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Original Research Article

Triterpenes and their Biological Activities from Propolis of Stingless Bee *Tetrigonilla collina*

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ABSTRACT

Propolis from stingless bees has been used in traditional medicine in many countries to treat diseases. Recently, chemical constituents and biological activities of stingless bee propolis have garnered increasing scientific attention. A phytochemical study of propolis of stingless bee *Tetrigonilla collina* collected in the Kon Ka Kinh forest-Vietnam, was performed using combined chromatographic methods. Six triterpenes including five oleanane-type triterpenes: —uncarinic acid M (**1**), 3-oxo-6*β*-hydroxy-12-oleanen-28-oic acid (**2**), 3*β*,6*β*,19*α*-trihydroxy-12-oleanen-28-oic acid (**3**), sumaresinolic acid (**4**), and siaresinolic acid (**5**),—and one dammarane-type triterpene isofouquirenone (**6**) were isolated. The chemical structures of the compounds **1**-**6** were elucidated by NMR and MS spectroscopic analysis. These triterpenes were identified for the first time in stingless bee propolis. The chemical composition suggests that *Styrax tonkinensis* is a botanical source of stingless bee propolis. The isolated compounds were evaluated for their cytotoxic, antimicrobial and anti-inflammatory effects. Compound **2** displayed antimicrobial activity against Gram-positive strains *Bacillus subtilis* and *Lactobacillus fermentum*. Sumaresinolic acid (**4**) exhibited anti-inflammatory activity with the IC_{50} values of 21.38±2.0 µg/mL. Compounds **1**, **2**, **4** and **6** showed weak to moderate cytotoxicity against HepG-2, KB, and A-549 cell lines.

Keywords: *Tetrigonilla collina*, Oleanane, Dammarane, Cytotoxic, Anti-inflammation.

Introduction

Stingless bees (Hymenoptera: Apidae: Meliponini) represent a diverse group of eusocial insects found predominantly in tropical and subtropical regions of the world. Unlike their stinging counterparts in the genus *Apis*, stingless bees lack a functional sting, relying instead on alternative defense mechanisms such as biting and the secretion of caustic substances. With over 500 described species distributed across South America, Africa, Asia, and Australia, stingless bees play a vital ecological role as pollinators of native flora and agricultural crops.[1-2] In addition, stingless bees, particularly from Southeast Asian countries and Australia, have garnered increasing scientific attention due to the medicinal properties of their hive products, particularly propolis—a resinous substance collected from plant exudates.[3-16] Stingless bee propolis has long been used in traditional medicine in various cultures to treat ailments and boost immunity. Propolis produced by stingless bees exhibits significant chemical diversity and biological activity. These characteristics are attributed to the diverse botanical sources utilized by different stingless bee species, which vary by geography and ecology.[3-4]

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Previous phytochemical studies have identified numerous natural compounds in stingless bee propolis, including flavonoids, coumarins, xanthones, phenolic acids, and terpenoids. Pharmacological studies demonstrated antimicrobial, antioxidant, anti-inflammatory, and anticancer properties.[3-16] *Tetrigonilla collina* (Smith, 1857) is a stingless bee species native to Southeast Asia, including Indonesia, Laos, Malaysia, Myanmar, and Vietnam. It typically builds underground nests, often within termite colonies.[17] However, no chemical or biological studies had been conducted on the propolis of *T. collina* until recently. Chemical studies of propolis of several stingless bee species in Vietnam have been reported by our research group.[18-24] Recently, we have documented preliminary results of *T. collina* propolis constituents, isolating six phenolic compounds: siamyl benzoate, pinoresinol, coniferyl aldehyde, *p*-coumaric aldehyde, *trans*-cinnamic acid, and vanillin.[25] In our continuing search for bioactive compounds from stingless bee propolis in Vietnam, in this investigation, we isolated six triterpenes **1**-**6** for the first time from propolis. The structures of isolated compounds were determined by NMR and MS spectral data. The plant sources for *T. collina* propolis were discussed and *Styrax tonkinensis* was suggested as a botanical origin for stingless bee propolis. The isolated compounds were evaluated for cytotoxic, antimicrobial and NO production inhibitory effects.

Materials and Methods

Propolis material

The propolis sample was obtained from a hive of stingless bee in the Kon Ka Kinh forest (14°12'45.6"N, 108°17'14.1"E), Gia Lai province, Vietnam, in 2023. The stingless bee species was determined as *Tetrigonilla collina* (Smith, 1857) by Institute of Biology, Vietnam Academy of Science and Technology (VAST). The specimen (voucher

SB-GL-01) of the stingless bee is preserved at the Institute of Biology, VAST.

General experimental procedures

NMR spectra were recorded using either Bruker AVANCE III HD 500 MHz or Bruker AVANCE NEO 600 MHz spectrometers (Bruker, Billerica, MA, USA), with tetramethylsilane (TMS) used as the internal standard. High-resolution mass spectra (HR-ESI-MS) were performed with a Thermo LTQ Orbitrap XL mass spectrometer (Thermo Fisher, Germany). ESI-MS analyses were obtained on an Agilent 1260 series single quadrupole LC/MS system (Agilent Technologies, Palo Alto, CA, USA). Column chromatography (CC) was conducted using normal phase silica gel (230-400 mesh, Merck, Darmstadt, Germany), YMC RP-18 (120 Å, 150 µm, Tokyo, Japan) and Sephadex® LH-20 (Sigma Aldrich). Thin layer chromatography (TLC) was performed on silica gel 60 GF_{254} and RP-18 GF_{254s} plates (Merck, Darmstadt, Germany). Compounds visualized by spraying with 10% sulfuric acid and heating. All solvents used were of laboratory grade and were distilled prior to use.

Extraction and Isolation

The propolis of *T. collina* (410 g) were extracted with 70% EtOH for four times (5 L, 2 days, each time) at room temperature to obtain a crude EtOH extract. The crude extract was suspended in water and extracted with ethyl acetate (EtOAc) to yield an EtOAc (252 g) fraction. The EtOAc fraction (250 g) was separated on a normal phase silica gel column and eluted with a gradient of *n*-hexane/EtOAc (from 100:1 to 0:100) to obtain 15 fractions (F1-F15). Fraction F7 (5.4 g) was purified by silica gel column chromatography (CC) using n-hexane/EtOAc (9:1, *v*/*v*) as the eluent to obtain 11 fractions (F7A-F7K). Compound **4** (15.3 mg) was obtained from Fr.F7J. Fr.F7I was further purified by silica gel CC and eluted with n-hexane/EtOAc (8:2, *v*/*v*) to afford six subfractions (F7I1-F7I6). Fr. F7I2 (22 mg) was separated by silica gel CC using n-hexane/EtOAc (8:2, *v*/*v*) as the eluent to afford **5** (3.2 mg). Fr.F7I6 was chromatographed on silica gel CC and eluted with CH_2Cl_2/EtOAc (9:1, *v*/*v*) to give 6 fractions (F7I6A-F7I6F). Fr. F7I6F (16 mg) was purified by silica gel CC using n-hexane/EtOAc (8:2, *v*/*v*) as the eluent to obtain **1** (4.2 mg). Fr.F7I7 (0.454 mg) was separated into 8 fractions (F7I7A-F7I7H) by silica gel CC, eluted with n-hexane/EtOAc (8:2, *v*/*v*). Fr.F7I7G was fractionated by Sephadex LH-20 CC eluted with MeOH to yield **6** (2.3 mg). Fraction F9 (5.0 g) was chromatographed by silica gel CC and eluted with CH_2Cl_2/EtOAc (50:1, *v*/*v*) to afford 8 fractions F9A-F9H. Fr.F9F (1.5 g) was separated by silica gel CC using CH_2Cl_2/EtOAc (40:1, v/v) to yield three fractions F9F1-F9F3. Fraction F91F3 (52 mg) was further purified by reversed-phase *silica gel* CC using acetone/H_2O (2:1, *v*/*v*) to give **3** (2.8 mg). Fraction F11 was applied to silica gel CC and eluted with a gradient elution of n-hexane/acetone (from 30:1 to 0:1) to afford 9 fractions (F11A-F11I). Fr.F11D was chromatographed on silica gel CC using CH_2Cl_2/EtOAc (20:1) to afford 4 fractions (F11D1-F11D4). Fraction F11D4 (52 mg) was purified by reversed-phase silica gel CC, eluted with acetone/H_2O (2:1, v/v) to give compound **2** (4.1 mg).

Assay for Inhibition of NO Production.

The anti-inflammatory activity of isolated triterpenes **1**–**6** was assessed by measuring the inhibition of NO production in LPS-stimulated RAW264.7 cells (ATCC®-TIB-71™).[26-27] RAW264.7 cells were seeded in 96-well plates at 2×10^5 cells/well and incubated for 24 hours. After pretreatment with various concentrations of the test samples for 30 minutes, the cells were further incubated for 24 hours with or without 1 µg/mL LPS. Nitrite concentration in the culture supernatant–an indicator of NO synthesis, was determined using Griess method. Briefly, 100 µL of the supernatant was transferred to a 96-well plate, mixed with 100 µL of Griess reagent (Sigma-Aldrich), and the absorbance was measured at 570 nm using a microplate reader. NG-Monomethyl-L-Arginine (L-NMMA, Sigma-Aldrich) served as the positive control.

Cytotoxicity assay

Cytotoxicity assays were performed on A-549, HepG-2 and KB cell lines (ATCC). Cells were cultured in Dulbecco's D-MEM medium (Gibco) supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 UI/mL penicillin G, 100 µg/mL streptomycin, and 10 µg/mL gentamicin. Stock solutions of compounds were prepared in DMSO/H_2O (1:9) (Merck, Germany), and the assays were conducted using a previously published method.[19] Cancer cells (3×10^3 cells/mL) were incubated for 72 hours at 37 °C in a 95:5 air/CO_2 atmosphere, with or without test compounds. Cell growth was assessed colorimetrically at 540 nm using a Titertek Multiskan photometer. Ellipticine (Sigma-Aldrich) used as the reference compound.

Antimicrobial activity assay

The antimicrobial activity of isolated triterpenes was tested against three Gram-positive bacterial strains (*Staphylococcus aureus*, *Lactobacillus fermentum, Bacillus cereus*), three Gram-negative bacterial strains (*Escherichia coli*, *Salmonella enterica*, *Pseudomonas aeruginosa*), and fungus *Candida albicans* (all obtained from ATCC), as previously reported.[19] Compound solutions were prepared in dimethyl sulfoxide (Merck, Germany) at varying concentrations (128 µg/mL, 32 µg/mL, 8 µg/mL, 2 µg/mL, and 0.5 µg/mL). Ampicillin, cefotaxim, and nystatin were used as positive controls for Gram-positive bacteria, Gram-negative bacteria, and *C. albicans*, respectively. The inhibitory effects of the isolated compounds were determined as IC_{50} values.

Statistical Analysis

The biological assays were performed in triplicate. IC_{50} values (half-maximal inhibitory concentration) are presented as mean ± SD (standard deviation), calculated using Microsoft Excel 2013.

Results and Discussion

Uncarinic acid M (**1**)*:* White amorphous powder, HR-ESI-MS: *m/z* 487.3399 $[M+H]^+$ (calcd. for $C_{30}H_{47}O_5^+$ 487.3418). ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H 4.49 (1H, br s, H-6), 5.49 (3H, s, H-12), 3.11 (1H, br s, H-18), 3.35 (1H, d, *J* = 4.2 Hz, H-19), 1.17 (3H, s, H-23), 1.42 (3H, s, H-24), 1.50 (3H, s, H-25), 1.13 (3H, s, H-26), 1.23 (3H, s, H-27), 0.97 (3H, s, H-29), 0.97 (3H, s, H-30). ^{13}C-NMR (125 MHz, $CDCl_3$) see Table 1.

3-Oxo-6*β*-hydroxylean-12-en-28-oic acid (**2**)*:* White solid, ESI-MS: *m/z* 471 $[M+H]^+$. ^{1}H-NMR (600 MHz, DMSO-d_6) δ_H 4.30 (1H, d, *J* = 3.6 Hz, H-6), 5.20 (1H, t, *J* = 3.6 Hz, H-12), 1.04 (3H, s, H-23), 0.87 (3H, s, H-24), 1.41 (3H, s, H-25), 1.03 (3H, s, H-26), 1.03 (3H, s, H-27), 0.86 (3H, s, H-29), 1.30 (3H, s, H-30). ^{13}C-NMR (125 MHz, DMSO-d_6) see Table 1.

3*β*,6*β*,19*α*-Trihydroxy-12-oleanen-28-oic acid (**3**)*:* White amorphous powder, ESI-MS: *m/z* 489 $[M+H]^+$. ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H 4.49 (1H, br s, H-6), 5.49 (1H, s, H-12), 3.11 (1H, br s, H-18), 3.35 (1H, d, *J* = 4.2 Hz, H-19), 1.17 (3H, s, H-23), 1.42 (3H, s, H-24), 1.50 (3H, s, H-25), 1.13 (3H, s, H-26), 1.23 (3H, s, H-27), 0.97 (3H, s, H-29), 0.97 (3H, s, H-30). ^{13}C-NMR (125 MHz, $CDCl_3$) see Table 1.

Sumaresinolic acid (**4**)*:* White amorphous powder, ESI-MS: *m/z* 473 $[M+H]^+$. ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H 3.15 (1H, dd, 10.8, 5.4 Hz, H-3), 4.54 (1H, br s, H-6), 5.33 (1H, t, *J* = 3.6 Hz, H-12), 2.84 (1H, dd, *J* =13.8, 3.6 Hz, H-18), 1.08 (3H, s, H-23), 1.18 (3H, s, H-24), 1.30 (3H, s, H-25), 1.08 (3H, s, H-26), 1.10 (3H, s, H-27), 0.90 (3H, s, H-29), 0.93 (3H, s, H-30). ^{13}C-NMR (125 MHz, $CDCl_3$) see Table 1.

Siaresinolic acid (**5**)*:* White solid, ESI-MS: *m/z* 473 $[M+H]^+$. ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H 3.22 (1H, dd, *J* = 10.8, 4.8 Hz, H-3) 5.20 (1H, t, H-12), 3.09 (1H, br s, H-18), 3.34 (1H, d, *J* = 3.6 Hz, H-19), 0.99 (3H, s, H-23), 0.79 (3H, s, H-24), 0.90 (3H, s, H-25), 0.72 (3H, s, H-26), 1.25 (3H, s, H-27), 0.98 (3H, s, H-29), 0.96 (3H, s, H-30). ^{13}C-NMR (125 MHz, $CDCl_3$) see Table 1.

Isofouquirenone (**6**)*:* White amorphous powder, ESI-MS: *m/z* 459 $[M+H]^+$. ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H 1.04 (3H, s, H-18), 0.95 (3H, s, H-19), 1.26 (3H, s, H-21), 5.70 (2H, m, H-23, H-24), 1.33 (6H, s, H-26, H-27), 1.14 (3H, s, H-28), 1.08 (3H, s, H-29), 0.88 (3H, s, H-30). ^{13}C-NMR (125 MHz, $CDCl_3$) see Table 1.

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Table 1: ^{13}C-NMR data of isolated compounds **1-6**

C	1	2	3	4	5	6
	δ_C[a]	δ_C[b]	δ_C[a]	δ_C[a]	δ_C[a]	δ_C[a]
1	41.3	42.0	40.5	40.6	38.2	39.8
2	34.3	34.1	27.3	27.3	28.0	34.0
3	216.4	216.0	79.1	79.1	79.0	217.7
4	48.8	48.4	39.6	39.6	39.6	47.3
5	56.9	55.9	55.9	55.7	55.3	55.3
6	69.4	67.2	68.7	68.7	18.5	19.6
7	40.5	39.9	40.3	40.5	32.5	34.5
8	38.9	38.3	38.8	38.4	38.8	40.2
9	47.6	46.8	48.2	48.0	47.8	49.9
10	36.6	36.1	36.7	36.5	37.2	36.8
11	23.7	22.8	23.6	23.3	23.6	21.9
12	125.1	121.8	125.4	122.9	125.4	27.4
13	142.0	143.5	141.7	142.8	142.5	43.3
14	42.1	42.0	41.9	42.3	41.2	50.2
15	27.8	27.3	28.1	27.6	27.5	31.0
16	27.5	23.1	27.5	23.0	23.8	27.5
17	45.3	45.7	45.2	46.5	45.3	49.8
18	43.6	41.0	43.6	41.1	43.5	15.9
19	81.6	45.8	81.6	45.9	81.6	15.1
20	34.7	30.5	34.6	30.9	34.6	74.9
21	27.9	33.5	27.9	33.9	27.1	25.8
22	32.3	32.3	32.4	32.3	32.5	43.3
23	25.6	25.7	27.8	27.9	28.1	122.2
24	23.9	23.5	17.1	17.1	15.5	141.9
25	16.3	15.9	16.8	16.9	15.1	70.6
26	18.0	18.2	17.8	18.3	17.2	29.8
27	24.9	25.4	25.0	25.9	25.0	29.9
28	181.5	178.8	181.6	182.2	183.2	26.6
29	28.1	33.0	27.9	33.1	28.0	20.9
30	24.5	23.5	24.5	23.5	24.4	16.2

[a]: measured in $CDCl_3$,125 MHz,; [b]: measured in DMSO-d_6,125 MHz

Identification of isolated compounds (Figure 1).

Compound **1** was isolated as a white powder. Its HR-ESI-MS spectrum (positive mode) showed a protonated molecular ion peak $[M+H]^+$ at *m/z* 487.3399 (calcd. *m/z* $[C_{30}H_{47}O_5]^+$ 487.3418), establishing a molecular formula of $C_{30}H_{47}O_5$. The ^{1}H, ^{13}C NMR and HSQC spectra displayed the characteristic signals of an oleanane-type triterpene with seven methyl singlets at δ_H 1.17 (3H, s, H-23)/δ_C 25.6 (C-23), 1.42 (3H, s, H-24)/δ_C 23.9 (C-24), 1.50 (3H, s, H-25)/δ_C 16.3 (C-25), 1.13 (3H, s, H-26)/δ_C 18.0 (C-26), 1.23 (3H, s, H-27)/δ_C 24.9 (C-27), 0.97 (3H, s, H-29)/δ_C 28.1 (C-29), and 0.97 (3H, s, H-30)/δ_C 24.5 (C-30). An olefinic proton at δ_H 5.49 (H, br s, H-12) indicated a double bond between C-12 (δ_C 125.1) and C-13 (δ_C 142.0). Additionally, two hydroxymethine groups were observed at δ_H 4.49 (1H, br s, H-6)/δ_C 69.4 (C-6) and 3.35 (1H, d, J = 4.2 Hz, H-19)/δ_C 81.6 (C-19). The ^{13}C NMR spectrum exhibited thirty carbon signals, including ketone and carboxylic carbons at C-3 (δ_C 216.4) and C-28 (δ_C 181.6), respectively.

Functional group locations in the oleanane skeleton were determined by ^{1}H-^{1}H COSY and HMBC spectra. HMBC correlations of H-23 (δ_H 1.17), H-24 (δ_H 1.42) and H-2 (δ_H 1.42) with C-3 (δ_C 216.4) confirmed the C-3 ketone. The C-19 hydroxyl group was identified through HMBC correlations of H-29 and H-30 (δ_H 0.97) to C-19 (δ_C 81.6) and a ^{1}H-^{1}H COSY correlation of H-18 (δ_H 3.11) and H-19 (δ_H 3.35). The ^{1}H-^{1}H COSY spectrum further showed the spin systems for H-1/H-2, H-5/H-6/H-7, H-15/H-16, H-18/H-19 and H-21/H-22 systems (Figure 2). The relative configurations of hydroxyl groups at C-6 and C-19 were established based on coupling constants and NOESY data. Proton H-6 appeared as broad singlet, and a NOESY cross-peak between H-6 (δ_H 4.49) and H-5 (δ_H 1.21) confirm the α-orientation. In contrast, the NOESY correlations of H-18 (δ_H 5.70) with H-19 (δ_H 5.32) and H-12 (δ_H 5.49), coupled with the small coupling constant of H-19 (J = 4.2 Hz) suggested β-orientation of proton H-19. Consequently, **1** was identified as 3-oxo-6β-19α-dihydroxy-olean-12-en-28-oic acid (uncarinic acid M).[28]

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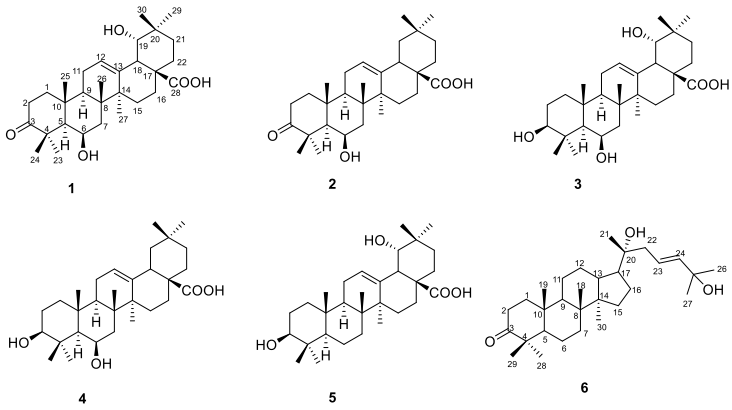

Figure 1: Chemical structures of isolated compounds **1-6** from *T. collina* propolis

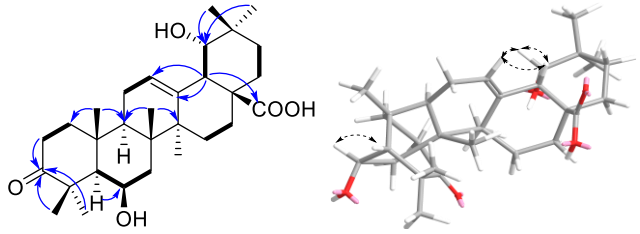

Figure 2: Selected COSY (▬), HMBC (⟶) and NOESY (⋯➤) correlations of **1**

Similarly, the chemical structures of oleanane-type triterpenes **2-5** were elucidated as 3-oxo-6β-hydroxy-12-oleanen-28-oic acid (**2**),[29] 3β,6β,19α-trihydroxy-12-oleanen-28-oic acid (**3**),[30] sumaresinolic acid (**4**),[31] and siaresinolic acid (**5**)[32] by comparison of their ^{1}H and ^{13}C NMR data (Table 1) with literatures values.

Compound **6** was isolated as a white powder. The ^{1}H, ^{13}C NMR and HSQC spectra showed signals of eight methyl singlets at δ_H 1.04 (3H, s, H-18)/δ_C 15.9 (C-18), 0.95 (3H, s, H-19)/δ_C 15.1 (C-19), 1.26 (3H, s, H-21)/δ_C 25.8 (C-21), 1.33 (6H, s, H-26, H-27)/δ_C 29.8 and 29.9 (C-26, C-27), 1.14 (3H, s, H-28)/δ_C 26.6 (C-28), 1.08 (3H, s, H-29)/δ_C 20.9 (C-29), and 0.88 (3H, s, H-30)/δ_C 16.2 (C-30). The ^{1}H spectrum exhibited two olefinic protons at δ_H 5.70 (2H, m, H-23, H-24), assigned to a double bond between C-23 (δ_C 122.2) and C-24 (δ_C 141.9). The ^{13}C NMR and HSQC spectra exhibited thirty carbon signals comprising eight methyl carbons, nine methylene carbons, six methine groups and seven quaternary carbons, including a ketone at δ_C 217.7 (C-3) and two oxygenated carbons at δ_C 74.9 (C-20) and 70.6 (C-25). The molecular formula of $C_{30}H_{50}O_3$ was deduced from NMR data and ESI-MS spectrum (protonated ion at *m/z* 459). HMBC spectroscopy determined functional group locations: correlations of H-28 (δ_H 1.14), H-29 (δ_H 1.08) and H-2 (δ_H 2.51-2.41) with C-3 (δ_C 217.7) indicated C-3 ketone group. Hydroxyl groups located at C-20 and C-25 were identified by HMBC correlations of H-21 (δ_H 1.26) and C-20 (δ_C 74.9), and H-26 and H-27 (δ_H 1.33) to C-25 (δ_C 70.6), respectively. The C-23/C-24 double bond was confirmed by HMBC cross-peaks of H-26 and H-27 (δ_H 1.33) to C-24 (δ_C 141.9) (Figure 3). Compound **6** was determined as isofouquirenone by comparison of its NMR data with those reported in the previous study.[33]

Plant sources for propolis

All the isolated triterpenes **1-6** were isolated from stingless bee propolis for the first time. Among the isolated oleanane-type triterpenes **1-5**, compound **1** was previously reported only in *Uncaria rhynchophylla* (Rubiaceae family)[28] while compound **3** was isolated from *Uncaria macrophylla*[30] and *Antirhea chinensis* (Rubiaceae family).[34] Triterpenes **2**, **4** and **5** are distributed across diverse plant families including Altingiaceae, Anacardiaceae, Adoxaceae, Celastraceae, Ericaceae, Fabaceae, Lamiaceae, Melastomataceae, Rubiaceae, Plantaginaceae, Rosaceae and Styracaceae.[28-52] Notably, compounds **2**, **4**, and **5** have been simultaneously identified from *Styrax tonkinensis* (Styracaceae family) (Table 2).[31] Although compounds **1** and **3** were exclusively reported in Rubiaceae species, both feature a C-6 hydroxy group – a structural characteristic of *Styrax* triterpenes.[53] In addition, our previous study of this propolis revealed phenolic compounds (e.g., siamyl benzoate, pinoresinol, coniferyl aldehyde and *trans*-cinnamic acid) also present in *S. tonkinensis*, which is widely distributed in many regions of Vietnam.[25,54-55] Collectively, these finding indicate *S. tonkinensis* as primary botanical source for the *T. collina* propolis. *S. tonkinensis* have been previously reported as the resin source for *Apis melifera* propolis in Thailand.[56]

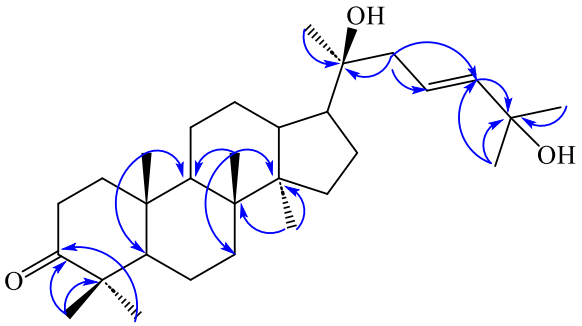

Figure 3: Selected HMBC (⟶) correlations of **6**

Dammarane triterpene isofouquirenone (**6**) has been identified in *Aglaia sp., Ailanthus altissima* and *Commiphora dalzielii.*[33,57-60] This finding aligns with our prior investigations of *Homotrigona apicalis* and *Tetragonula iridipennis* propolis from Binh Dinh province, which established *Aglaia sp.* or *Ailanthus sp.*, respectively, as botanical sources for stingless bee propolis.[19-20] *Aglaia* and *Ailanthus* species also occur in Kon Ka Kinh National park (Gia Lai province, Vietnam),[55] where the studied *T. collina* propolis was collected. Thus, *Aglaia sp.* or *Ailanthus sp.* likely present additional resin sources for stingless bees in this region.

All isolated compounds were evaluated for cytotoxic, anti-inflammatory and antimicrobial activities. The cytotoxicity of isolated compounds was tested against KB, HepG-2, and MCF-7 cancer cell lines (Table 3). Compound **1**, **2**, **4** and **5** showed weak to moderate activity (IC_{50} range from 70.92±3.67 µg/mL to 113.45±4.11 µg/mL). Compound **2** was the most potent on KB cell lines with IC_{50} of 70.92±3.67 µg/mL. Several previous studies have described the cytotoxicity of isolated compounds **1**–**6**. Compound **1** was inactive in the cytotoxic assay against human cancer cells U251, HepG2, HGC27, MCF7, and HCT-116.[28] Compound **3** exhibited weak cytotoxicity on HepG-2 and MCF-7 cell lines in the Yuan's study.[30] Jing *et al*. have reported that compounds **2**, **4** and **5** demonstrated moderate cytotoxic activities to human leukemia HL-60 cells with IC_{50} values from 29.0 µM to 41.0 µM.[31]

In NO production assay (LPS-stimulated RAW264.7 cells), only sumaresinolic acid (**4**) showed significant activity (IC_{50} = 21.38±2.0 µg/mL, vs. L-NMMA positive control IC_{50} = 4.08±0.5 µg/mL). Other compounds were weakly active or inactive (IC_{50} > 128 µg/mL). To the best of our knowledge, this is the first report of NO inhibitory activity of compounds **1**–**6**.

Table 2: The distribution of triterpenes **1**-**6** in plant species and families

Compounds	Plant name	Family	References
1	*Uncaria rhynchophylla*	Rubiaceae	[28]
2	*Liquidamber styraciflua*	Altingiaceae	[29]
	Semiliquidambar cathayensis	Altingiaceae	[35]
	Entada phaseoloides	Fabaceae	[36]
	Orthopterygium huancuy	Anacardiaceae	[37]
	Styrax tonkinensis	Styracaceae	[31]
	Rhus chinensis	Anacardiaceae	[38]
3	*Uncaria macrophylla*	Rubiaceae	[30]
	Antirhea chinensis	Rubiaceae	[34]
4	*Styrax tonkinensis*	Styracaceae	[31]
	Miconia fallax	Melastomataceae	[39]
	Morinda officinalis var. officinalis	Rubiaceae	[40]
	Miconia albicans	Melastomataceae	[41]
	Spermacoce latifolia	Rubiaceae	[42]
	Celastrus angulatus	Celastraceae	[43]
	Salvia sclareoides	Lamiaceae	[44]
	Viburnum aboricolum	Adoxaceae	[45]
	Limnophila geoffrayi	Plantaginaceae	[46]
	Enkianthus campanulatus	Ericaceae	[47]
	Coffea canephora	Rubiaceae	[48]
5	*Sanguisorba officinalis*	Rosaceae	[31]
	Ilex cornuta	Aquifoliaceae	[49]
	Ilex kudingcha	Aquifoliaceae	[50]
	Styrax tonkinensis	Styracaceae	[31]
	Entada phaseoloides	Fabaceae	[36]
	Callicarpa cathayana	Lamiaceae	[51]
	Santisukia pagetii	Bignoniaceae	[52]
6	*Commiphora dalzielii*	Burseraceae	[33]

Aglaia perviridis	Meliaceae	[57]
Aglaia rubiginosa	Meliaceae	[58]
Ailanthus altissima	Simaroubaceae	[59]
Aglaia odorata var. microphyllina	Meliaceae	[60]

Table 3: Anti-inflammatory and cytotoxic activity of isolated compounds

Compounds	IC_{50} (µg/mL)	IC_{50} (µg/mL)		
	NO production inhibition	KB	HepG2	A549
1	>128	107.79±7.15	102.96±5.90	113.45±4.11
2	>128	70.92±3.67	101.52±4.68	100.57±2.77
3	>128	>128	>128	>128
4	21.38±2.0	101.82±4.11	102.15±5.22	85.05±3.57
5	112.52±5.56	>128	>128	>128
6	>128	110.32±3.57	104.53±3.02	113.07±3.02
L-NMMA	4.08±0.5			
Ellipticine		0.46±0.02	0.46±0.02	0.45±0.02

As shown in Table 4, only compound **2** exhibited antimicrobial activity against Gram-positive strains *B. subtilis* and *L. fermentum* with IC_{50} values of 20.24±1.45 and 15.54±0.98 µg/mL. Although Zhang *et al*. reported sumeresinolic (**4**) has mild antibacterial activity against *Mycobacterium smegmatis* and *B. subtilis* with MIC values of 39.1 and 21.0 µM,[36] it was inactive in our assay. Compounds **1**, **3**, **5** and **6** were inactive in the test (IC_{50} >128 µg/mL).

Table 4: Antimicrobial activity of isolated compounds **1**-**6**

Compounds	IC_{50} (µg/mL)						
	S. aureus	*B. subtilis*	*L. fermentum*	*S. enterica*	*E. coli*	*P. aeruginosa*	*C. albican*
1	>128	>128	>128	>128	>128	>128	>128
2	>128	20.24 ± 1.45	15.54± 0.98	>128	>128	>128	>128
3	>128	>128	>128	>128	>128	>128	>128
4	>128	>128	>128	>128	>128	>128	>128
5	>128	>128	>128	>128	>128	>128	>128
6	>128	>128	>128	>128	>128	>128	>128
Ampicillin	0.02 ± 0.005	3.62± 0.15	1.03 ± 0.07				
Cefotaxime				0.43 ± 0.05	0.007 ± 0.002	4.34 ± 0.15	
Nystatin							1.32 ± 0.05

Conclusion

Six known triterpenes–uncarinic acid M (**1**), 3-oxo-6*β*-hydroxy-olean-12-en-28-oic acid (**2**), 3*β*,6*β*,19*α*-trihydroxy-olean-12-en-28-oic acid (**3**), sumaresinolic acid (**4**), siaresinolic acid (**5**) and isofouquirenone (**6**)–were isolated from *Tetrigonilla collina* propolis. These compounds were identified for the first time in stingless bee propolis. Our findings confirmed that *S. tonkinensis* (Styraceae) is the primary botanical resin source for *T. collina* propolis, with *Aglaia sp.* (Meliaceae) or *Ailanthus sp.* (Simaroubaceae) as the potential secondary source. Compound **2** showed antimicrobial activity against Gram-positive strains (*B. subtilis*: IC_{50} = 20.24±1.45 µg/mL and *L. fermentum*: IC_{50} = 15.54± 0.98 µg/mL), while compound **4** inhibited NO production in LPS-stimulated RAW264.7 cells (IC_{50} = 21.38±2.0 µg/mL). Propolis of *T. collina* and

compounds **2/4** show potential as sources for developing natural antimicrobial and anti-inflammatory agents.

Conflicts of Interest
The authors declare no conflicts of interest.

Authors' Declaration
The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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