GC-MS and Bioassay-Guided Isolation of Xanthones from *Mammea siamensis*

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Abstract: Mammea siamensis *(Miq.) T. Anders. (Calophyllaceae) plants have long been employed as an active integral composition in Thai traditional medicine. Additionally, phenylcoumarins and triterpenes were reported as major components in phytochemical research. This work explored the various parts of* M. siamensis*; barks, flowers, twigs, leaves, and young leaves; to determine their bioactive compounds. By using the GC-MS and bioassay guidance, two xanthones, 6-deoxyisojacareubin (**1**) and 1,5-dihydroxyxanthone (**2**), together with a mixture of phenylcoumarins, mammea A/AA cyclo D (**3**) and mammea A/AB cyclo D (**4**) have been isolated from the methanolic extract of young leaves. Their structures were identified by means of spectroscopic technique and by comparison with literature data. In particular, the current study was the first exposed report of xanthones **1** and **2** from the genus Mammea. Furthermore, compounds **1** and **2** and the methanolic young leaf extract had high antioxidant efficiency on DPPH and ABTS assays. The young leaf extract provided mild toxicity on the brine shrimp lethality test (BSLT) with LC_{50} value of 93.11 ± 1.37 µg/mL. In addition, the isolated compounds **1** and **2** were non-toxicity in BSLT assay. Therefore, the young leaf extract and the purified constituents **1** and **2** should be further studied and developed for using in pharmaceutical industries.*

Keywords: *antioxidant activity;* Mammea siamensis*; phenylcoumarins; toxicity; xanthones*

■ INTRODUCTION

Medicinal plants are employed as ingredients in several traditional remedies due to their phytochemical metabolites, such as terpenoids, flavonoids, alkaloids, and polyphenols. These significant bioactive components revealed diverse efficiency, for example, anticancer [1-3], antioxidant [3-5], antimicrobial [4-6], and antileishmanial activities [5,7]. Accordingly, various Thai medicinal plants are famous for acting as active elements in several folk medicines, including *Mammea siamensis* [8-9].

The genus *Mammea* belongs to the family Guttiferae, recently assigned to the Calophyllaceae [10]. This genus consists of approximately 75 species overspreading throughout the tropics in Africa, Central America, Madagascar, and tropical Asia. Three species, *M. brevipes*, *M. harmandii*, and *M. siamensis*, have been reported in Thailand [11].

M. siamensis (Miq.) T. Anders, named "Saraphi", is a Thai botanical medicine. Its flowers have long been traditionally employed as an active, integral composition in Thai herbal prescriptions as a heart tonic and promoting of appetite [12-13]. Several parts of this plant, such as flowers [12,14], twigs [15], seeds [16-18], and barks [19], have been phytochemically investigated. For example, many bioactive compounds separated from the methanol extract of *M. siamensis* flowers showed potent cytotoxic activity, such as mammeasin A and surangin B [20]. The extensive literature review has investigated only one phytochemical constituent from *M. siamensis* leaves. Proanthocyanidin, a condensed tannin, has been isolated from 95% ethanol extract of the leaves, and it showed molluscicidal activity at its sufficient concentration [21]. In particular, the prior reports determined higher effectiveness of shoot or young leaves than mature leaves in various bioactivities, anticancer [1,22], antioxidant, and total phenolic contents [23-24]. In the present work, gas chromatography-mass spectrometry (GC-MS) analytical method and bioactivity evaluation, antioxidative activity and *in vivo* toxicity on brine shrimp lethality assays were performed to examine bioactive compounds from the leaf, young leaf (first 3-5 leaves) [25-26], twig, bark and flower extracts of *M. siamensis*. As guided by such techniques, the methanolic young leaf extract was further investigated, leading to the isolation of two bioactive xanthones, 6-deoxyisojacareubin (**1**) and 1,5-dihydroxyxanthone (**2**) and a mixture of mammea A/AA cyclo D (**3**) and mammea A/AB cyclo D (**4**). Their structures were identified by NMR and mass spectroscopic data, with this work providing the first report of compounds **1** and **2** from the medicinal plant in *Mammea* genus. Furthermore, these two isolated compounds were tested for their antioxidative ability and *in vivo* toxicity using brine shrimp lethality assay and the results were discussed.

■ EXPERIMENTAL SECTION

Materials

The barks, flowers, twigs, leaves, and young leaves of *Mammea siamensis* were collected in February 2019 from Chachoengsao province, Thailand. The plant materials were identified by SH and a voucher specimen with the plant code RRU-SH-009 was collected at the Faculty of Science and Technology, Rajabhat Rajanagarindra University, Chachoengsao, Thailand.

Instrumentation

The ^{1}H-, ^{13}C-, and 2D-NMR spectra were recorded with a Bruker Ascend™ 400 spectrometer in acetone-d_6 (CD_3COCD_3), dimethyl sulfoxide-d_6 (CD_3SOCD_3) and chloroform-d ($CDCl_3$) (were acquired from Merck, Germany) solutions by using an internal standard as either tetramethylsilane (TMS) or residual non-deuterated solvent peak. High-resolution mass spectra were recorded with a Bruker micro TOF spectrometer. Agilent 5977B GC/MSD was employed for GC-MS technique evaluation. Analytical purposes were performed by using silica gel 60 PF_{254}-pre-coated TLC aluminum sheets of (20 × 20 cm, layer thickness of 0.2 mm, Merck, Germany). Spraying with 12% H_2SO_4 in ethanol or anisaldehyde reagent and visualization under ultraviolet light were used for chemical composition monitoring. Column chromatography was implemented using Merck silica gel 60 (60–200 μm or 70–230 mesh ASTM) and Merck Sephadex LH-20. Distillation technique was employed for solvent preparation prior to use in extraction, chromatography, and crystallization processes. Analytical grade solvents, ethanol and methanol, were obtained from Fisher Scientific Korea Ltd.

Procedure

Extraction

The air-dried powdered materials from the barks, flowers, twigs, leaves and young leaves of *M. siamensis* (10 g each) were macerated at room temperature with methanol (200 mL × 7 d × triplicates) for each extraction. Removal of the solvents under reduced pressure and subsequent freeze-drying were performed after filtration. Then the crude methanol extracts were obtained and kept at −4 °C until further analysis.

GC-MS analysis

GC-MS analysis was implemented by employing an Agilent 5977B GC/MSD with an HP5MS column (30 m × 0.25 mm × 0.25 mm) under Helium as carrier gas with a flow rate of 1.3 mL/min. Samples were

analyzed in the column held at an initial temperature of 50 °C for 3 min after injection. Then, increasing temperature was carried out to 280 °C at a program rate of 10 °C/min and then held for 20 min. The injections were performed at 250 °C in splitless mode. The pressure was 10 psi, the run time was 37.5 min, and the temperatures of injector and detector were 250 °C. Before submission to GC-MS analysis, each crude extract (2.0 mg) was dissolved in 1 mL of methanol and subjected to exhaustive filtration. The isolated constituents were examined to compare with the authentic samples by referring to their retention times and mass weights available in GC-MS NIST library.

Antioxidant activity

The free radical scavenging ability of the extracts and the isolated compounds were evaluated on the basis of DPPH and ABTS free radical scavenging assays.

The DPPH inhibition of each sample was determined according to the modified procedure [27]. Briefly, varied concentrations of the samples; 6.25, 12.5, 25, 50, and 100 μg/mL; were prepared by dissolution and dilution in methanol. To analyze, 0.2 mM DPPH solution in methanol (4 mL) was mixed with the sample solutions (1 mL) and the mixtures were shaken intensely. Instantaneously, incubation of the reaction mixtures at room temperature was operated in the absence of light for 30 min before the absorbance measurement at 517 nm. All assays were executed in triplicate to afford accurate data. The percentage of DPPH free radical inhibition was determined by using Eq. (1):

$$\text{Percentage of DPPH inhibition} = \left(\frac{A_0 - A_1}{A_0}\right) \times 100 \qquad (1)$$

where A_0 and A_1 correspond to the absorbance at 517 nm of the DPPH radical in control and in the presence of samples, respectively. Additionally, the 50% inhibitory concentration (IC_{50}) value indicating the least sample concentration inhibiting 50% of free radicals was calculated. The quercetin and butylated hydroxytoluene (BHT) solutions were employed as reference antioxidants.

For ABTS assay, the procedure was modified according to the literature [28]. In brief, 7 mM ABTS solution (10 mL) was added to 2.45 mM potassium persulfate ($K_2S_2O_8$, 176 μL) and the mixture was immediately kept in the dark condition at room temperature for 12–16 h prior to use. Subsequently, the ABTS working solution was prepared to obtain the appropriate absorbance of 0.700 ± 0.020 at 734 nm by dilution with 95% ethanol. Then, 100 μL of the sample solutions with different concentrations: 6.25, 12.5, 25, 50, and 100 μg/mL, were mixed with 900 μL of the ABTS working solution and the reaction was allowed to leave for 6 min at room temperature. Ethanol was set as the standard blank for absorbance measurement at 734 nm. Quercetin and BHT were used as references. The Eq. (2) evaluated the percentage of free radical inhibition of the extracts:

$$\text{Percentage of ABTS inhibition} = \left(\frac{A_0 - A_1}{A_0}\right) \times 100 \qquad (2)$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the tested sample or standard after treatment.

In vivo *toxicity on brine shrimp lethality assay*

The prepared extracts and the isolated compounds were examined by employing the brine shrimp lethality test (BSLT) [29]. Briefly, brine shrimp cysts (0.25 g) were hatched in an Erlenmeyer flask containing 1 L of well-aerated artificial seawater (26.29 g of NaCl, 0.74 g of KCl, 0.99 g of $CaCl_2$, 2.86 g of $MgCl_2$, and 3.94 g of $MgSO_4{\cdot}7H_2O$, with adjusted pH 7.8) under lighted conditions for 48 h. The extracts were prepared by dissolving in 1% v/v of DMSO in artificial seawater in concentrations ranging from 62.5, 125, 250, 500, and 1000 μg/mL. Simultaneously, more than 30 *Artemia* larvae were transferred from the hatching flask into each concentration of sample during the preparation to avoid dilution from transferring larvae medium to the test samples. Also, potassium dichromate ($K_2Cr_2O_7$) solution with the same preparation was employed as a positive control in 6.25, 12.5, 25, 50, and 100 μg/mL concentrations. All experiments were determined in triplicate by only dividing 10 nauplii per sample tube. Furthermore, nauplii was examined as blank in 1% v/v of DMSO in artificial seawater. Then, the incubation of all samples was investigated under proper light for 24 h at room temperature. The percentage lethality was

evaluated from the counted alive nauplii by utilizing Eq. (3):

$$\% \text{ Mortality} = \left(\frac{\text{Total nauplii} - \text{Alive nauplii}}{\text{Total nauplii}} \right) \times 100 \qquad (3)$$

The data were processed using a Probit analysis program to calculate the lethal concentration of half of the test organisms (LC_{50}).

Extraction, purification, and spectroscopic data of isolated compounds

Air-dried and finely powdered young leaves (1.5 kg) of *M. siamensis* were macerated with methanol (4 L × 7 d × triplicates) at room temperature. Then, the methanolic crude extract (34.9 g) was obtained by filtration and solvent removal under reduced pressure, respectively. A vacuum liquid column chromatography (VCC) technique was employed to isolate the pure compounds. Gradient elution of EtOAc–hexane solution (0, 10, 20, 40, 60, 80, 100%, 2 L each) followed by MeOH–EtOAc solution (10, 20, 50, 100%, 1 L each) was conducted for VCC. Based on TLC characteristics, the fractions (1 L each) were collected and combined to yield seven fractions (F1–F7). Fraction F3 (1.3 g) was purified employing silica gel column chromatography (Si-gel CC) with the gradient solvent system elution of EtOAc–hexane (0–100%) and MeOH–EtOAc (0–100%) to provide subfractions F3.1–F3.4. Sephadex LH-20 was presented for gel filtration of subfraction F3.2 (242.0 mg) to isolate a pure constituent, xanthone **1** (6.0 mg), together with a mixture of mammea A/AA cyclo D (**3**) and mammea A/AB cyclo D (**4**) (4.2 mg) in the ratio of 1:2. After purification of fraction F4 (2.3 g) using Si-gel CC with MeOH-CH_2Cl_2 (0–100%), gradient as eluent, subfractions F4.1–F4.4 were obtained**.** Chromatography of subfraction F4.2 (177.0 mg) was performed on Sephadex LH-20 with MeOH elution to afford compound **2** (18.0 mg).

6-Deoxyisojacareubin (1). A yellowish solid; m.p. 220.1–222.0 °C; UV (MeOH) λ_{max} (log ε) 329 (3.78), 267 (4.23), 251 (4.27) nm; IR (KBr) ν_{max} 3467, 3402, 3023, 2978, 1651, 1619, 1573, 1485, 1344, 1278, 1159, 1114, 762 cm^{-1}; ^{13}C-NMR (acetone-d_6, 100 MHz) and ^{1}H-NMR (acetone-d_6, 400 MHz) data, see Table 2; HR-EI-MS *m/z* 311.0907 $[M+H]^+$ (calc. for $C_{18}H_{15}O_5$, 311.0914).

1,5-Dihydroxyxanthone (2). A brownish solid; m.p. 198.2–199.0 °C; UV (MeOH) λ_{max} (log ε) 370 (3.94), 312 (4.15), 348 (4.88) nm; IR (KBr) ν_{max} 3424, 1651, 1611, 1579, 1497, 1462, 1279, 1240, 1144, 1067, 793, 724 cm^{-1}; ^{13}C-NMR (DMSO-d_6, 100 MHz) and ^{1}H-NMR (DMSO-d_6, 400 MHz) data, see Table 2; HR-EI-MS *m/z* 288.0428 $[M]^+$ (calc. for $C_{13}H_8O_4$, 288.0423).

A mixture of mammea A/AA cyclo D (3) and mammea A/AB cyclo D (4). (ratio 1:2 by ^{1}H-NMR data); ^{13}C-NMR ($CDCl_3$, 100 MHz): 206.7 (C-1”), 164.4 (C-5), 159.6 (C-2), 156.4 (C-4), 154.9 (C-10b), 153.1 (C-6a), 139.3 (C-1’), 128.8 (C-4’), 127.6 (C-3’, C-5’), 127.2 (C-2’, C-6’), 126.3 (C-9), 115.0 (C-10), 112.7 (C-3), 107.2 (C-6), 102.0 (C-4a), 101.0 (C-10a), 79.9 (C-8), 53.6 (C-2”), 28.7 (C-1’”, C-2’”), 25.5 (C-3”), 22.7 (C-4”, C-5”) for **3**; 211.5 (C-1”), 164.4 (C-5), 159.7 (C-2), 157.8 (C-10b), 156.4 (C-4), 154.4 (C-6a), 139.2 (C-1’), 128.2 (C-4’), 127.6 (C-3’, C-5’), 127.1 (C-2’, C-6’), 126.3 (C-9), 115.5 (C-10), 112.7 (C-3), 107.3 (C-6), 102.0 (C-4a), 101.5 (C-10a), 79.8 (C-8), 46.6 (C-2”), 28.6 (C-1’”, C-2’”), 25.5 (C-3”), 16.8 (C-4”), 11.6 (C-5”) for **4**; ^{1}H-NMR ($CDCl_3$, 400 MHz):14.10 (1H, *s*), 7.43 (3H, *m*, H-3’, H-4’, H-5’), 7.35 (2H, *m*, H-2’, H-6’), 6.92 (1H, *d*, 10.0, H-10), 6.03 (1H, *s*, H-3), 5.67 (1H, *d*, 10.0, H-9), 3.00 (2H, *d*, 7.2, H-2”), 2.26 (1H, *m*, H-3”), 1.60 (6H, *s*, H-1’”, H-2’”), 0.98 (6H, *d*, 6.8, H-4”, H-5”) for **3**; 14.54 (1H, *s*), 7.31 (3H, *m*, H-3’, H-4’, H-5’), 7.24 (2H, *m*, H-2’, H-6’), 6.81 (1H, *d*, 10.0, H-10), 5.91 (1H, *s*, H-3), 5.55 (1H, *d*, 10.0, H-9), 3.67 (2H, *sextet*, 6.6, H-2”), 1.69 (1H, *m*, Ha-3”), 1.48 (6H, *s*, H-1’”, H-2’”), 1.26 (1H, *m*, Hb-3”), 1.12 (3H, *d*, 6.9, H-5”), 0.84 (3H, *t*, 7.3, H-4”) for **4**; HR-EI-MS *m/z* 404.1631 $[M]^+$ (calc. for $C_{25}H_{24}O_5$, 404.1624).

■ RESULTS AND DISCUSSION

After extraction of several parts of *M. siamensis*; barks, flowers, twigs, leaves, and young leaves, with methanol as the eluent followed by filtration and evaporation under reduced pressure, each crude extract was analyzed using GC-MS and the constituents are shown in Fig. 1. The tentative scanning of the GC-MS chromatogram of barks, flowers, twigs, leaves, and young leaves revealed the signal of several phenolic components by comparison with the NIST database (see

Supplementary information). After comparison of GC-MS chromatograms, it was indicated that various phenolic components were contained in crude methanol extract of the leaves and young leaves. Antioxidant activities in both DPPH and ABTS radical scavenging assays evaluated in the extracts from various parts of *M. siamensis* are summarized in Table 1.

As shown in Table 1, the antioxidant activity of the

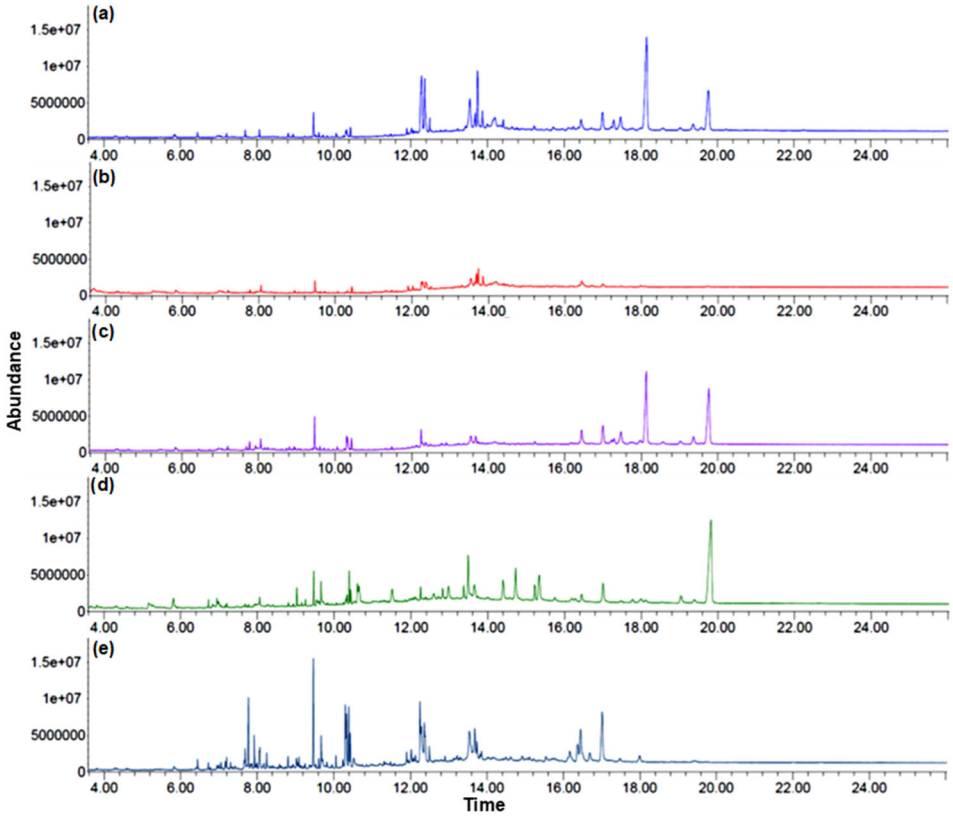

Fig 1. Total ion GC-MS chromatograms of different extracts from *M. siamensis*, (a) barks, (b) flowers, (c) twigs, (d) leaves, and (e) young leaves

Table 1. Antioxidant activity and *in vivo* toxicity on BSLT of *M. siamensis* extracts and isolated compounds **1** and **2**

Sample	IC_{50} (µg/mL)		LC_{50} (µg/mL)
	DPPH assay	ABTS assay	BSLT assay
Bark extract	55.11 ± 2.18	103.69 ± 1.21	>100
Flower extract	50.38 ± 2.90	69.58 ± 0.88	70.14 ± 0.79
Twig extract	53.91 ± 0.55	76.67 ± 1.23	76.59 ± 1.27
Leave extract	66.31 ± 1.24	47.71 ± 0.67	8.37 ± 1.22
Young leaf extract	59.04 ± 1.92	47.22 ± 1.09	93.11 ± 1.37
1	78.16 ± 1.32	40.42 ± 0.59	> 100
2	70.16 ± 0.97	38.86 ± 1.66	> 100
Quercetin	14.02 ± 0.86	14.02 ± 0.86	ND
BHT	ND	23.23 ± 1.50	ND
$K_2Cr_2O_7$	ND	ND	12.62 ± 0.72

IC_{50} and LC_{50} results are average of three independent experiments ± standard deviation. Quercetin and BHT were used as positive controls for the antioxidant assay and $K_2Cr_2O_7$ was used as positive control for BSLT assay. ND = not determined

extracts exhibited free radical scavenging activity with IC_{50} values ranging from 50.38 ± 2.90 to 66.31 ± 1.24 µg/mL for DPPH assay and from 47.22 ± 1.09 to 103.69 ± 1.21 µg/mL for ABTS assay. The results indicated strong effective antioxidant ability of whole parts according to the literature [30]; at lower than 50 µg/mL of IC_{50} values shows very strong antioxidant properties, strong antioxidant characterization is exhibited as IC_{50} values of 50–100 µg/mL, IC_{50} values of moderate ability are provided at 100–150 µg/mL, and weak efficiency shows the IC_{50} value of 150–200 µg/mL. Furthermore, the toxicity assay based on the BSLT at different concentrations (Table 1) revealed that all the extracts except from the barks showed toxicity at LC_{50} ranging from 8.37 ± 1.22 to 93.11 ± 1.37 µg/mL [31-32]. Based on the antioxidant results of the extracts, the young leaf extract provided free radical scavenging activity apparently in both DPPH and ABTS assays at 59.04 ± 1.92 and 47.22 ± 1.09 µg/mL, respectively. Additionally, the cytotoxic effect in BSLT from the young leaf extract afforded mild toxicity with LC_{50} value of 93.11 ± 1.37 µg/mL.

In accordance with the combination of GC-MS profiling and the biological activities of the various parts of *M. siamensis* extracts, the young leaf extract exhibited more interesting efficiency than the others; therefore, it was chosen to isolate its attractively bioactive constituents. After the isolation and purification by chromatography techniques, compounds **1** and **2** along with a mixture of coumarins **3** and **4** were obtained (Fig. 2). All compounds were elucidated and identified based on spectroscopic techniques.

Compound **1** was obtained as a yellowish solid. The molecular formula $C_{18}H_{14}O_5$ was determined by HR-EI-MS at *m/z* 311.0907 $[M+H]^+$ (calc. for $C_{18}H_{15}O_5$, 311.0914). Its ^1H and ^{13}C-NMR data are summarized in Table 2. The ^{13}C-NMR and DEPT135 spectra analysis identified 17 signals for 18 carbons, 2 methyls, 6 methines and 10 quaternary carbons. The ^1H-NMR spectrum exhibited low-field broad signals at δ 12.11 and 9.14 ppm, indicating the hydroxyl chelated group at C-1 and free hydroxyl group at C-5, respectively. Three aromatic protons at δ 7.67 (1H, dd, *J* = 7.8, 1.6 Hz), 7.35 (1H, dd, *J* = 7.8, 1.6 Hz), and 7.30 (1H, t, *J* = 7.8 Hz) ppm indicated the presence of the ABC-type aromatic protons H-8, H-6, and H-7, respectively. A singlet signal at δ 6.19 (1H) ppm was assigned to the aromatic proton H-2. A sharp singlet at δ 1.50 (CH_3-14 and CH_3-15) ppm and a pair of doublets at δ 7.09 (1H, H-11) and 5.75 (1H, H-12) ppm with coupling constant of 10.1 Hz suggested the presence of a 2,2-dimethylchromene ring. To confirm the structure of **1**, the chemical shifts of each carbon and proton were deduced based on the 2D NMR information (COSY, HMQC, and HMBC correlations), along with comparisons of the data to those in the literature [33-35]. Thus, compound **1** was identified as 6-deoxyisojacareubin.

Compound **2** was a brownish solid with a molecular formula of $C_{13}H_8O_4$ associated with the HR-EI-MS *m/z* 288.0428 (calc. for $[M]^+$, 288.0423). The comparison between ^{13}C and DEPT135 NMR spectra indicated 13 signals for 13 carbons, 4 methine carbons, and 9 quaternary carbons, as summarized in Table 2. The ^1H-NMR spectrum of **2** showed the characteristics of a chelated hydroxyl proton at C-1 and of a free hydroxyl group at C-5 at δ 12.62 (1H, s) and δ 10.55 (1H, s) ppm, respectively. The aromatic protons at δ 7.70 (1H, dd, *J* = 8.3, 8.3 Hz, H-3), 7.05 (1H, d, *J* = 8.3 Hz, H-4), and

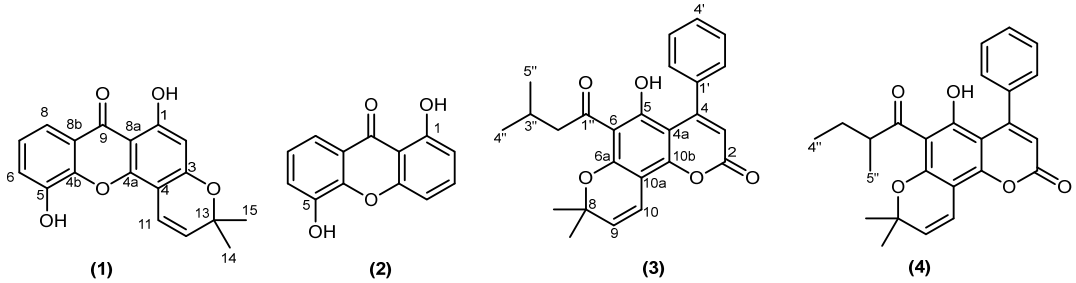

Fig 2. Structures of compounds **1**–**4** isolated from young leaves of *M. siamensis*

Table 2. ^{13}C (100 MHz) and ^{1}H-NMR (400 MHz) data of compounds **1** and **2**

Position	**1**[a]		**2**[b]	
	δ_C	δ_H (mult., *J* (Hz))	δ_C	δ_H (mult., *J* (Hz))
1	164.20 (C)		161.00 (C)	
2	99.80 (CH)	6.19 (s)	110.50 (CH)	6.77 (d, 8.3)
3	161.90 (C)		137.40 (C)	7.70 (dd, 8.3, 8.3)
4	102.20 (C)		107.60 (C)	7.05 (d, 8.3)
4a	152.50 (C)		155.80 (C)	
4b	147.00 (C)		146.00 (C)	
5	147.10 (C)		146.40 (C)	
6	122.20 (CH)	7.35 (dd, 7.8, 1.6)	121.00 (CH)	7.55 (d, 7.7)
7	125.70 (CH)	7.30 (t, 7.8)	124.90 (CH)	7.25 (dd, 7.7, 7.7)
8	116.90 (CH)	7.67 (dd, 7.8, 1.6)	114.60 (CH)	8.33 (d, 7.7)
8a	122.60 (C)		121.30 (C)	
8b	104.40 (C)		108.90 (C)	
9	182.00 (C=O)		182.10 (C=O)	
11	115.90 (CH)	7.09 (d, 10.1)		
12	129.50 (CH)	5.75 (d, 10.1)		
13	79.20 (C)			
14	28.40 (CH_3)	1.50 (s)		
15	28.40 (CH_3)	1.50 (s)		
1-OH		12.11 (s)		12.62 (s)
5-OH		9.14 (br. s)		10.55 (br. s)

[a]The spectral data were recorded in deuterated acetone (CD_3COCD_3). [b]The spectral data were recorded in deuterated dimethyl sulfoxide (CD_3SOCD_3). Carbon types were deduced by DEPT135 experiment. mult. = multiplicity

6.77 (1H, d, *J* = 8.3 Hz, H-2) ppm were assigned to be 1,2,3-trisubstituted benzene ring A. The three aromatic signals at δ 8.33 (1H, d, *J* = 7.7 Hz, H-8), 7.55 (1H, d, *J* = 7.7 Hz, H-6), and 7.25 (1H, dd, *J* = 7.7, 7.7 Hz, H-7) ppm were also indicated as 1,2,3-trisubstituted benzene ring C. In addition, the chemical structure of compound **2** was evaluated using 2D (COSY, HMQC, and HMBC correlations) NMR spectroscopy. Finally, the data were intensively compared with the literature data [36]. Hence, compound **2** was identified as 1,5-dihydroxyxanthone.

Although compounds **3** and **4** were isolated as the mixture, their chemical structural identification was determined using the ^{1}H and ^{13}C-NMR data together with HR-EI-MS data and comparison to the human metabolome database (HMDB). The ^{1}H and ^{13}C chemical shifts of each compound were clearly identified by comparing them with the previous report by Verotta, L. and co-workers [37]. Based on the intensive comparison of the spectroscopic data of the mixture with the literature data, the mixture of coumarins **3** and **4** was identified as mammea A/AA cyclo D and mammea A/AB cyclo D, respectively (see supplementary information).

The GC-MS chromatograms of the methanolic extracts of young leaves and leaves were identified to contain compounds **1** and **2** by comparing the molecular mass and retention time of each pure isolated compound, as shown in Fig. 3. This result might indicate that both compounds are the remarkably important secondary metabolites for the plant leaves.

The biological activities of the isolated compounds **1** and **2** were examined by using DPPH and ABTS radical scavenging assays along with *in vivo* toxicity in the BSLT assay. Both compounds exhibited strong antioxidant characteristics in DPPH assay with IC_{50} values of 78.16 ± 1.32 and 70.16 ± 0.97 μg/mL, respectively [30].

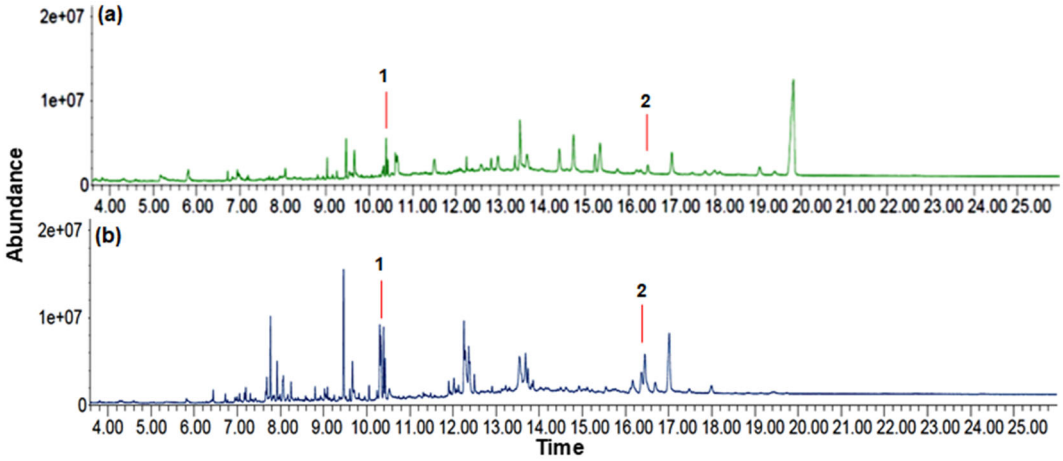

Fig 3. GC-MS chromatograms of compounds **1** and **2** in crude extracts, (a) leaves; (b) young leaves

In ABTS assay, the IC_{50} values of **1** and **2** were 40.42 ± 0.59 and 38.86 ± 1.66 µg/mL, respectively, which also showed very strong antioxidative levels [30]. After comparing the data, compounds **1** and **2** have the coordinately antioxidative results. In addition, they were non-toxic in BSLT with LC_{50} values > 100 µg/mL [31]; thus, both compounds were not active regarding *in vivo* toxicity. This result agreed with the toxicity of the related xanthone derivatives, 1,7-dihydroxyxanthone and 1-hydroxy-5-methoxyxanthone, previously isolated from seed of *M. siamensis* [16].

According to the previous study, the crude extracts and isolated compounds from several parts of *M. siamensis* were evaluated for biological cytotoxicity [14,16-17,20] and antibacterial activity [38], and most of them revealed coumarins being the major constituents [17,39-40]. However, xanthones are a small group of phytochemical constituents in *M. siamensis*. To the best our precedent search from SciFinder database, we first found xanthone derivatives **1** and **2** isolated from *Mammea* plants in the present study.

■ CONCLUSION

Mammea siamensis is an important medicinal plant containing notable bioactive compounds. Based on GC-MS and bioassay-guided isolation screening, the present study isolated two naturally occurring xanthone derivatives, 6-deoxyisojacareubin (**1**) and 1,5-dihydroxyxanthone (**2**), together with a mixture of phenylcoumarins, namely mammea A/AA cyclo D (**3**) and mammea A/AB cyclo D (**4**) from the methanolic young leaf extract of *M. siamensis*. The structures of the two isolated compounds were confirmed based on spectroscopic data and comparison with the literature. Furthermore, isolation of the secondary metabolites **1** and **2** has not been previously reported from this plant or the *Mammea* genus. Additionally, both isolated constituents provided efficient scavenging activity on DPPH and ABTS radicals, whereas they were no *in vivo* toxicity based on the brine shrimp lethality assay. Hence, isolated constituents, **1** and **2**, and the young leaf extract should be further studied for employment in anti-aging cosmetics and pharmaceutical industries in traditional plant-based medicines.

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AUTHOR CONTRIBUTIONS

Wiyarat Kumutanat, Sakchai Hongthong, Sariyarach Thanasansurapong, and Napasawan Chumnanvej conducted the experiment in isolation of the isolated compounds and analysis using GC-MS spectrometry. Naowarat Kongkum and Napasawan Chumnanvej conducted the experiment of DPPH and ABTS radical scavenging inhibition and *in vivo* toxicity by BSLT assay. Napasawan Chumnanvej and Wiyarat Kumutanat wrote and revised the manuscript. All authors agreed to the final version of this manuscript.

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