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Contributed Paper

Antitubercular and Antiplasmodial Prenylated Flavones from the Roots of *Artocarpus altilis*

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ABSTRACT

Antitubercular and antimalarial activity-guided study of the roots of *Artocarpus altilis* led to the isolation of nine prenylated flavones. Cycloartocarpin (**1**), artocarpin (**2**), and chaplashin (**3**) were isolated from the dichloromethane extract of the root stems, whereas morusin (**4**), cudraflavone B (**5**), cycloartobiloxanthone (**6**), artonin E (**7**), cudraflavone C (**8**) and artobiloxanthone (**9**) were found in the root barks. The isolated compounds exhibited antitubercular and antiplasmodial activities, and also showed moderate cytotoxicity against KB (human oral epidermoid carcinoma) and BC (human breast cancer) cell lines.

Keywords: *Artocarpus altilis,* prenylated flavones, antituberculosis, antimalarial activity.

1. INTRODUCTION

The incidence of malaria and tuberculosis is a major problem occurring in many tropical and subtropical regions. The rapid increase in the resistance of the responsible strains to conventional treatments necessitates urgent search for new drug development.

Artocarpus altilis (family Moraceae) is widely distributed over the tropical regions of Asia including Thailand. In addition to the major growing for edible fruits, its parts are also utilized as traditional medicine. The roots have been used as a component in Thai folk remedies for venereal diseases and cancer [1] whereas in Taiwan, the stems and roots have been used traditionally for the treatment of liver cirrhosis and hypertension [2].

Preliminary bioactivity study of the crude CH_2Cl_2 extract of the roots of *A. altilis* revealed that it possessed antitubercular activity against *Mycobacterium tuberculosis* with a minimum inhibitory concentration (MIC) of 25 μg/mL. Furthermore, a moderate antiplasmodial (IC_{50} 3.5 μg/mL) activity against the parasite *Plasmodium falciparum* was also observed. This prompted us to further investigate for bioactive constituents of this plant.

2. MATERIALS AND METHODS

2.1 General Experimental Procedures

^{1}H, ^{13}C, DEPT (135), ^{1}H-^{1}H COSY, NOESY, HMQC (optimized for $^1J_{HC}$ = 45 Hz) and HMBC (optimized for $^nJ_{HC}$ = 8 Hz) experiments were carried out on Bruker AV400 (or Bruker AV500) spectrometer, operating at 400 (or 500) MHz for proton

and 100 (or 125) MHz for carbon, respectively. The ESITOF mass spectra were obtained from a Micromass LCT mass spectrometer. IR spectra were recorded on a FT-IR Perkin-Elmer spectrum GX spectrometer, and UV spectra were recorded on an Analytikjena AG UV-VIS spectrophotometer. Optical rotations were measured on a Jasco DIP 370 polarimeter. Melting points were recorded using a Stuart Scientific melting point apparatus SMP2 and uncorrected.

2.2 Plant Material

The roots of *A. altilis* were collected in March 2002 from Phitsanulok Province, Thailand. The voucher specimen (no. W129) was deposited at the Department of Biology, Faculty of Science, Naresaun University, Phitsanulok, Thailand.

2.3 Extraction and Isolation

Air dried root stems (3.4 kg) and root barks (1.1 kg) of *A. altilis* were chopped and macerated in CH_2Cl_2 for 2 days. The CH_2Cl_2 extract was filtered and the solvent was evaporated to dryness to yield a crude extract of root stems (8.60 g) and root barks (9.35 g). The crude extract of root stems was dissolved in MeOH (250 mL); the insoluble gummy residue was filtered off (1.75 g), while the MeOH soluble part was chromatographed on Sephadex LH-20 (250 g, MeOH as eluent) to yield 18 fractions (50 mL each). Fractions 7-12 (1.25 g) were combined and rechromatographed (silica gel 100 g, eluted with EtOAc in CH_2Cl_2 ranging from 1-5% of EtOAc by increasing 1 % of EtOAc for every 250 mL), yielding compounds **1** (30 mg), **2** (82 mg) and **3** (8 mg). The root bark crude extract (9.35 g) was dissolved in MeOH (250 mL), and again the insoluble residue was removed by filtration, while the soluble material was chromatographed on Sephadex LH-20 (250 g, MeOH as eluent) to yield 18 fractions (50 mL each). Fractions 6-7 (526 mg) were combined and purified by column chromatography (silica gel 50 g, elulent 1% EtOAc/CH_2Cl_2 750 mL) to yield compounds **4** (50 mg) and **5** (6 mg). Fractions 8-9 (352 mg) were combined and subjected to column chromatography (silica gel 50 g, elulent 3% EtOAc/CH_2Cl_2 750 mL) to yield compounds **6** (7 mg) and **7** (57 mg). Combination of fractions 10-11 (238 mg) followed by column chromatography (silica gel 50 g, elulent 5% EtOAc/CH_2Cl_2 750 mL) afforded compounds **8** (10 mg) and **9** (25 mg).

Cycloartocarpin (**1**): yellow amorphous powder; m.p. 248-250 °C; $[\alpha]^{27}_{D}$ -60.98 (*c* 0.03, CH_3OH); ESITOF MS: *m/z* 435.1802 $(M+H)^+$, calcd. for $(C_{26}H_{26}O_6+H)^+$, 435.1807.

Artocarpin (**2**): yellow amorphous powder; m.p. 160-163 °C; ESITOF MS: *m/z* 437.1971 $(M+H)^+$, calcd. for $(C_{26}H_{28}O_4+H)^+$, 437.1964.

Chaplashin (**3**): pale yellow amorphous powder; m.p. 200-203 °C; $[\alpha]^{27}_{D}$ -4.04° (*c* 0.02, CH_3OH); ESITOF MS: *m/z* 453.1912 $(M+H)^+$, calcd. for $(C_{26}H_{28}O_7+H)^+$, 453.1913; ^{1}H-NMR (acetone-d_6): δ 13.90 (1H, s, 5-OH), 9.20 (1H, bs, 4′-OH), 8.03 (1H, d, *J* = 8.9, 6′-H), 6.80 (1H, s, 8-H), 6.75 (1H, dd, *J* = 8.9, 2.4, 5′-H), 6.73 (1H, dd, *J* = 16.3, 7.1, 2′-H), 6.68 (1H, d, *J* = 2.4, 3′-H), 6.60 (1H, d, *J* = 16.3, 1′-H), 4.01 (3H, s, 7-OCH_3), 4.00 (1H, dd, *J* = 9.8, 1.9, 2′-H), 3.55 (1H, dd, *J* = 16.8, 1.9, 1′-H), 2.61 (1H, dd, *J* = 16.8, 9.8, 1′-H), 2.44 (1H, m, 3′-H), 1.40 (3H, s, 4′-CH_3), 1.34 (3H, s, 5′-CH_3), 1.09 (6H, d, *J* = 6.7, 4′-, 5′-CH_3); ^{13}C-NMR (acetone-d_6): δ 181.7 (C-4), 163.7 (C-7), 162.2 (C-2′) and 160.9 (C-4′), (159.4 (C-5), 158.6 (C-2), 156.4 (C-9), 142.0 (C-2′), 130.8 (C-6′), 117.3 (C-3), 116.7 (C-1′), 114.3 (C-1′), 111.6 (C-5′), 109.6 (C-6), 108.3 (C-3′), 104.2 (C-10), 90.6 (C-2′), 90.2 (C-8), 72.1 (C-3′), 56.4 (7-OCH_3), 33.7 (C-3′), 27.0 (C-5′), 25.1 (C-1′), 24.8 (C-4′), 22.8 (C-4′, C-5′).

Morusin (**4**): pale yellow prisms; m.p. 147-149 °C; ESITOF MS: *m/z* 421.1652 $(M+H)^+$,

calcd. for $(C_{25}H_{24}O_6+H)^+$, 421.1651.

Cudraflavone B (**5**): pale yellow amorphous powder; m.p. 136-139 °C; ESITOF MS: *m/z* 421.1645 $(M+H)^+$, calcd. for $(C_{25}H_{24}O_6+H)^+$, 421.1651.

Cycloartobiloxanthone (**6**): yellow needles; m.p. 283-285 °C; $[\alpha]^{27}_D$ +31.34° (*c* 0.03, CH_3OH); ESITOF MS: *m/z* 435.1451 $(M+H)^+$, calcd. for $(C_{25}H_{22}O_7+H)^+$, 435.1444.

Artonin E (**7**): yellow needles; m.p. 242-244 °C; ESITOF MS: *m/z* 437.1572 $(M+H)^+$, calcd. for $(C_{25}H_{24}O_7+H)^+$, 437.1601.

Cudraflavone C (**8**): yellow amorphous powder; m.p. 135-137 °C; ESITOF MS: *m/z* 423.1811 $(M+H)^+$, calcd. for $(C_{25}H_{26}O_6+H)^+$, 423.1807.

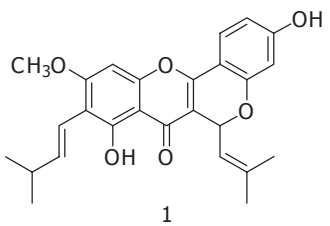
Artobiloxanthone (**9**): yellow amorphous powder; m.p. 145-147 °C; $[\alpha]^{26}_D$ +24.64° (*c* 0.02, CH_3OH); ESITOF MS: *m/z* 435.1443 $(M+H)^+$, calcd. for $(C_{25}H_{22}O_7+H)^+$, 435.1444.

2.4 Biological Activities

The antitubercular activity was performed against *Mycobacterium tuberculosis* H37Ra using the microplate Alamar blue assay (MABA) as described by Collins and Franzblau [18]. The standard drugs, *viz.* rifampicin, kanamycin and isoniazide were employed as references. The quantitative assessment of antimalarial activity against the parasite *Plasmodium falciparum* (K1, multidrug-resistant strain) was conducted using the microculture radioisotope technique based upon the method described by Desjardins et al. [19]. The standard compound employed was dihydroartemisinin. The cytotoxicity assay against the KB (human oral epidermoid carcinoma), BC (human breast cancer) and Vero (African green monkey kidney fibro-blasts) cell lines was determined employing the colorimetric method described by Skehan and co-workers [20]. The reference substance was ellipticine.

3. RESULTS AND DISCUSSION

The roots of *A. altilis* were separated into two parts, the root stems and the root barks. The former was extracted with CH_2Cl_2 and subsequently subjected to chromatographic analysis. Three known prenylated flavones, *viz.* cycloartocarpin (**1**) [3,4], artocarpin (**2**) [4,5], and chaplashin (**3**) [6] were obtained after chromatographic separation, and their NMR spectra were identical to those published. Although chaplashin (**3**) is a known compound, its ^1H and ^{13}C NMR data have never been assigned; this is the first report on the assignment of proton and carbon resonances in **3**. The investigation of the CH_2Cl_2 extract of the root barks was carried out in the same manner as that for the former, resulting in the isolation of six prenylated flavones, *viz.* morusin (**4**) [7], cudraflavone B (**5**) [7,8], cycloartobiloxanthone (**6**) [9,10], artonin E (**7**) [10,11], cudraflavone C (**8**) [4,12] and artobiloxanthone (**9**) [9,11]. Spectral data of compounds **4**-**9** were in good agreement with those in the literatures [7-12]. In spite of previous reports on the isolation of **1-9** from various plants especially the ones belonging to the genus *Artocarpus*, which are known as abundant sources of prenylated flavonoids, compounds **3, 5**, **6** and **8** were isolated for the first time from *A. altilis*. Surprisingly, to the best of our knowledge, cudraflavone B (**5**) has never been found in the *Artocarpus* genus before.



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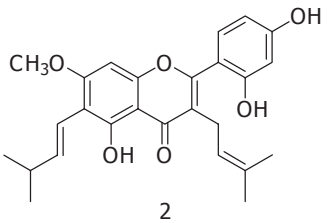

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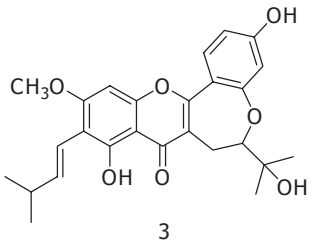

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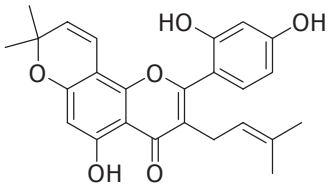

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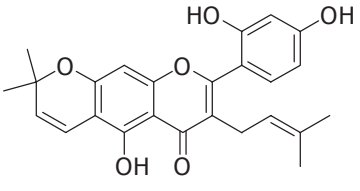

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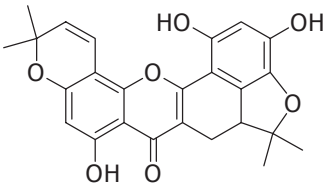

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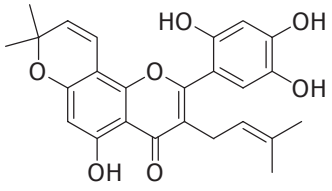

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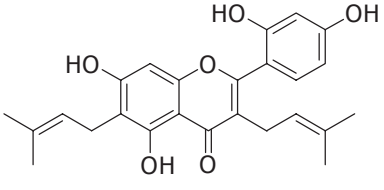

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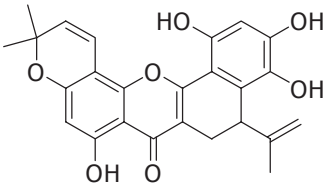

9

All nine compounds isolated exhibited antitubercular activity with a minimum inhibitory concentration (MIC) ranging from 3.12 to 100 μg/mL (Table **1**). Among the isolated metabolites, compounds **2** and **3** were the most potent antitubercular agents possessing the MIC value of 3.12 μg/mL comparable to that of the standard drug, kanamycin (MIC value of 2.5 μg/mL) (Table 1). Compounds **1-9**, except **8**, exhibited moderate antiplasmodial activity with the IC_{50} values ranging from 1.9 to 4.3 μg/mL (Table 1). Cytotoxicities of compounds **1-9** against KB (human oral epidermoid carcinoma), BC (human breast cancer), and Vero cell lines were similar, with the IC_{50} values of 2.9-14.7 μg/mL (Table 1). Though prenylated flavones from *Artocarpus* plants were found to exhibit various biological activities, for example, as potential anti-cancer drugs [13], inhibitors of

Table 1. Biological activities of prenylated flavones 1-9.

Compound	Antitubercular activity; MIC (μg/mL)	Antiplasmodial activity; IC_{50} (μg/mL[b])	Cytotoxicity; IC_{50} (μg/mL)[b]		
			KB cells	BC cells	Vero cells
1	12.5[a]	4.3±0.7	5.5±1.1	3.1±0.1	5.5±1.6
2	3.12[a]	3.0±0.2	5.1±1.0	3.3±0.6	5.6±2.0
3	3.12[a]	3.5±0.4	13.4±0.4	5.5±0.05	8.0±1.9
4	25[a]	1.9±0.2	6.9±2.1	3.3±1.1	10.1±3.2
5	25[a]	2.2±0.3	7.9±1.8	5.2±0.4	14.7±6.2
6	50[a]	2.3±0.6	8.7±0.8	4.1±0.04	7.2±1.5
7	100[a]	2.8±0.3	9.8±0.6	3.5±0.2	6.1±2.8
8	12.5[a]	Not determined	8.5±2.5	2.9±0.5	7.4±2.5
9	50[a]	3.0±0.4	9.3±1.6	4.9±0.2	7.4±1.7
rifampicin	0.0023	-	- -	-	
kanamycin	2.5	-	- -	-	
isoniazide	0.1	-	- -	-	
dihydroartemisinin	-	$1.2x10^{-3}$±$0.2x10^{-3}$	- -	-	
ellipticine	-	-	0.21±0.1	0.27±0.1	0.4±0.1

[a] Values from the dilution technique of triplicate experiments.
[b] Mean ± standard deviation (n=3)

melanin biosynthesis [14], 5-α-reductase inhibitor [15], skin-lightener with effects on UVB-induced pigmentation [16] and antiinflammatory agent [17], yet this is the first report of their antitubercular and antiplasmodial activities.

4. CONCLUSIONS

Nine known prenylated flavones (**1**-**9**) had been isolated from the dichloromethane extract of the roots of *Artocarpus altilis*. The ^{1}H and ^{13}C NMR spectral data of chaplashin (**3**) is fully assigned for the first time in this report. The first finding of cudraflavone B (**5**) in the *Artocarpus* plant is also noted. The bioactivity study of the isolated flavones revealed that all nine compounds exhibited interesting antitubercular and antiplasmodial activities, whereas the cytotoxic activity towards KB and BC cell lines was moderate. The results extend the potentials of the prenylated flavones for use as alternative substances for treatment of various deceases, especially malaria and tuberculosis, which present some of the major problems occurring nowadays.

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