.91 and 3.94. These methoxy groups were placed at C-7 and C-3, respectively, due to the NOESY correlations (H-4↔3-OCH$_3$ (δ_H 3.91), H-8↔7-OCH$_3$ (δ_H 3.94)) as well as the carbon chemical shifts in the dioxysubstituent aromatic ring of compound **2** at C-1 (δ_C 161.7), C-2 (δ_C 94.8), C-3 (δ_C 165.3) and C-4 (δ_C 92.7) corresponded to those for 1,3-dimethoxyxanthone at δ_C 161.7, 94.8, 164.6 and 92.5, respectively (26) (Figure 2). Therefore, the structure of **2** was determined to be 5-hydroxy-1,3,6,7-tetramethoxyxanthone.

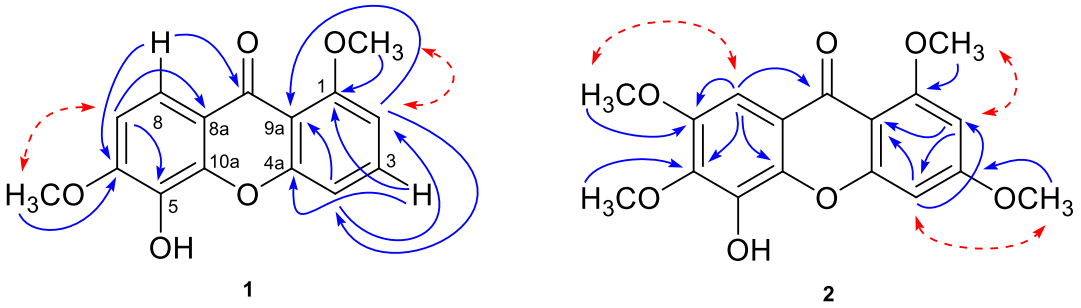


Figure 2 Selected HMBC ( ) and NOESY ( ) correlations of **1** and **2**.

Mammea A/AA cyclo F, compound **9**, is a 6-acyl-7,8-dihydrofurano derivative of 4-phenyl-5,7-dihydroxycoumarin previously isolated from *Mammea americana* (15) and *M. ferrea* blossoms (14). In this study, the 4-phenylcoumarin, **9**, was isolated as a colourless solid which was recrystallized using EtOAc to yield colourless single crystals. The structure of the crystal was analysed by single-crystal X-ray diffraction (Figure 3).

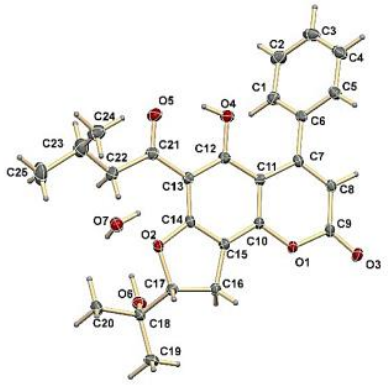


Figure 3 X-ray ORTEP diagram of **9**.

Compounds **3–5** and **9** were evaluated for their antioxidant activities (Table 1). Xanthone **3** displayed significant antioxidant activity with IC$_{50}$ values of 45.0 µg/mL and 197.0 µg/mL in the DPPH and ABTS assays, respectively. Compounds **4** and **5** showed inhibitory activity in an ABTS assay with IC$_{50}$ values of 264.0 µg/mL and 212.0 µg/mL, respectively. All these values were lower than the IC$_{50}$ values obtained from the standard antioxidant agent, trolox, in the same assays (77.6±1.0 µg/mL in DPPH and 282.9±1.2 µg/mL in ABTS). Besides, the xanthone, **4**, and the 4-phenylcoumarin, **9**, showed weak antibacterial activities against *Staphylococcus aureus* ATCC23235 with MIC value of 62.5 µg/mL and 125.0 µg/mL, respectively, compared to the antibiotic vancomycin (MIC 0.97 µg/mL) (Table 1).

Table 1 Biological activities of isolated compounds.

Compounds	Radical scavenging properties IC_{50} (μg/mL)		Antibacterial activity MIC (μg/ml)
	DPPH	**ABTS**	***S. aureus* ATCC 23235**
3: 1,5,6-trihydroxyxanthone	45.0±0.001	197.0±0.002	>1,000
4: 1,3,7-trihydroxyxanthone	8513±0.350	264.0±0.002	62.5
5: 1,7-dihydroxy-3-methoxyxanthone	8903±0.890	212.0±0.002	>1,000
9: mammea A/AA cyclo F	-	-	125.0
trolox[a]	77.6±1.06	282.9±1.29	
vancomycin[a]			0.97

[a] Trolox and vancomycin were used as the standard drugs.

4. Conclusions

In summary, five xanthones and four phenylcoumarins were isolated from twigs of *M. ferrea* and structure elucidated. The ^{1}H and ^{13}C-NMR spectra of compounds **1** and **2** were completely assigned since there are no NMR data that had been previously published from these compounds. The antioxidant properties of compounds **3–5** and **9** were assessed using DPPH and ABTS scavenging capacity assays. Compound **3** was found to be more active than the other compounds with IC_{50} values of 45.0 μg/mL and 197.0 μg/mL in DPPH and ABTS assays, respectively. Compounds **4–7** were isolated from this plant for the first time. The structure of 4-phenylcoumarin **9** was analysed by single-crystal X-ray diffraction.

Acknowledgements

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Declaration of conflicting interests

The authors declared that they have no conflicts of interest in the research, authorship, and this article's publication.

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