

Further Studies of Bioactive Flavonoids from *Kaempferia parviflora*

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Abstract

Five flavonoids (1-5) have been isolated from the rhizomes of *Kaempferia parviflora* and their structures were identified on the basis of spectral data. Among these, 5,7-dimethoxyflavone (1) exhibited antifungal activity against *Candida albicans* with an IC₅₀ value of 19.98 µg/ml and antimycobacterial activity with a MIC value of 50 µg/ml. However, none of the isolated compounds showed antiplasmodial activity and cytotoxicity against KB, BC and NCI-H187 cell lines. This is the first report of the isolation of compounds 3-5 from this plant.

Key Words: *Kaempferia parviflora*, Flavonoids, Antiplasmodial activity, Antifungal activity, Antimycobacterial activity, Cytotoxicity

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Introduction

Kaempferia parviflora (Zingiberaceae), known in Thai as “Kra-Chai-Dam” and formerly known as *Boesenbergia pandurata* black rhizome, has been widely used as a health-promoting herb and for the treatment of anti-gastrointestinal disorders. In Thailand, a commercial tonic drink made from *K. parviflora* rhizomes has been believed to relieve impotent symptoms. In our previous report on this plant, nine flavonoids were isolated and evaluated for their biological activities (Yenjai et al., 2004). In continuation of our research on this plant, we now report the isolation, identification and some biological evaluation of five additional flavonoids.

Materials and Methods

General Experimental Procedures

Column chromatography was carried out on silica gel 60 (0.040-0.063 mm). NMR spectra were recorded in $\text{CDCl}_3/\text{DMSO-d}_6$ on a Varian Mercury Plus 400 spectrometer, using residual CHCl_3 (δ 7.26) and DMSO (δ 2.50) as an internal standard. IR spectra were carried out on a Tensor 27 Bruker (OPUS version 4) or Perkin-Elmer Spectrum One spectrophotometers. ESI-TOF mass spectra were obtained from a Micromass LCT mass spectrometer, and the lock mass calibration was applied for the determination of the accurate mass. Melting points were determined using a Gallenkamp melting point apparatus and were uncorrected.

Plant material

Fresh rhizomes of *K. parviflora* were collected from Loei province, Thailand in May 2003. Plant specimen was verified by Dr. Varima Wongpanich, Faculty of Pharmaceutical Sciences,

Khon Kaen University. A voucher specimen (CY 4303) has been deposited at the herbarium of the Faculty of Pharmaceutical Sciences, Khon Kaen University.

Extraction and isolation

Dried rhizome powder of *K. parviflora* (1 kg) was macerated twice with CH_2Cl_2 (3 L) at room temperature for three days and the solvent was removed in vacuo to give a CH_2Cl_2 extract (54 g). Then, a portion of this extract (30 g) was subjected to column chromatography on silica gel (300 g) and subsequently eluted with gradient of three solvents (hexane, CH_2Cl_2 and MeOH) by gradually increasing the polarity of eluting solvent system. The eluates were collected and examined by TLC resulting in 35 groups of eluting fractions. Further chromatographic separation of fractions 28, 30 and 31 eluting with hexane-EtOAc gradient yielded 5,7-dimethoxyflavone (**1**, 123 mg), 3,5,7,3',4'-pentamethoxyflavone (**2**, 38 mg), and 5,3'-dihydroxy-3,7,4'-trimethoxyflavone (**3**, 15 mg), respectively. Similarly, fraction 33 was chromatographed using EtOAc-MeOH as eluting solvent to give 5 subfractions. Subfractions 2 and 5 were separately rechromatographed using EtOAc-MeOH and CH_2Cl_2 - CH_3CN as eluting solvents to afford 5,4'-dihydroxy-7-methoxyflavone (**4**, 7 mg) and 4'-hydroxy-5,7-dimethoxyflavone (**5**, 4 mg), respectively.

Compound **1**: colorless crystals (CH_2Cl_2 -hexane): m.p. 150.8-151.0 °C; IR(KBr) $\bar{\nu}_{\text{max}}$ 1644, 1605, 1351, 1164 cm^{-1} ; $^1\text{H NMR}$ (CDCl_3 , 400 MHz) δ 3.90 (3H, s, OCH_3), 3.94 (3H, s, OCH_3), 6.37 (1H, d, $J = 2.2$ Hz, H-6), 6.56 (1H, d, $J = 2.2$ Hz, H-8), 6.67 (1H, s, H-3), 7.49 (3H, m, Ar-H), 7.86 (2H, m, Ar-H); $^{13}\text{C NMR}$ (CDCl_3 , 100 MHz) δ 55.7 (OCH_3),

56.3 (OCH₃), 92.9 (C-8), 96.2 (C-6), 109.1 (C-3), 109.4 (C-10), 125.9 (C-2'), 128.9 (C-3'), 131.1 (C-4'), 131.6 (C-1'), 159.9 (C-9), 160.7 (C-2), 161.0 (C-5), 164.1 (C-7), 177.5 (C-4); ESI-TOF MS *m/z* 283 [M+1]⁻.

Compound 2: colorless crystals (CH₂Cl₂-hexane): m.p. 156.0-156.5 °C; IR(KBr) $\bar{\nu}_{\max}$ 1625, 1604, 1163 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) δ 3.86 (3H, s, OCH₃), 3.89 (3H, s, OCH₃), 3.95 (9H, s, OCH₃), 6.33 (1H, d, *J* = 1.8 Hz, H-6), 6.49 (1H, d, *J* = 1.8 Hz, H-8), 6.97 (1H, d, *J* = 8.8 Hz, H-5'), 7.73 (2H, m, H-2',6'); ¹³C NMR (CDCl₃, 100 MHz) δ 55.7 (OCH₃), 55.9 (OCH₃), 56.0 (OCH₃), 56.4 (OCH₃), 59.9 (OCH₃), 92.4 (C-8), 95.7 (C-6), 109.5 (C-10), 110.8 (C-5'), 111.3 (C-2'), 121.6 (C-6'), 123.4 (C-1'), 141.2 (C-3), 148.7 (C-3'), 150.8 (C-4'), 152.6 (C-2), 158.8 (C-9), 161.0 (C-5), 163.9 (C-7), 174.0 (C-4); ESI-TOF MS *m/z* 373 [M+1]⁻.

Compound 3: colorless crystals (CH₂Cl₂-MeOH): m.p. 175.0-176.0 °C; IR(KBr) $\bar{\nu}_{\max}$ 3399, 1650, 1593, 1497, 1159 cm⁻¹; ¹H NMR (DMSO-d₆, 400 MHz) δ 3.80 (3H, s, OCH₃), 3.82 (3H, s, OCH₃), 3.90 (3H, s, OCH₃), 6.23 (1H, d, *J* = 2.2 Hz, H-6), 6.41 (1H, d, *J* = 2.2 Hz, H-8), 6.92 (1H, d, *J* = 8.7 Hz, H-5'), 7.56 (1H, dd, *J* = 8.7, 2.2 Hz, H-6'), 7.61 (1H, d, *J* = 2.2 Hz, H-2'); ¹³C NMR (DMSO-d₆, 100 MHz) δ 55.5 (2xOCH₃), 59.7 (OCH₃), 91.7 (C-8), 97.4 (C-6), 105.4 (C-10), 110.9 (C-5'), 115.0 (C-2'), 120.4 (C-6'), 122.4 (C-1'), 138.3 (C-3), 146.1 (C-3'), 149.9 (C-4'), 155.7 (C-2), 156.3 (C-9), 161.3 (C-5), 165.0 (C-7), 178.3 (C-4); ESI-TOF MS *m/z* 345 [M+1]⁻.

Compound 4: colorless crystals (CH₂Cl₂-MeOH): m.p. 293.0-294.0 °C; IR(KBr) $\bar{\nu}_{\max}$ 3277, 1667, 1605, 1590, 1501, 1377 cm⁻¹; ¹H NMR

(DMSO-d₆, 400 MHz) δ 3.89 (3H, s, OCH₃), 6.33 (1H, d, *J* = 2.2 Hz, H-6), 6.55 (1H, d, *J* = 2.2 Hz, H-8), 6.60 (1H, s, H-3), 6.94 (2H, d, *J* = 9.0 Hz, H-3',5'), 7.81 (2H, d, *J* = 9.0 Hz, H-2',6'); ¹³C NMR (DMSO-d₆, 100 MHz) δ 55.9 (OCH₃), 92.5 (C-8), 98.1 (C-6), 103.4 (C-3), 105.3 (C-10), 116.3 (C-3',5'), 121.6 (C-1'), 128.4 (C-2',6'), 157.7 (C-9), 161.6 (C-4'), 161.8 (C-5), 164.6 (C-2), 165.4 (C-7), 182.3 (C-4); ESI-TOF MS *m/z* 285 [M+1]⁻.

Compound 5: colorless crystals (CH₂Cl₂-MeOH): m.p. 289.0-290.0 °C; IR(KBr) $\bar{\nu}_{\max}$ 3435, 2920, 1608, 1414, 1384 cm⁻¹; ¹H NMR (DMSO-d₆, 400 MHz) δ 3.79 (3H, s, OCH₃), 3.86 (3H, s, OCH₃), 6.46 (1H, d, *J* = 2.0 Hz, H-6), 6.52 (1H, s, H-3), 6.81 (1H, d, *J* = 2.0 Hz, H-8), 6.87 (2H, d, *J* = 8.7 Hz, H-3',5'), 7.82 (2H, d, *J* = 8.7 Hz, H-2',6'); ¹³C NMR (DMSO-d₆, 100 MHz) δ 61.1 (OCH₃), 61.2 (OCH₃), 98.5 (C-8), 101.3 (C-6), 110.9 (C-3), 113.4 (C-10), 121.3 (C-3',5'), 125.6 (C-1'), 133.0 (C-2',6'), 164.3 (C-5), 165.4 (C-4'), 165.6 (C-2), 166.9 (C-9), 168.8 (C-7), 180.9 (C-4); ESI-TOF MS *m/z* 321.0737 [M+Na]⁺ (calcd for C₁₇H₁₄O₅+Na, 321.0739).

Bioassay

Antimalarial Assay. Antimalarial activity was performed against *Plasmodium falciparum* (K1, multidrug resistant strain) which was cultured continuously according to the method of Trager and Jensen (1976). Quantitative assessment of antimalarial activity *in vitro* was determined by means of the microdilution radioisotope technique based on the method described by Desjardins et al. (1979). The inhibitory concentration (IC₅₀) represents the concentration that causes 50% reduction in parasite growth as indicated by the *in vitro* uptake of [³H] hypoxanthine by *P. falciparum*. The standard compound was artemisinin.

Antifungal Assay. The antifungal activity was assessed against *Candida albicans* using method modified from the soluble formazan assay (Scudiero et al., 1988). The number of living cells was determined by measuring the absorbance of 2,3-bis (2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide (XTT) formazan at 450 nm. The standard drug was amphotericin B.

Antimycobacterial Assay. The antimycobacterial activity was assessed against *Mycobacterium tuberculosis* H₃₇Ra using the Microplate Alamar Blue Assay (MABA) (Collins and Franzblau, 1997). The standard drugs, isoniazid and kanamycin sulfate, were used as the reference compounds.

Cytotoxicity Assay. Cytotoxicity assay against human epidermoid carcinoma (KB), human breast cancer cells (BC) and human small cell lung cancer (NCI-H187) cell lines were performed employing the colorimetric method (Skehan et al., 1990). Ellipticine was included as a reference substance.

Results and Discussion

Chromatographic separation of the CH₂Cl₂ extract of the rhizomes of *K. parviflora* gave five flavonoids, 5,7-dimethoxyflavone (**1**) (Jaipetch et al., 1983), 3,5,7,3',4'-pentamethoxyflavone (**2**) (Herunsalee et al., 1987), 5,3'-dihydroxy-3,7,4'-trimethoxyflavone (**3**) (Guerrero et al., 2002; Matsuda et al., 2002), 5,4'-dihydroxy-7-methoxyflavone (**4**) (Jamzad et al., 2003; Skoula et al., 2005) and 4'-hydroxy-5,7-dimethoxyflavone (**5**) (Lin et al., 2002). Their structures were identified on

the basis of spectral data. Compounds 3-5 were isolated from this plant for the first time.

Biological activity testing of the isolated compounds may help to establish a scientific basis for the utilization of *K. parviflora*. Our work focused on antiplasmodial, antifungal, antimycobacterial and cytotoxic activity tests of all isolates, and the results are shown in Table 1. Compound **1** showed antifungal activity against *Candida albicans* with an IC₅₀ value of 19.98 µg/ml and antimycobacterial activity with a MIC value of 50 µg/ml. In our point of view, the mono-substitution at the aromatic ring B is likely essential for antimycobacterial and antifungal activities. All of the isolated compounds (**1-5**) possessed no antiplasmodial activity and cytotoxicity against KB, BC and NCI-H187 cell lines.

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