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Anthraquinone and xathone derivatives from propolis of *Tetragonula laeviceps* stingless bee

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Abstract. Stingless bees, which lack a functional sting, are distributed throughout most of the tropical and subtropical regions of the world, such as South America, Africa, Australia and Southeast Asia. *Tetragonula laeviceps* (Smith, 1857) is a species of stingless bee found in Southeast Asian countries. The extracts of *T. laeviceps* propolis showed many biological effects, but the chemical study of *T. laeviceps* propolis has been very limited. Chemical investigation of *T. laeviceps* propolis collected in Hoa Binh province (Viet Nam) led to the isolation of nine known compounds, including emodin (**1**), 3-geranyloxy emodin (**2**), 1,3,7-trihydroxy-2,4-bis(3-methylbut-2-enyl)xanthone (**3**), cudratricusxanthone F (**4**), 9-hydroxycalabaxanthone (**5**), *α*-mangostin (**6**), γ-mangostin (**7**), 1,3,5-trihydroxy-4-geranylxanthone (**8**) and pruniflorone I (**9**). Their structures were elucidated by MS and NMR spectral analysis and compared with those reported in the literature. This is the first investigation of *T. laeviceps* propolis in Viet Nam. Compounds **1**–**4**, **8** and **9** were found in stingless bee propolis for the first time.

Keywords: Tetragonula laeviceps, anthraquinones, xanthones.

Classification numbers: 1.1.1, 1.1.6.

1. INTRODUCTION

Stingless bees, belonging to the tribe Meliponini of the family Apidae (Hymenoptera), are eusocial bees that have evolved without a functional sting. Stingless bees can be found in various tropical and subtropical regions, including South America, Africa, Australia and

Southeast Asia. In Southeast Asian countries, there are over 60 species, of which around 15 stingless bee species are distributed in Viet Nam [1]. Similar to honeybees (*Apis mellifera*), stingless bees also engage in the collection of plant resins to produce propolis. Propolis is well-known for its medicinal properties, possessing a wide range of biological effects, including antibacterial, anticancer, antioxidant, immune-stimulating, and antiviral activities, making it an important natural remedy for enhancing health and preventing diseases [2].

Tetragonula laeviceps (Smith, 1857) (synonym: *Trigona (Tetragonula) pagdeniformis*) is a species of stingless bee distributed in Southeast Asian countries such as Indonesia, Malaysia, Thailand, and Viet Nam [1]. The alcoholic extracts of *T. laeviceps* propolis showed many pharmacological activities, such as anticancer, antibacterial and antifungal effects [3-6]. However, only a chemical study of *T. laeviceps* propolis has been reported so far. Bankova *et al.* have isolated eight compounds from a sample in Thailand, including six xanthones: α-mangostin, 9-hydroxycalabaxanthone, 8-deoxygartanin, gartanin, γ-mangostin and garcinone B, a triterpene dipterocarpol and a lignan methylpinoresinol [7].

Recently, we have investigated the chemical composition of the propolis of several stingless bees in Viet Nam, such as the propolis of *Lisotrigona spp.* from Binh Dinh, Khanh Hoa and Hoa Binh provinces, and propolis of *Lepidotrigona ventralis* from Tuyen Quang province [8-12]. In our continuing study of stingless bee propolis in Viet Nam, we described herein the isolation and elucidation of nine phenolic constituents from *Tetragonula laeviceps* propolis, including two anthraquinones: emodin (**1**), and 3-geranyloxy emodin (**2**), and seven xanthones: 1,3,7-trihydroxy-2,4-bis(3-methylbut-2-enyl)xanthone (**3**), cudratricusxanthone F (**4**), 9-hydroxycalabaxanthone (**5**), α-mangostin (**6**), γ-mangostin (**7**), 1,3,5-trihydroxy-4-geranylxanthone (**8**), and pruniflorone I (**9**).

2. MATERIALS AND METHODS

2.1. Propolis materials

The propolis sample was collected from a stingless bee-keeping house in Tan Lac, Hoa Binh province, Viet Nam, in November 2018. The stingless bee species was identified as *Tetragonula laeviceps* (Smith, 1857) by Prof. N.T.P. Lien and MSc. T.T. Ngat, Department of Insect Ecology, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology.

2.2. General experimental procedures

NMR spectra were taken on a Bruker AVANCE III HD 500 MHz or Bruker AVANCE NEO 600 MHz spectrometers using TMS as an internal standard. The mass spectra (ESI-MS) were obtained on an Agilent 1260 series single quadrupole LC/MS system. Column chromatography (CC) was carried out on silica gel (Merck, 230 - 400 mesh) or Sephadex® LH-20. Thin-layer chromatography was performed using precoated silica gel plates (Merck 60 F_{254}). Compounds were visualized by spraying with 10 % sulfuric acid and heating.

2.3. Extraction and isolation

The propolis of *Tetragonula laeviceps* (150 g) was macerated with ethanol (EtOH) 90 % at room temperature (4 times × 1.5 L for one day). The extracts were filtered, and EtOH was removed under reduced pressure. The residue was suspended in water (500 mL), and extracted with ethyl acetate (EtOAc). Evaporation of the organic solvent under vacuum afforded an EtOAc residue (103 g). The EtOAc residue (100 g) was subjected to silica gel CC and eluted

with a gradient solvent system of *n*-hexane/EtOAc (100:1 – 0:1) to give twelve fractions E1–E12, respectively. Fraction E2 (500 mg) was separated by silica gel CC and eluted with *n*-hexane/EtOAc (19:1, v/v) to give five fractions E2.1-E2.5. Fraction E2.4 (82 mg) was purified by silica gel CC using *n*-hexane/EtOAc (19:1, v/v) as eluent to yield compound **2** (5.9 mg). Fraction E6 (3.0 g) was chromatographed on silica gel CC eluting with *n*-hexane/acetone (9:1, v/v) to afford eight fractions E6.1–E6.8. Fraction E6.7 (150 mg) was purified by Sephadex® LH-20 CC and eluted with MeOH to give compound **5** (3.2 mg). Fraction E8 (9.2 g) was separated on silica gel CC and eluted with *n*-hexane/EtOAC (8: 2, v/v) to afford nine fractions E8.1–E8.9. Compound **3** (30.6 mg) was obtained from fraction E8.5 by crystallization in *n*-hexane. Fraction E9 (5.3 g) was subjected to silica gel CC and eluted with *n*-hexane/acetone (8:2, v/v) to give seven fractions E9.1–E9.7. Fraction E9.5 (1.88 g) was purified by Sephadex® LH-20 CC and eluted with MeOH/CH_2Cl_2 (8:2, v/v) to give compound **1** (5.2 mg). Fraction E9.6 (448 mg) was fractionated by Sephadex® LH-20 CC and eluted with MeOH/CH_2Cl_2 (8:2, v/v) to afford two sub-fractions E9.6.1-E9.6.2. The sub-fraction E9.6.2 (35.1 mg) was separated by silica gel CC and eluted with *n*-hexane/acetone (8:2, v/v) to yield compounds **8** (4.7 mg) and **9** (4.4 mg). Fraction E9.7 (173 mg) was separated on silica gel CC and eluted with *n*-hexane/acetone (9:1, v/v) to yield compound **6** (3.5 mg). Fraction E12 (4.5 g) was chromatographed on silica gel CC eluting with *n*-hexane/acetone (9:1, v/v) to afford sixteen fractions E12.1–E12.16. Fraction E12.12 (400 mg) was fractionated by silica gel CC eluting with *n*-hexane/acetone (8:2, v/v) to afford five sub-fractions E12.1-E12.5. Fraction E12.3 (180 mg) was purified by Sephadex® LH-20 CC and eluted with MeOH to yield compounds **4** (5 mg) and **7** (10.4 mg).

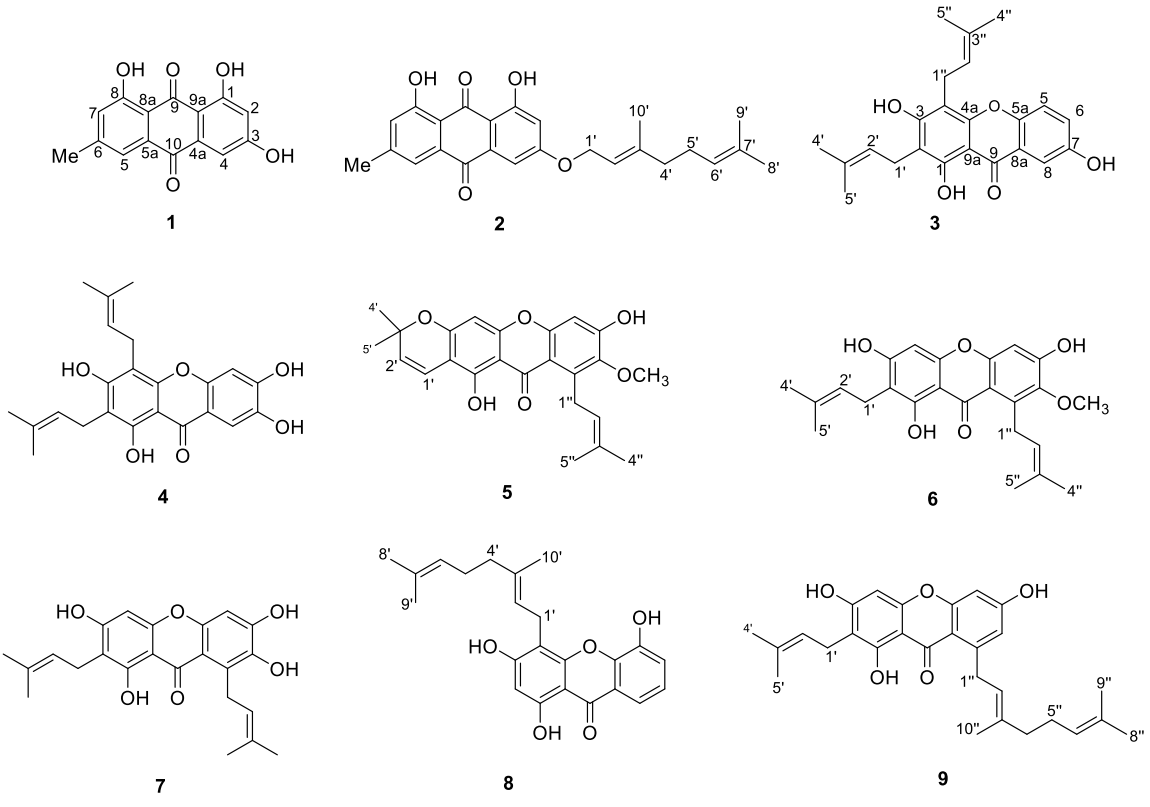

Figure 1. Chemical structures of compounds **1–9**.

Emodin (**1**): Red solid; ESI-MS *m/z*: 271 $[M+H]^+$, (calcd. for $C_{15}H_{11}O_5$ 271). ^1H and ^{13}C NMR (acetone-d_6): see Table 1.

3-Geranyloxyemodin (**2**): Brown solid; ESI-MS *m/z*: 407 $[M+H]^+$, (calcd. for $C_{25}H_{27}O_5$ 407). ^1H and ^{13}C NMR ($CDCl_3$): see Table 1.

1,3,7-Trihydroxy-2,4-*bis*(3-methylbut-2-enyl)xanthone (3): Yellow solid; ESI-MS *m/z*: 381 $[M+H]^+$, (calcd. for $C_{23}H_{25}O_5$ 380). ^1H and ^{13}C NMR ($CDCl_3$): see Table 2.

Cudratricusxanthone F (4): Yellow solid; ESI-MS *m/z*: 397 $[M+H]^+$, (calcd. for $C_{23}H_{25}O_6$ 396). ^1H and ^{13}C NMR (acetone-d_6): see Table 2.

9-Hydroxycalabaxanthone (5): Yellow solid; ESI-MS *m/z*: 409 $[M+H]^+$, (calcd. for $C_{24}H_{25}O_6$ 408). ^1H and ^{13}C NMR ($CDCl_3$): see Table 2.

***α*-Mangostin (6)**: Yellow solid; ESI-MS *m/z*: 411 $[M+H]^+$, (calcd. for $C_{24}H_{27}O_6$ 410). ^1H and ^{13}C NMR (CD_3OD): see Table 2.

γ-Mangostin (7): Yellow solid; ESI-MS *m/z*: 397 $[M+H]^+$, (calcd. for $C_{23}H_{25}O_6$ 396). ^1H and ^{13}C NMR (CD_3OD): see Table 3.

1,3,5-Trihydroxy-4-geranylxanthone (8): Yellow solid; ESI-MS *m/z*: 381 $[M+H]^+$, (calcd. for $C_{23}H_{25}O_5$ 380). ^1H and ^{13}C NMR (CD_3OD): see Table 3.

Pruniflorone I (9): Yellow solid; ESI-MS *m/z*: 449 $[M+H]^+$, (calcd. for $C_{28}H_{33}O_5$ 448). ^1H and ^{13}C NMR ($CDCl_3$): see Table 3.

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a red solid. The ^1H-NMR spectrum showed signals of four aromatic protons at δ_H 7.57 (1H, s, H-5), 7.26 (1H, d, J = 2.4 Hz, H-4), 7.14 (1H, s, H-7) and 6.65 (1H, d, J = 2.4 Hz, H-2); and a methyl singlet at δ_H 2.47 (3H, s). The ^{13}C-NMR spectrum revealed the presence of fifteen carbon signals, including two carbonyl signals at δ_C 191.5 (C-9) and 182.3 (C-10); twelve aromatic carbons from δ_C 167.0 to 108.8 ppm, and the methyl group at δ_C 21.9. The molecular formula of **1** was indicated as $C_{15}H_{10}O_5$ based on the *quasi*-molecular ion peak at *m/z* 271 $[M+H]^+$ in the ESI-MS spectrum and NMR data. Compound **1** was assigned as emodin (Figure 1) by comparison of the NMR and MS spectral data with those reported [13].

Compound **2** was isolated as a brown solid. The NMR spectra of **2** showed similar signals to those of compound **1** except for the addition of one geranyl group. The ^1H-NMR spectrum showed signals of four aromatic protons at δ_H 7.62 (1H, d, J = 1.0 Hz, H-5), 7.37 (1H, d, J = 2.4 Hz, H-4), 7.07 (1H, d, J = 1.2 Hz, H-7) and 6.68 (1H, d, J = 2.4 Hz, H-2); a methyl singlet at δ_H 2.44 (3H, s) and signals of a geranyl group at δ_H 4.68 (2H, d, J = 6.6 Hz, H-1′), 5.47 (1H, t, J = 6.6 Hz, H-2′), 2.17-2.10 (4H, m, H-4′, H-5′), 5.09 (1H, t, J = 6.6 Hz, H-6′), 1.78, 1.67 and 1.60 (each 3H, s, H-8′, H-9′, H-10′). The ^{13}C-NMR exhibited twenty-five carbon signals, including fifteen carbons of the emodin skeleton and ten signals of the geranyl group. The HMBC correlation of H-1′ (δ_H 4.68) to C-3 (δ_C 165.9) confirmed that the oxygeranyl group was linked to C-3 (Figure 2). The ESI-MS spectrum exhibited a *quasi*-molecular ion peak $[M+H]^+$ at *m/z* 407, corresponding to a molecular formula of $C_{25}H_{26}O_5$. Based on the spectral data, **2** was identified as 3-geranyloxyemodin or 3-geranyloxy-6-methyl-1,8-dihydroxyanthraquinone (Figure 1). The NMR data of **2** agreed with those published [14, 15].

Compound **3** was obtained as a yellow solid. The ^1H-NMR spectrum revealed signals of a hydroxyl group at δ_H 13.13 (1H, s, 1-OH), three protons of an ABX system at δ_H 7.60 (1H, d, J = 3.0 Hz, H-8), 7.37 (1H, d, J = 9.0 Hz, H-8), 7.26 (1H, dd, J = 9.0, 3.0 Hz, H-6) and proton

signals of two prenyl groups at δ_H 5.28 (2H, m, H-2′, H-2″), 3.54 (1H, d, J = 7.0 Hz, H-1′), 3.47 (1H, d, J = 7.0 Hz, H-1″), 1.88, 1.85, 1.77 and 1.73 (each 3H, s, H-4′, H-5′, H-4″, H-5″). The ^{13}C-NMR spectrum showed the presence of twenty-three carbon signals of a prenylated xanthone, including a carbonyl carbon at δ_C 180.9 (C-9), twelve carbon signals at the aromatic region, and ten carbon signals of the two prenyl groups at δ_C 22.1 (C-1′), 123.0 (C-2′), 132.5 (C-3′), 25.8 (C-4′), 18.0 (C-5′) and δ_C 22.4 (C-1″), 123.0 (C-2″), 132.3 (C-3″), 25.8 (C-4″), 18.1 (C-5″), respectively. The molecular formula of **3** was suggested as $C_{23}H_{24}O_5$ based on the *quasi*-molecular ion peak at *m/z* 365 $[M+H]^+$. The substitutions of the xanthone structure by the two prenyl groups at C-2 and C-4 were confirmed by HMBC correlations of H-1′ (δ_H 3.48) to C-2 (δ_C 109.2), C-3 (δ_C 161.1), C-1 (δ_C 159.2) and H-1″ (δ_H 3.54) to C-4 (δ_C 106.7), C-3, and C-4a (δ_C 153.9) (Figure 2). Compound **3** was identified as 1,3,7-trihydroxy-2,4-bis(3-methylbut-2-enyl)xanthone (Figure 1) by comparison of NMR data with those published in the literature [16, 17].

Table 1. ^{1}H and ^{13}C NMR data of anthraquinones **1** and **2**.

	1		**2**			**2**	
C	δ_H; mult.; J Hz	δ_C	δ_H; mult.; J Hz	δ_C	C	δ_H; mult.; J Hz	δ_C
1		166.3		165.1	1′	4.68; d, 6.6	65.8
2	6.65, d; 2.4	108.9	6.68, d; 2.4	107.5	2′	5.47; t; 6.6	118.0
3		167.0		165.9	3′	-	142.9
4	7.26; d; 2.4	109.9	7.37; d; 2.4	108.8	4′	2.17-2.10; m	39.5
4a		136.7		135.2	5′	2.17-2.10; m	26.2
5	7.57; s	121.4	7.62; d; 1.2	121.2	6′	5.09; t; 6.6	123.6
6		149.4		148.4	7′	-	132.0
7	7.14; s	124.9	7.07; d; 1.2	124.5	8′	1.74; s	25.6
8		162.3		162.5	9′	1.78; s	16.8
8a		114.5		113.3	10′	1.60; s	17.7
9		191.5		190.7			
9a		134.3		133.2			
10		182.3		182.0			
10a		110.2		110.1			
Me	2.47; s	21.9	2.47; s	22.9			

Compound **4** was isolated as a yellow solid. The ^{1}H-NMR spectrum revealed signals of a hydroxyl group at δ_H 13.13 (1H, s, 1-OH), two singlet protons at δ_H 7.54 (1H, s, H-8), 6.97 (1H, s, H-5), and proton signals of two prenyl groups at δ_H 5.23 (2H, m, H-2′, H-2″), 3.57 (1H, d, J = 7.2 Hz, H-1′), 3.43 (1H, d, J = 7.2 Hz, H-1″), 1.88, 1.78, 1.65 and 1.65 (each 3H, s, H-4′, H-5′, H-4″, H-5″). The ^{13}C-NMR spectrum also displayed twenty-three carbon signals of a prenylated xanthone, including a carbonyl carbon at δ_C 180.9 (C-9), twelve carbon signals in the aromatic region, and ten carbon signals of the two prenyl groups. The ESI-MS spectrum showed a protonated molecular ion peak at *m/z* 397 $[M+H]^+$, corresponding to a molecular formula of $C_{23}H_{24}O_6$. The NMR data of **4** were similar to those of compound **3**, except an aromatic proton signal (δ_H 7.26) in **3** was absent and replaced by a hydroxyl group in **4**. Compound **4** was determined as cudratricusxanthone F (Figure 1). Its NMR data followed those reported in a previous paper [18].

Compound **5** was isolated as a yellow solid. The ^{1}H-NMR spectrum exhibited signals of a prenylated xanthone with two aromatic protons at δ_H 6.83 (1H, s, H-5) and 6.24 (1H, s, H-4), proton signals of a prenyl group at δ_H 4.09 (2H, d, J = 6.0 Hz, H-1″), 5.26 (1H, m, H-2″), 1.83 and 1.69 (each 3H, s, H-4″, H-5″) and a methoxy group at δ_H 3.80 (3H, s). In addition, signals of a chromene moiety were observed at δ_H 6.73 (1H, d, J = 10.0 Hz, H-1′), 5.56 (1H, d, J = 10.0 Hz, H-2′), 1.46 (6H, s, H-4′, H-5′). The ^{13}C-NMR spectrum showed twenty-four carbon signals, including thirteen carbons of a xanthone skeleton, five signals of the prenyl group at δ_C 26.5 (C-1″), 123.1 (C-2″), 132.2 (C-3″), 25.8 (C-4″), 18.2 (C-5″), five signals of the chromene ring at δ_C 115.7 (C-1′), 127.1 (C-2′), 77.9 (C-3′), 26.3 (C-4′, C-5′) and the methoxy group at δ_C 62.0 ppm. Compound **5** had the molecular formula of $C_{24}H_{24}O_6$ based on the protonated ion peak at *m/z* 409 $[M+H]^+$ in the ESI-MS spectrum. The HMBC spectrum showed the correlations of 1-OH (δ_H 13.68) to C-1 (δ_C 157.9) and C-2 (δ_C 104.5); correlations of H-1′ (δ_H 6.73), H-2′ (δ_H 5.56) to C-2 (δ_C 104.5); correlations of H-1″ (δ_H 4.09), H-2″ (δ_H 5.26) to C-8 (δ_C 137.0); and the correlation of the methoxy group to C-7 (δ_C 137.0) (Figure 2). Compound **5** was assigned as 9-hydroxycalabaxanthone (Figure 1). Its NMR data matched well with previous data [7].

Table 2. ^{1}H and ^{13}C NMR data of xanthones **3–6**.

	3		**4**		**5**		**6**	
C	δ_H; mult.; J Hz	δ_C	δ_H; mult.; J Hz	δ_C	δ_H; mult.; J Hz	δ_C	δ_H; mult.; J Hz	δ_C
1		159.2		159.1		157.9		163.6
2		109.2		110.7		104.5		111.4
3		161.1		160.3		159.9		161.5
4		106.7		106.4	6.24; s	94.2	6.36; s	93.1
4a		153.9		153.8		156.3		157.9
5	7.37; d; 9.0	119.8	6.97; s	103.4	6.83; s	101.7	6.80; s	102.7
5a		150.8		152.7		151.7		156.7
6	7.26; dd; 9.0, 3.0	125.0		154.5		154.6		156.1
7		154.6		144.1		142.7		144.8
8	7.60; d; 3.0	110.8	7.54; s	109.0		137.0		138.4
8a		121.6		113.4		112.2		112.1
9		181.6		180.9		182.0		183.1
9a		103.6		103.3		103.8		103.7
1′	3.48; d; 7.0	22.1	3.43; d; 7.2	22.1	6.73;d;10.0	115.7	3.30; d; 7.5	22.2
2′	5.28; m	123.0	5.23; m	123.2	5.56;d;10.0	127.1	5.25; m	123.8
3′		132.5		132.3		77.9		131.6
4′	1.77 s	25.8	1.65; s	25.8	1.46; s	28.3	1.67; s	25.9
5′	1.85 s	18.0	1.78; s	17.9	1.46; s	28.3	1.79; s	17.9
1″	3.54; d; 7.0	22.4	3.57; d; 7.2	22.4	4.09; d; 6.0	26.5	4.09; d; 6.5	27.1
2″	5.25; m	123.0	5.23; m	123.2	5.26; m	123.1	5.25; m	125.1
3″		132.3		132.1		132.2		131.8
4″	1.74; s	25.8	1.65; s	25.8	1.69; s	25.8	1.69; s	25.8
5″	1.88; s	18.1	1.88; s	18.0	1.83; s	18.2	1.84; s	18.3
1-OH	13.13; s	-	13.51; s	-	13.68; s	-		
OMe					3.80; s	62.0	3.77; s	61.3

Compound **6** was obtained as a yellow solid. The NMR spectra also showed signals of a prenylated xanthone. The NMR data were similar to those of compound **5**, except the chromene ring was opened to the prenyl group. The ^{1}H-NMR spectrum showed signals of two aromatic protons at δ_H 6.80 (1H, s, H-5) and 6.36 (1H, s, H-4), a methoxy group at δ_H 3.77 (3H, s) and proton signals of two prenyl groups at δ_H 3.30 (2H, d, J = 6.5 Hz, H-1′), 5.25 (1H, m, H-2′), 1.79 (3H, s, H-5′), 1.67 (3H, s, H-4′) and 4.09 (2H, d, J = 6.5 Hz, H-1″), 5.25 (1H, m, H-2″), 1.84 (3H, s, H-5″), and 1.69 (3H, s, H-4″), respectively. The ^{13}C-NMR spectrum showed twenty-four carbon signals, including thirteen carbons of a xanthone skeleton, ten signals of two prenyl groups and the methoxy group at δ_C 61.3 ppm. The ESI-MS spectrum showed a protonated molecular ion peak at *m/z* 411 $[M+H]^+$, corresponding to a molecular formula of $C_{24}H_{26}O_6$. Compound **6** was identified as α-mangostin (Figure 1) by comparison of the NMR data with the previous paper [19].

The NMR spectra of compound **7** were similar to those of compound **6**, except a hydroxy group was placed at the C-6 position in **7** instead of the methoxy group in **6**. The ^{1}H-NMR spectrum showed signals of two aromatic protons at δ_H 6.67 (1H, s, H-5) and 6.26 (1H, s, H-4), and proton signals of two prenyl groups at δ_H 3.30 (2H, d, J = 7.0 Hz, H-1′), 5.27 (1H, m, H-2′), 1.80 (3H, s, H-5′), 1.68 (3H, s, H-4′) and δ_H 4.13 (2H, d, J = 6.5 Hz, H-1″), 5.25 (1H, m, H-2″), 1.85 (3H, s, H-5″), and 1.68 (3H, s, H-4″), respectively. The ^{13}C-NMR spectrum showed twenty-three carbon signals, including thirteen carbons of a xanthone skeleton and ten signals of two prenyl groups. Compound **7** was assigned as γ-mangostin (Figure 1). The NMR data were identical to those reported [20].

Compound **8** was obtained as a yellow solid. The ^{1}H-NMR spectrum displayed signals of a geranyl xanthone with four aromatic protons at δ_H 7.21 (1H, dd, J = 7.8, 1.8 Hz, H-8), 7.37 (1H, dd, J = 7.8, 1.8 Hz, H-6), 7.25 (1H, t, J = 7.8 Hz, H-7), 6.37 (1H, s, H-2), a signal of the hydroxyl group at δ_H 13.13 (1H, s, 1-OH) and signals of a geranyl group at δ_H 3.61 (2H, d, J = 7.2 Hz, H-1′), 5.40 (1H, t, J = 7.2 Hz, H-2′), 2.05-1.95 (4H, m, H-4′, H-5′), 5.05 (1H, m, H-6′), 1.86, 1.50 and 1.53 (each 3H, s, H-8′, H-9′, H-10′). The ^{13}C-NMR spectrum confirmed the presence of twenty-three carbon signals, including thirteen carbons of the xanthone structure and ten signals of the geranyl moiety. The ESI-MS spectrum showed a protonated molecular ion peak at *m/z* 381 $[M+H]^+$, corresponding to a molecular formula of $C_{23}H_{24}O_5$. Thus, compound **8** was elucidated as 1,3,5-trihydroxy-4-geranylxanthone by comparison of its NMR data with the previous literature [21].

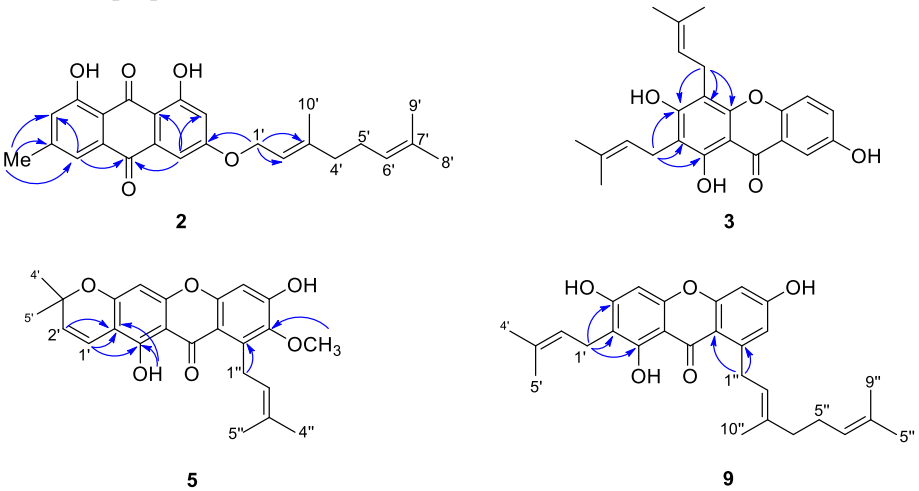

Figure 2. Key HMBC correlations of compound **2, 3, 5** and **9**.

Compound **9** was isolated as a yellow solid. The ^{1}H-NMR spectrum also revealed signals of a substituted xanthone with a signal of the hydroxyl group at δ_H 13.63 (1H, s, 1-OH); three aromatic protons at δ_H 7.21 (2H, s, H-5, H-7), 6.31 (1H, s, H-4); signals of a prenyl group at δ_H 3.46 (1H, d, J = 6.6 Hz, H-1′), 5.28 (1H, m, H-2′), 1.77 (3H, s, H-5′) and 1.65 (3H, s, H-5′); signals of a geranyl group at δ_H 4.32 (2H, d, J = 7.2 Hz, H-1″), 5.29 (1H, m, H-2″), 2.08-2.04 (4H, m, H-4″, H-5″), 5.04 (1H, m, H-6″), 1.86, 1.84 and 1.58 (each 3H, s, H-8″, H-9″, H-10″). The ^{13}C-NMR spectrum exhibited twenty-eight carbon signals, including thirteen carbons of the xanthone structure and fifteen signals of the prenyl and geranyl groups. The ESI-MS spectrum showed a protonated molecular ion peak at *m/z* 449 $[M+H]^+$, corresponding to a molecular formula of $C_{28}H_{32}O_5$. The locations of the prenyl and geranyl groups at C-2 and C-8 of the xanthone structure, respectively, were confirmed by HMBC correlations of H-1′ (δ_H 3.46) to C-2 (δ_C 108.4) and H-1″ (δ_H 4.32) to C-8 (δ_C 127.1) (Figure 2). The NMR and MS data analysis indicated that the chemical structure of **9** was pruniflorone I. The NMR data were in accordance with the previous study [22].

Table 3. ^{1}H and ^{13}C NMR data of xanthones **7**–**9**.

	7			**8**			**9**	
C	δ_H; mult.; J Hz	δ_C	C	δ_H; mult.; J Hz	δ_C	C	δ_H; mult.; J Hz	δ_C
1		161.5	1		162.3	1		160.7
2		111.1	2	6.36; s	98.6	2		108.4
3		163.3	3		164.2	3		162.2
4	6.26; s	92.9	4		107.8	4	6.31; s	93.2
4a		156.3	4a		155.5	4a		155.3
5	6.67; s	101.0	5		147.2	5	7.21; s	116.7
5a		154.3	5a		146.5	5a		151.3
6		153.3	6	7.37; dd; 7.8, 1.8	121.2	6		152.0
7		142.3	7	7.25; t; 7.8	125.1	7	7.21; s	123.7
8		129.1	8	7.65; dd, 7.8, 1.8	116.2	8		127.1
8a		111.8	8a		122.2	8a		118.5
9		183.5	9		183.5	9		183.5
9a		103.9	9a		103.6	9a		104.1
1′	3.30; d; 7.0	22.2	1′	3.61; d; 7.2	22.1	1′	3.46; d; 6.6	21.4
2′	5.27; m	124.9	2′	5.40; t; 7.2	123.4	2′	5.28; m	121.4
3′		131.7	3′		135.4	3′		135.7
4′	1.68; s	17.9	4′	1.96; m	40.5	4′	1.77; s	25.8
5′	1.80; s	26.0	5′	2.05; m	27.3	5′	1.84; s	17.9
1″	4.13; d; 6.5	26.6	6′	5.04; m	124.5	1″	4.32; d; 7.2	25.8
2″	5.25; m	123.9	7′		131.5	2″	5.29; m	121.4
3″		131.6	8′	1.53; s	25.7	3″		138.7
4″	1.68; s	18.3	9′	1.86; s	16.3	4″	2.08-2.05; m	39.7
5″	1.85; s	25.9	10′	1.50; s	17.6	5″	2.08-2.05; m	26.4
						6″	5.04; m	123.8
						7″		132.0
						8″	1.65; s	25.6
						9″	1.87; s	16.4
						10″	1.58; s	17.7

Among nine isolated compounds, xanthones **5**–7 were found in *T. laeviceps* propolis in Thailand [7], while xanthones **3**, **4**, **8** and **9** were isolated for the first time. Emodin (**1**) has been found in honeybee propolis in Cyprus and Greece [23], but anthraquinones emodin (**1**) and 3-geranyloxyemodin (**2**) were discovered in stingless bee propolis for the first time.

4. CONCLUSIONS

A chemical study of the propolis of *Tetragonula laeviceps* stingless bee collected in Hoa Binh province led to the isolation of nine phenolic compounds **1**–**9**. This is the first study of *T. laeviceps* propolis in Viet Nam. Anthraquinones **1**, **2** and xanthones **3**, **4**, **8** and **9** were found in stingless bee propolis for the first time. Further studies on the chemical composition and biological activity of different kinds of stingless bee propolis in Viet Nam will be reported in due course.

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Declaration of competing interest. The authors declare that they have no conflict of interests.

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