Preynlated Flavone from Roots of a Hybrid between
*Artocarpus heterophyllus* and *Artocarpus integer* and its Biological Activities

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Uma nova flavona prenilada, 2,8-dihidroxi-3,10-dimetoxy-6-(2-metil-1-propeno-1-il)-6H,7H-[1]benzopirano[4,3-b][1]-benzopirano-7-on, juntamente com 24 compostos conhecidos foram isolados do extrato bruto em acetona das raízes de um híbrido entre *Artocarpus heterophyllus* e *Artocarpus integer*. Suas estruturas foram determinadas pelos dados de espectroscopia da ressonância magnética nuclear (NMR) 1D e 2D. Avaliaram-se as atividades antioxidante e bactericida dos compostos isolados. O novo composto mostrou atividade antioxidante potente com relação a DPPH e superóxido com valores de IC50 de 0,033 e 0,125 mg mL\(^{-1}\), respectivamente.

One new prenylated flavone, 2,8-dihydroxy-3,10-dimethoxy-6-(2-methyl-1-propen-1-yl)-6H,7H-[1]benzopyran[4,3-b][1]-benzopyran-7-one, together with 24 known compounds were isolated from crude acetone extract from the roots of a hybrid between *Artocarpus heterophyllus* and *Artocarpus integer*. Their structures were determined by 1D and 2D nuclear magnetic resonance (NMR) spectroscopic data. The antioxidant and antibacterial activities of the isolated compounds were evaluated. The new compound showed potent antioxidant activity against DPPH and superoxide with IC50 values of 0.033 and 0.125 mg mL\(^{-1}\), respectively. Significant antibacterial activity against *Acinetobacter baumannii* was observed with MIC value of 50 µg mL\(^{-1}\).

**Keywords:** *Artocarpus*, Moraceae, prenylated flavone, antioxidant activity, antibacterial activity

**Introduction**

*Artocarpus* plants, belonging to the family Moraceae, are distributed in tropical and subtropical regions. Plants in this genus are rich in phenolic compounds, flavonoids, stilbenoids and arylbenzofurans.1 Experimental studies performed in the past suggest that *A. heterophyllus* possesses diverse medicinal uses including antioxidant, anti-inflammatory, antibacterial, anticariogenic, antifungal, antineoplastic and hypoglycemic effects.1,2 Norartocarpetin and artocarpsin isolated from the twigs and woods of *A. heterophyllus* showed good tyrosinase inhibitory activity.3 Artocarpalpanone from the roots of *A. heterophyllus* significantly inhibited the nitric oxide (NO) production and inducible nitric oxide synthase (iNOS) in RAW 264.7 cell.4 Artocarpin isolated from the wood of *A. heterophyllus* showed potent cytotoxic activity on human T47D breast cancer cells.5 Prenylflavones, cycloartocarpusl and artonins A and B from *A. heterophyllus* showed lipid oxidation inhibitory activity,6 whereas prenylated flavones from *A. champeden* (syn. *A. integer* Merr.) showed antimalarial activity.7

In Thailand, a hybrid between *A. heterophyllus* (jackfruit) and *A. integer* (champedak) has been developed for disease resistance, larger size of fruit with more firm aril texture and better taste than *A. integer*. There have been some reports on chemical studies on *A. heterophyllus* and *A. integer*, however, very little information is documented on *A. heterophyllus* root.4 In our preliminary study, the crude acetone extract from the roots of a hybrid between *A. heterophyllus* and *A. integer* exhibited antioxidant activity with IC50 (IC95; sample concentration that produced 50% inhibition of the radical) values of 0.11 and 0.66 mg mL\(^{-1}\) in DPPH (1,1-diphenyl-2-picrylhydrazyl) and superoxide anion assays, respectively, and antibacterial activity against *Acinetobacter baumannii* and *Escherichia coli* with MIC

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(minimum inhibitory concentration) value lower than 3.125 µg mL⁻¹. Such results prompted us to investigate its chemical constituents in order to isolate bioactive constituents from this plant.

**Experimental**

**General procedures**

Melting points were recorded on an Electrothermal 9100 melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded using a FTS 165 FT-IR spectrometer (Perkin-Elmer 783). UV spectra were recorded on a SPECORD S 100 (Analytikjena), and optical rotations on a JASCO P-1020 polarimeter. ¹H and ¹³C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker FT-NMR Ultra Shield™ 300 MHz spectrometer using CDCl₃ solution unless otherwise stated with TMS (tetramethylsilane) as the internal standard. HRESIMS and LREIMS mass spectra were recorded on a Waters micromass and a Finnigan MAT 95 XL spectrometer, respectively. Vacuum liquid column chromatography (VLC) and column chromatography (CC) were carried out on 60H and 100 (70-230 mesh ASTM) silica gel (Merck) types, respectively, or on Sephadex LH-20 (GE Healthcare). Thin layer chromatograph (TLC) (thickness 200 μm) and precoated TLC (thickness 250 μm) were performed on F₂₅₄ silica gel 60 (Merck).

**Plant material**

The roots of a hybrid between *A. heterophyllus* and *A. integer* were collected from Nakhon Si Thammarat Province, in the Southern part of Thailand, in July 2009. The plant was identified by Prof. Kitichate Sridith and a voucher specimen (No. T. Kanogwan) has been deposited at the Herbarium of the Department of Biology, Faculty of Science, Prince of Songkla University, Songkhla, Thailand.

**Extraction and isolation**

The dried and chopped roots of a hybrid between *A. heterophyllus* and *A. integer* (1.0 kg) were extracted with 100% acetone (15 L) for 7 days at room temperature three times to give the acetone extract as a red-brown gum (47.0 g). The crude extract was subjected to VLC, eluting with *n*-hexane, acetone and MeOH in a polarity gradient (*n*-hexane-acetone from 100:0 to 10:90, and acetone-MeOH from 100:0 to 0:100, 1000 mL each) to give 7 fractions (1-7). Fraction 2 (1.32 g) was further purified by recrystallization from acetone-*n*-hexane to afford 19 (108.0 mg). The mother liquor of fraction 2 (1.21 g) was further separated by silica gel CC using acetone-*n*-hexane (from 2:98 to 4:96) to 100% MeOH (350 mL each) to give 4 subfractions (A1-A4). Subfraction A2 (489.7 mg) was further separated by silica gel CC using acetone-petroleum ether (1:4) to acetone to give 6 (4.8 mg), 12 (9.7 mg) and 13 (1.0 mg), respectively. Subfraction A3 (447.6 mg) was further purified by recrystallization from acetone-*n*-hexane to afford 14 (15.0 mg). The mother liquor of subfraction A3 (432.6 mg) was repeatedly separated by silica gel CC using MeOH-CH₂Cl₂ (2:98) to MeOH and finally with precoated TLC using MeOH-CH₂Cl₂ (2:98 × 3) to yield 1 (32.3 mg), 15 (1.5 mg), 17 (15.0 mg) and 20 (2.8 mg), respectively. Fraction 3 (562.0 mg) was further purified by recrystallization from acetone-hexane to afford 12 (9.4 mg). The mother liquor of fraction 3 (552.6 mg) was further separated by silica gel CC using a gradient system (CH₂Cl₂-acetone) to give 5 subfractions (B1-B5). Subfraction B2 (20.3 mg) was further purified by precoated TLC using CH₂Cl₂-*n*-hexane (4:1 × 3) to yield 12 (9.4 mg). Subfraction 13 (139.2 mg) was further purified by silica gel CC using acetone-CH₂Cl₂ (1:4) to MeOH and followed by precoated TLC using EtOAc-*n*-hexane (1:4 × 11) to give 2 (2.4 mg) and 16 (2.4 mg). Subfraction B4 (26.0 mg) was further purified by precoated TLC using acetone-CH₂Cl₂ (6:94 × 3) to give 9 (1.2 mg). Subfraction 15 (63.3 mg) was further purified by silica gel CC using EtOAc-*n*-hexane (3:7) to MeOH and followed by precoated TLC using EtOAc-*n*-hexane (3:7 × 3) to yield 18 (20.4 mg). Fraction 4 (1.87 g) was further separated by silica gel CC using a gradient system (CH₂Cl₂-MeOH) to give 6 subfractions (C1-C6). Subfraction C5 gave 8 (306.8 mg). Subfraction C2 (179.4 mg) was further purified by silica gel CC using MeOH-CH₂Cl₂ (2:98) to MeOH to give 7 (2.6 mg). Subfraction C2 (368.8 mg) was further purified by silica gel CC using MeOH-CH₂Cl₂ (5:95) to MeOH and followed by recrystallization from acetone-hexane to give 21 (16.0 mg). Subfraction C4 (264.4 mg) was further purified by recrystallization from acetone-*n*-hexane to give 21 (74.8 mg). The mother liquor of subfraction C4 (189.6 mg) was further separated by silica gel CC using acetone-CH₂Cl₂ (2:98) to MeOH to give 2 subfractions (C4A-C4B). Subfraction C4B (82.5 mg) was further purified by silica gel CC using MeOH-CH₂Cl₂ (1:9) to MeOH and followed by precoated TLC using acetone-petroleum ether (3:7 × 2) to give 22 (5.6 mg) and 23 (6.2 mg). Fraction 5 (3.94 g) was separated by Sephadex LH-20 CC using MeOH-CH₂Cl₂ (1:1) to give 3 subfractions (D1-D3). Subfraction D2 (1.09 g) was further purified by silica gel CC using MeOH-CH₂Cl₂ (2:98) to MeOH to give 3 (12.0 mg) and 10 (8.0 mg). Subfraction D3 (1.51 g) was further purified by silica gel CC using MeOH-CH₂Cl₂ (2:98) to MeOH to give 4 (4.5 mg), and 11 (10.5 mg).
Fraction 6 (7.45 g) was separated by Sephadex LH-20 CC using MeOH-CH₂Cl₂ (1:1) to afford 4 subfractions (E1-E4). Subfraction E2 (1.68 g) was repeatedly separated by silica gel CC using MeOH-CH₂Cl₂ (2:98) to MeOH to yield 24 (4.5 mg). Subfraction E3 (2.70 g) was further purified by silica gel CC using a gradient of CH₂Cl₂-MeOH and subsequent precoated TLC using acetone-petroleum ether (3:7 x 2) to afford 25 (5.6 mg) and 5 (8.0 mg).

2,8-Dihydroxy-3,10-dimethoxy-6-(2-methyl-1-propen-1-yl)-6H,7H-[1]benzopyran[4,3-b][1]benzopyran-7-one (1)

Yellow solid; mp 201.2-202.2 °C; [α]D26 +41.2° (c 0.05, acetone); UV (MeOH) λmax/nm (log e) 264 (4.28), 305 (4.05), 394 (4.13); IR (neat) νmax/cm⁻¹ 3409, 1656; "H NMR (CDCl₃, 300 MHz) and ¹³C NMR (CDCl₃, 75 MHz) see Table 1; HRESIMS m/z 397.1297 [M + H]+ (calcd. for C₁₇H₂₀O₇, 397.1287); LREIMS m/z (rel. int.) 396 [M⁺]² (26), 381 [M-C₂H₅]+ (37), 341 [M-C₂H₅]²+ (55), 98 (100).

Table 1. "H and ¹³C NMR (300 and 75 MHz, respectively) data and major HMBC correlations for compound 1 in CDCl₃

Position δH (m, J / Hz) δC HMBC
1 7.29 (s) 108.4 C-2, C-3, C-12a, C-13
2 – –
2-0H 5.35 (br s) – C-1, C-2, C-3
3 – 151.6 –
3-OMe 3.92 (s) 56.4 C-3
4 6.48 (s) 100.9 C-2, C-4a, C-13
4a – 151.4 –
6 6.22 (d, J 9.6) 69.8 C-4a, C-6a, C-7, C-12a, C-14, C-15
6a – 110.5 –
7 – 178.7 –
7α – 106.1 –
8 – 162.4 –
8-0H 12.76 (s) - C-7a, C-8, C-9
9 6.33 (d, J 2.4) 98.2 C-7a, C-8, C-10, C-11
10 – 165.3 –
10-0Me 3.86 (s) 56.0 C-10
11 6.44 (d, J 2.4) 92.9 C-7a, C-9, C-10, C-11a
11a – 157.2 –
12a – 155.8 –
12 – 108.2 –
14 5.47 (d, J 9.6) 121.1 C-16, C-17
15 – 139.4 –
16 1.98 (s) 18.9 C-14, C-15, C-17
17 1.71 (s) 26.2 C-14, C-15, C-16

Free radical scavenging activity

In this study, DPPH, hydroxyl and superoxide radical scavenging assays were carried out, according to those previously described.⁸

Antibacterial activity

A modified broth microdilution method⁹ was used to obtain the MIC values of the tested materials. 20 µL of a 3-5 h culture of each bacterial strain (multidrug-resistant Acinetobacter baumannii and Escherichia coli), containing approximately 5 x 10⁹ CFU mL⁻¹, were applied into Muller-Hinton broth (MHB, Merck, Germany) supplemented with the plant extracts. 100 µg of the extract and pure compounds were diluted in 1 mL of dimethyl sulfoxide (DMSO, Merck, Germany) and two-fold dilutions were made to obtain concentrations ranging from 0.05-100 µg mL⁻¹. The microtiter plates were incubated at 37 °C for 18 h. MICs were observed at least in duplicate as the lowest concentration of the plant extracts that produced a complete suppression of the bacterial growth.

Results and Discussion

The crude acetone extract (47.0 g) from the roots of a hybrid between A. heterophyllus and A. integer was investigated by chemical method and chromatographic techniques. In this work, it is described the isolation and structural elucidation of one new prenylated flavone, 2,8-dihydroxy-3,10-dimethoxy-6-(2-methyl-1-propen-1-yl)-6H,7H-[1]benzopyran[4,3-b][1]benzopyran-7-one (1), as well as twenty-four known compounds: flavokawain-A (2),⁰¹ gemichalcone A (3),¹¹ cyanoclerodane (4),¹² dihydromorin (5),¹³ sakuranetin (6),¹⁴ naringenin (7),¹⁵ artocarpane (8),⁴ isoscinensetin (9),¹⁴ norartocarpetin (10),¹⁵ artonin Y (11),¹⁵ artoindonesianin S (12),¹⁶ artopened A (13),¹⁷ artonin A (14),⁴ artonin F (15),¹⁸ 5,3′,4′-trimethoxy-6,7-methylenedioxyisoflavone (16),¹⁹ morusin (17),²⁰ artocarpin (18),¹⁵ cycloheptaphyllin (19),⁴ isocyclomulberrin (20),²¹ artoindonesianin A-2 (21),²² cyclocommunol (22),²¹ 1,3,6-trihydroxy-2-(3-methyl-2-butenyl)xanthone (23),²⁴ 1,3,6-trihydroxyxanthone (24)²⁵ and 1,3,5,6-tetrahydroxyxanthone (25)²⁶ (Figure 1). Their structures were elucidated by spectroscopic data, especially using 1D and 2D NMR. The structure of known compounds (2-25) were confirmed by comparison of the "H and ¹³C NMR spectroscopic data with those reported in the literature.

Compound 1 was obtained as a yellow solid. Its molecular formula was determined as C₂₂H₂₀O₇ by HRESIMS measurement for C₂₂H₂₀O₇ at m/z 397.1297 [M + H]+ (calcd. 397.1287). The UV spectrum showed maximum absorption bands at 264, 305 and 394 nm, which is typical of a pyranoflavone chromophore.²⁶ The IR spectrum exhibited absorption bands for a hydroxyl group at 3409 cm⁻¹ and a conjugated C=O group at 1656 cm⁻¹. The
Figure 1. Structures of isolated compounds 1-25.

1. $R^1 = R^3 = R^4 = H, R^2 = R^6 = OMe, R^5 = OH$

19. $R^1 + R^2 = \text{prenyl, } R^3 = \text{H, } R^5 = R^6 = OH$

20. $R^1 = \text{prenyl, } R^2 = R^6 = OH, R^3 = R^4 = R^5 = H$

21. $R^1 = R^3 = R^4 = \text{H, } R^2 = R^5 = OH, R^6 = OMe$

22. $R^1 = R^3 = R^4 = R^5 = \text{H, } R^2 = R^6 = OH$

5. $R^1 = R^2 = R^3 = OH$

6. $R^1 = OMe, R^2 = R^3 = \text{H}$

7. $R^1 = OH, R^2 = R^3 = \text{H}$

8. $R^1 = OMe, R^2 = OH, R^3 = \text{H}$

13. $R^1 = R^3 = \text{H, } R^2 = R^4 = OMe$

14. $R^1 + R^2 = \text{prenyl, } R^4 = OH$

15. $R^1 = \text{prenyl, } R^2 + R^3 = OH$

23. $R^1 = \text{prenyl, } R^2 = R^4 = \text{H, } R^3 = OH$

24. $R^1 = R^2 = R^4 = \text{H, } R^3 = OH$

25. $R^1 = R^4 = \text{H, } R^2 = R^3 = OH$
The 1H NMR spectrum (Table 1) showed one chelated hydroxyl signal at δ 12.76 (s), one non chelated hydroxyl signal at δ 5.35 (br s), two meta coupled aromatic signals at δ 6.33 (d, J 2.4 Hz) and 6.44 (d, J 2.4 Hz), two methoxyl groups at δ 3.86 (s) and 3.92 (s), an isoprenyl group at δ 1.98 (s, 3H), 1.71 (s, 3H), 6.22 (d, J 9.6 Hz) and 5.47 (d, J 9.6 Hz) and characteristic signals for a 1,2,4,5-tetrasubstituted aromatic ring at δ 7.29 (s) and 6.48 (s). The 13C NMR spectrum (Table 1) showed signals for 22 carbons, one of which corresponds to a conjugated carbonyl carbon (δ 178.7). The substitution pattern of the tricyclic flavonoid skeleton A-B-C was supported from the long-range (HMBC) 1H-13C NMR correlations indicated in Figure 2 and Table 1. The aromatic ring B and isoprenyl group were linked with an ether linkage at C-4a to C-6, as confirmed by HMBC cross-peaks between an oxymethine proton at δ 6.22 (H-6) with C-4a, C-6a, C-7, C-12a, C-14 and C-15, and that of proton at δ 5.47 (H-14) with C-16 and C-17. Comparison of its NMR data with that of 21 indicated that a hydroxy signal (δ 9.72, br s) at C-10 of 21 was replaced by a methoxy signal (δ 3.86, s) of 1. Thus, compound 1 was identified as 2,8-dihydroxy-3,10-dimethoxy-6-(2-methyl-1-propen-1-yl)-6H,7H-[1]benzopyran-4,3-b][1]-benzopyran-7-one.

Figure 2. Key HMBC (H → C) correlations of compound 1.

All isolated compounds of sufficient amount (1-6, 8, 9, and 11-25) were assayed for their antioxidant and antibacterial activities (Table 2). Only compounds 1, 3, 13, 14, 15, 19, 24 and 25 exhibited DPPH· scavenging activity with IC_{50} values of 0.033, 0.14, 0.14, 0.033, 0.04, 0.032, 0.039 and 0.034 mg mL\(^{-1}\), respectively (standard BHA, 0.02 mg mL\(^{-1}\)). Compounds 3, 13, 15, 20, 24 and 25 exhibited OH· scavenging activity with IC_{50} values of 0.008, 0.005, 0.0024, 1.56, 0.01 and 0.009 mg mL\(^{-1}\), respectively (standard tannin, 0.44 mg mL\(^{-1}\)). The finding that compound 20 did not scavenge DPPH· but showed its activity in OH· assay suggests that this compound might inhibit the formation of OH· by chelating iron. Compounds 1 and 19 also exhibited superoxide scavenging activity with IC_{50} values of 0.125 and 0.05 mg mL\(^{-1}\), respectively (standard trolox, 0.39 mg mL\(^{-1}\)). In vitro antioxidant activities of compounds 14, 19 and 25 have been previously reported.\(^6,27,28\) Only compounds 1, 14, 24 and 25 showed antibacterial activity against Acinetobacter baumannii ATCC 19606 with MIC values of 50, 25, 50 and 25 μg mL\(^{-1}\), respectively (standard gentamicin, 2 μg mL\(^{-1}\)). Compounds 23 and 24 showed antibacterial activity against Escherichia coli ATCC 25922 with the same MIC value of 25 μg mL\(^{-1}\) (standard gentamicin, 1 μg mL\(^{-1}\)).

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<th>Compound</th>
<th>DPPH· IC_{50} (mg mL(^{-1}))</th>
<th>OH· IC_{50} (mg mL(^{-1}))</th>
<th>Superoxide IC_{50} (mg mL(^{-1}))</th>
<th>Antibacterial activity, MIC / (μg mL(^{-1}))</th>
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IC_{50}: sample concentration that produced 50% inhibition of that radical; NA: no activity; ND: not determined; **+: very good activity; ***: good; **: fair; +: weak; -: very weak.

**Conclusions**

This is the first report on the chemical constituents and biological activities from the roots of a hybrid between Artocarpus heterophyllus and Artocarpus integer. A new prenylated flavone (compound 1) was isolated from the roots of a hybrid between Artocarpus heterophyllus and Artocarpus integer together with 24 known compounds. Compound 1 demonstrated potent antioxidant activity against DPPH· and superoxide with IC_{50} values of 0.033 and 0.125 mg mL\(^{-1}\), respectively and strong
antibacterial activity against \textit{Acinetobacter baumannii} with MIC value of 50 \textmu g mL$^{-1}$. Among the known compounds, compounds 2, 3, 6, 7, 9, 16, 17, 23 and 25 are firstly reported from \textit{Artocarpus} genus.

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\section*{Supplementary Information}

Supplementary data (Figures S1-S5) are available free of charge at http://jbcs.sbq.org.br as PDF file.

\section*{References}


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Figure S1. ¹H NMR (300 MHz, CDCl₃) spectrum of compound 1.
Figure S2. $^{13}$C NMR (75 MHz, CDCl$_3$) spectrum of compound 1.

Figure S3. HMQC (300 MHz, CDCl$_3$) spectrum of compound 1.
Figure S4. HMBC (300 MHz, CDCl₃) spectrum of compound 1.

Figure S5. HRESIMS spectrum of compound 1.