

The isolation of bioactive flavonoids from *Jacaranda obtusifolia* H. B. K. ssp. *rhombifolia* (G. F. W. Meijer) Gentry

SORACHAI KHAMSAN¹
SAISUNEE LIAWRUANGRATH²
APHIWAT TEERAWUTKULRAG²
STEPHEN G. PYNE³
MARY J. GARSON⁴
BOONSOM LIAWRUANGRATH^{1,*}

¹ Faculty of Pharmacy, Department of Pharmaceutical Science, Chiang Mai University, Chiang Mai, 50200 Thailand

² Faculty of Science, Department of Chemistry and Center for Innovation in Chemistry, Chiang Mai University Chiang Mai, 50200 Thailand

³ School of Chemistry, University of Wollongong, Wollongong, NSW 2522 Australia

⁴ School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, QLD, 4072 Australia

The paper describes the bioassay-guided isolation, structure elucidation and anticancer evaluation of five flavonoids (–)-liquiritigenin (1), (–)-neoliquiritin (2), isoliquiritigenin (3), isoliquiritin (4) and formononetin (5) from the twigs of *Jacaranda obtusifolia* H. B. K. ssp. *rhombifolia* (G. F. W. Meijer) Gentry. The structures were elucidated based on ¹H, ¹³C NMR, comprehensive 2D NMR, MS analyses and comparison with previously reported spectral data. Compounds 1 and 3 were demonstrated to be inhibitory *in vitro* against NCI-H187 (small cell lung cancer) with IC₅₀ values of 30.1 and 16.6 μg mL⁻¹, respectively. The isolates were non-cytotoxic to Vero cells (African green monkey kidney).

Keywords: *Jacaranda obtusifolia* (Bignoniaceae), flavonoids, anticancer activity

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Jacaranda is one of the genera of *Bignoniaceae* endemic to South America and is now widely distributed in the tropical and subtropical areas of the world. Some species are used in traditional medicine in various countries for the treatment of wounds, rheumatism, colds and skin diseases (1). More recently, a review of the ethnobotanical and pharmacological uses of *Jacaranda* species has pointed out its interesting phytochemical constituents and biological activities such as anti-dyspeptic, cytotoxic, anti-malarial and lipooxygenase inhibitory activity as well as antimicrobial activities (2). *Jacaranda obtu-*

* Correspondence; e-mail: liawruangrath@gmail.com

sifolia H. B. K. ssp. *rhombofolia* (G. F. W. Meijer) Gentry is a medium-sized, deciduous tree with a variably shaped crown and is now commonly cultivated in Thailand (3). Previously, we reported the isolation and structure elucidation of a 7-hydroxy-3'-methoxy-4'-*O*- β -D-glucoside isoflavone, daidzein, and genistin from *J. obtusifolia*. The isolated compounds possess significant anticancer activity (4). Therefore, in continuation of this work we focused on the isolation of different fractions and subfractions of *J. obtusifolia* and evaluation of their anticancer activity.

EXPERIMENTAL

General experimental procedures

All NMR spectra were recorded on a Bruker Avance (500 MHz) spectrometer (USA). ^1H and ^{13}C spectra were recorded relative to $\text{MeOH-}d_4$ ($\delta = 3.30$ and 49.0 ppm, respectively). Low resolution electrospray ionization mass spectra (LR-ESIMS) were measured on a Finnigan MAT 900 XL double focusing magnetic sector mass spectrometer (Finnigan, Germany). Optical rotations were measured on a Perkin-Elmer 241 MC polarimeter (USA) with a 10-cm path length. Reverse phase TLC was carried out on precoated silica gel aluminium-backed plates (RP-18 F_{254s}, 0.25 mm thick, Merck, Germany). The plates were first viewed under UV light at 254 and 365 nm, then spotted using 3 % cerium sulfate in 2 mol L⁻¹ H₂SO₄, followed by heating. Flash column chromatography was carried out using RP-C₁₈ silica gel (40–60 μm , Merck). Reverse phase high performance liquid chromatography (RP-HPLC) was performed on an Agilent 1100 system (USA) with UV detection at 254 nm using a Phenomenex Gemini (5 μm) C₁₈ (10 mm \times 250 mm) semipreparative column.

Plant material

Twigs of *J. obtusifolia* were collected in July 2008. The plant material was identified by J. F. Maxwell (Department of Biology, Faculty of Science, Chiang Mai University, Thailand), and a voucher specimen was deposited at the CMU Herbarium and Faculty of Pharmacy, of the same university.

Extraction and isolation

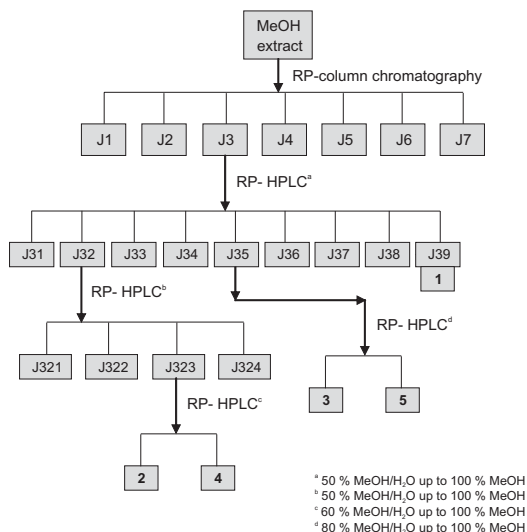
Dried twigs of *J. obtusifolia* (1 kg) were ground and extracted successively with 2.5 L of *n*-hexane, CHCl₃ and MeOH at room temperature, followed by filtration through a Whatman No. 1 filter paper. The hexane, CHCl₃ and MeOH extracts were concentrated under vacuum using a rotary evaporator (Büchi, Switzerland) to afford crude extracts as follows: crude hexane extract (dark green syrup 2.8 g), crude CHCl₃ extract (brownish sticky solid 7.6 g) and crude MeOH extract (dark brownish sticky solid 9.3 g), and were then stored in the refrigerator.

The methanolic extract (0.88 g) was purified by reverse-phase flash column chromatography on RP-C₁₈ silica gel (160 g) with gradient elution using MeOH/H₂O; 25 : 75, 50 : 50, 75 : 25 and 100 : 0, *V/V*, each 300 mL (each collection was 100 mL) in the order of

decreasing polarity. The eluents were collected, monitored by TLC and ^1H NMR, producing 7 groups of eluting fractions. The solvents were evaporated to dryness *in vacuo* to afford 7 major fractions: **J1** (0.15 g), **J2** (0.03 g), **J3** (0.34 g), **J4** (0.02 g), **J5** (0.05 g), **J6** (0.02 g) and **J7** (0.02 g). Fraction **J3** was further purified by RP-HPLC. Elution was conducted initially with 50 % MeOH/ H_2O up to 100 % MeOH (for 40 min, flow rate 1.5 mL min^{-1} , UV detection at 254 nm) to give 9 subfractions: **J31** to **J39** (compound **1**, $t_{\text{R}} = 20.2 \text{ min}$). Subfraction **J32** (5 mg) was purified by RP-HPLC using the same conditions to give 4 subfractions **J321**–**J324**. **J323** (2.2 mg) was subjected to further RP-HPLC purification (60 % MeOH/ H_2O up to 100 % MeOH) to afford compounds **2** (1.2 mg, $t_{\text{R}} = 12.7 \text{ min}$) and **4** (0.75 mg, $t_{\text{R}} = 19.0 \text{ min}$). Fraction **J5** (30 mg) was purified by RP-HPLC using gradient elution from MeOH/water (80 : 20) to 100 % MeOH to afford compounds **3** (1.5 mg, $t_{\text{R}} = 11.6 \text{ min}$) and **5** (0.8 mg, $t_{\text{R}} = 12.8 \text{ min}$) (Scheme 1).

Anticancer activity

Cytotoxic assays of crude extracts and the isolated compounds against three cancerous human-cell lines: KB cell line (epidermoid carcinoma of oral cavity, ATCC CCL-17), MCF-7 cell line (breast adenocarcinoma, ATCC HTB-22) and NCI-H 187 cell line (small cell lung carcinoma, ATCC CRL-5804) were performed employing the resazurin microplate assay (REMA) as described by Brien *et al.* (5) with suitable modification. Briefly, cells in a logarithmic growth phase were harvested and diluted to $7 \times 10^4 \text{ cells mL}^{-1}$ for KB and $9 \times 10^4 \text{ cells mL}^{-1}$ for MCF-7 and NCI-H 187, in fresh medium. Successively, 5 μL of the essential oil was diluted in 5 % DMSO, and 45 μL of cell suspension was added to 384-well plates, incubated at 37°C in a 5 % CO_2 incubator. After the incubation period (3 days for KB and MCF-7, and 5 days for NCI-H187), 12.5 μL of $62.5 \mu\text{g mL}^{-1}$ resazurin so-



Scheme 1. Isolation scheme for flavonoids from *J. obtusifolia*.

lution was added to each well, and the plates were then incubated at 37 °C for 4 hours. The fluorescence signal was measured using a SpectraMax M5 multi-detection microplate reader (Molecular Devices, USA) at excitation and emission wavelengths of 530 nm and 590 nm. The inhibitory concentration (IC_{50}) represented the concentration that caused 50 % reduction in cancer cell line growth. The cytotoxicity assay against Vero cells (African green monkey kidney) was performed using a green fluorescent protein (GFP)-based assay (6).

RESULTS AND DISCUSSION

The methanolic extract of *J. obtusifolia* twigs was purified by reverse phase flash column chromatography on RP-C₁₈ silica gel, followed by reverse phase HPLC purification resulting in the isolation of five flavonoids. The isolated compounds were identified as: (–)-liquiritigenin (1), (–)-neoliquiritin (2), isoliquiritigenin (3), isoliquiritin (4) and formononetin (5). The isolated compounds were identified by spectroscopic analysis (¹H, ¹³C NMR, 2D NMR and MS) (Fig. 1, Tables I and II). This data was also confirmed by comparison with previously reported spectral data. This extract was selected for its anticancer activity (small cell lung cancer with IC_{50} value of 23.2 $\mu\text{g mL}^{-1}$) (Table III).

Chemistry

Compound 1 was isolated as an amorphous yellowish solid. The LR-ESIMS mass spectrum showed the molecular ion peak at m/z 279.1 [$M + \text{Na}^+$]. The ¹H NMR (Table I) showed signals for a dihydroflavone: ABX-type aromatic unit at δ (ppm) of 7.68 (1H, d, $J = 8.8$ Hz, H-5), 6.43 (1H, dd, $J = 2.3, 8.8$ Hz, H-6) and 6.27 (1H, d, $J = 2.3$ Hz, H-8) due to the A ring; AA'BB'-type aromatic unit at δ 7.31 (2H, d, $J = 8.6$ Hz, H-2', 6') and 6.81 (2H, d, $J = 8.6$ Hz, H-3', 5') due to the B ring. The aliphatic proton signals at δ (ppm) of 5.35 (1H, dd, $J = 3.0, 13.0$ Hz, H-2), 3.01 (1H, dd, $J = 13.0, 16.9$ Hz, H-3) 2.66 (1H, dd, $J = 3.0,$

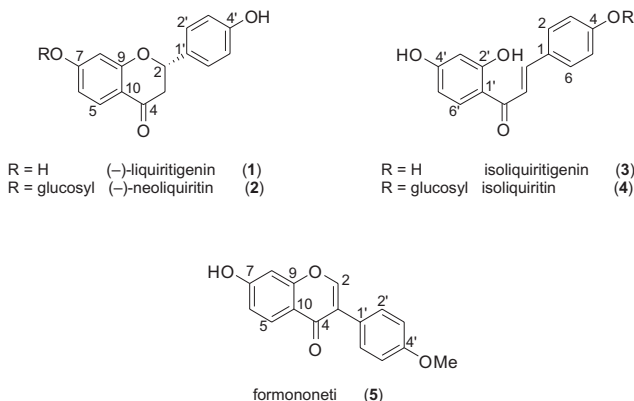


Fig. 1. Structures of flavonoids isolated from the twigs of *J. obtusifolia*.

16.9 Hz, H-3) were attributed to a CH-CH₂ system. On this basis and comparison of ¹H (Table I) and ¹³C NMR data (Table II) with published values (7) suggested that compound 1 was liquiritigenin. The specific rotation of compound 1 ($[\alpha]_D^{21} -11$ (γ 0.09 g mL⁻¹, MeOH) was comparable to the reported value, ($[\alpha]_D -10$ (γ 1.00 g mL⁻¹, EtOH) of (-)-li-

Table I. ¹H NMR data of flavonoids from *J. obtusifolia*

Position	Chemical shift (δ , ppm) ^{a,b}				
	1	2	3	4	5
2	5.35 (dd, 3.0, 13.0)	5.42 (dd, 2.5, 12.9)	7.59 (d, 8.6)	7.61 (d, 8.6)	8.02 (s)
3	3.01 (dd, 13.0, 16.9)	3.10 (dd, 12.9, 16.9)	6.83 (d, 8.6)	6.80 (d, 8.6)	
	2.66 (dd, 3.0, 16.9)	2.73 (dd, 2.7, 16.9)			
4					
5	7.68 (d, 8.8)	7.80 (d, 8.8)	6.83 (d, 8.6)	6.80 (d, 8.6)	7.91 (d, 8.9)
6	6.43 (dd, 2.3, 8.8)	6.77 (dd, 2.2, 8.8)	7.59 (d, 8.6)	7.61 (d, 8.6)	6.75 (dd, 2.2, 8.9)
7					
8	6.27 (d, 2.3)	6.70 (d, 2.2)	7.58 (d, 15.4)	7.60 (d, 15.4)	6.58 (d, 2.2)
9			7.74 (d, 15.4)	7.83 (d, 15.4)	
10					
1'					
2'	7.31 (d, 8.6)	7.32 (d, 8.6)			7.44 (d, 8.7)
3'	6.81 (d, 8.6)	6.80 (d, 8.6)	6.20 (d, 2.4)	6.61 (d, 2.5)	6.96 (d, 8.7)
4'					
5'	6.81 (d, 8.6)	6.80 (d, 8.6)	6.35 (dd, 2.4, 9.0)	6.69 (dd, 2.5, 8.9)	6.96 (d, 8.7)
6'	7.31 (d, 8.6)	7.32 (d, 8.6)	7.90 (d, 9.0)	8.06 (d, 8.9)	7.44 (d, 8.7)
1''		5.00 (d, 7.5)		5.02 (d, 7.5)	
2''		3.47 (m)		3.49 (m)	
3''		3.45–3.48 (m)		3.46–3.51 (m)	
4''		3.41 (m)		3.41 (m)	
5''		3.45–3.48 (m)		3.46–3.51 (m)	
6''					
6a		3.87 (dd, 2.2, 12.2)		3.90 (dd, 12.0, 2.2)	
6b		3.67 (dd, 5.5, 12.2)		3.70 (dd, 12.0, 5.4)	
OMe					3.82 (s)

^a Spectra obtained in CD₃OD.

^b Coupling constant in Hz.

quiritigenin and so compound **1** had the same configuration as liquiritigenin previously published in the literature (8). Flavanones with a 2*S* configuration and having a C-2 equatorial aryl group are known to be laevorotatory (9). The C-2 aryl substituent was in the equatorial orientation, as evident from the large $J_{2,3ax}$ (13.0 Hz). Thus, the absolute configuration at C-2 of compound **1** was confirmed as *S*.

The ^1H NMR (Table I) data for compound **2** suggested a structure similar to that of compound **1**, differing only in the sugar moiety. An analysis of the sugar unit was completed using DQF-COSY, 1D-TOCSY, HSQC and HMBC data and led to its identification as β -glucose. The doublet of doublets at δ 3.87 ppm (dd, $J = 2.2, 12.2$ Hz) and at 3.67 ppm (dd, $J = 5.5, 12.2$ Hz), each integrating for one proton, were characteristic of the H-6'' pro-

Table II. ^{13}C NMR data of flavonoids from *J. obtusifolia*

Position	Chemical shift (δ , ppm) ^a				
	1	2	3	4	5
1			127.8	125.0	
2	80.8	79.8	131.7	131.1	152.6
3	44.9	43.3	117.0	116.4	123.7
4	193.3	192.5	161.8	163.5	176.6
5	129.8	128.1	117.0	116.4	126.0
6	113.1	110.9	131.7	131.1	118.6
7	165.7	164.1	192.7	192.4	171.0
8	104.2	103.7	118.5	115.5	102.5
9	169.9	163.6	145.0	145.5	159.5
10	113.7	115.8			113.9
1'	131.1	129.8	113.6	115.0	124.7
2'	128.8	127.8	167.8	164.5	129.9
3'	116.2	114.9	104.4	103.4	113.4
4'	159.0	153.7	169.7	165.3	159.7
5'	116.2	114.9	110.8	108.0	113.4
6'	128.8	127.8	133.2	131.3	129.9
1''		100.1		99.8	
2''		73.5		73.3	
3''		76.5		76.1	
4''		69.8		71.6	
5''		76.5		78.4	
6''					
6a		60.9		61.8	
6b		60.9		61.8	
OMe					54.2

^a Spectra obtained in CD₃OD.

tons of the sugar and these signals correlated by HSQC with the carbon at δ 60.9 ppm. The linkage position of the sugar was established by a heteronuclear multiple bond correlation (HMBC) experiment (Fig. 2), which showed the correlation of the anomeric proton signal at δ 5.00 ppm to C-7 at δ 164.1 ppm. The specific rotation of **2** [α]_D²¹ -14 (γ 0.04 g mL⁻¹, MeOH) was comparable to the reported value, [α]_D -26.7 (γ 0.5 g mL⁻¹, MeOH) (10), despite the different concentrations used. Compound **2** was identified as (-)-neoliquiritin and indicated that C-2 had the *S* configuration. In nature, only D sugars occur in abundance. From the negative specific rotation value found, the sugar unit should be β -D-glucopyranoside (11). The LR-ESIMS displayed a molecular ion peak at m/z 441.0 [M+Na⁺] in agreement with the structure of compound **2**.

The ¹H NMR data (Table I) of compound **3** exhibited signals for an ABX-type aromatic unit at δ 7.90 ppm (1H, d, *J* = 9.0 Hz, H-6'), 6.35 ppm (1H, dd, *J* = 2.4, 9.0 Hz, H-5') and 6.20 ppm (1H, d, *J* = 2.4 Hz, H-3') due to the A ring; AA'BB'-type aromatic unit at δ 7.59 ppm (2H, d, *J* = 8.6 Hz, H-2, 6) and 6.83 ppm (2H, d, *J* = 8.6 Hz, H-3, 5) due to the B ring; the olefinic proton signals at δ 7.74 ppm (1H, d, *J* = 15.4 Hz, H-9), and 7.58 ppm (1H, d, *J* = 15.4 Hz, H-8) were attributed to positions β and α of a chalcone skeleton, and the coupling constant of 15.4 Hz (in MeOH-*d*₄) between H-8 and H-9 revealed that the olefinic system had a *trans* geometry. Comparison of the ¹H (Table I) and ¹³C NMR data (Table II) with previously published spectral data (7) suggested that compound **3** was

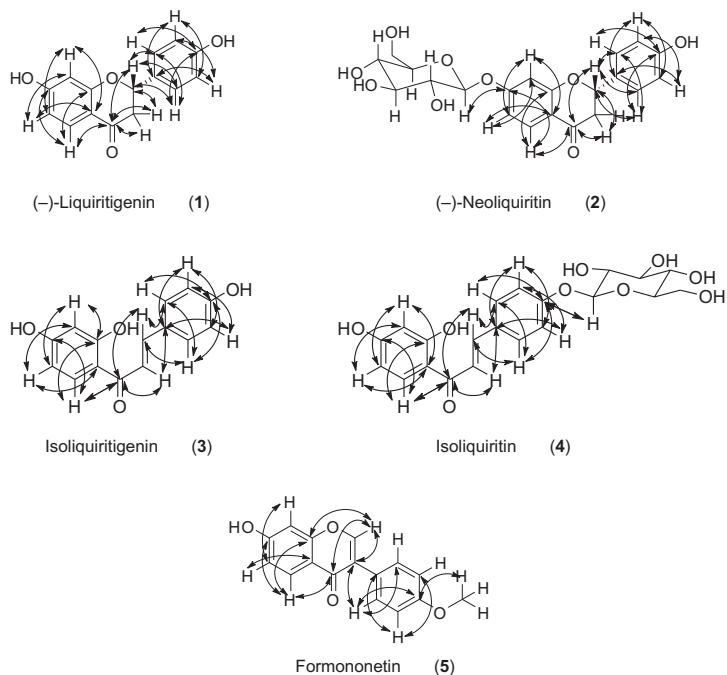


Fig. 2. Significant HMBC correlations of the isolated flavonoids.

Table III. Anticancer activity of flavonoids isolated from *J. obtusifolia*

Sample	Vero cells	IC ₅₀ ^a (μg mL ⁻¹)		NCI-H187
		KB	MCF-7	
Hexane extract	Non-cytotoxic	Inactive ^b	Inactive ^b	Inactive ^b
CHCl ₃ extract	Non-cytotoxic	Inactive ^b	Inactive ^b	Inactive ^b
MeOH extract	Non-cytotoxic	Inactive ^b	Inactive ^b	23.15
(–)-Liquiritigenin (1)	Non-cytotoxic	Inactive ^b	Inactive ^b	30.11
(–)-Neoliquiritin (2)	Non-cytotoxic	Inactive ^b	Inactive ^b	Inactive ^b
Isoliquiritigenin (3)	Non-cytotoxic	–	–	16.55
Isoliquiritin (4)	Non-cytotoxic	–	–	Inactive ^b
Formononetin (5)	Non-cytotoxic	–	–	Inactive ^b
Ellipticine ^c	1.67	0.51		1.18
Doxorubicine ^d	–	0.34	1.18	0.058

^a Concentration that killed 50 % of cell lines.

^b Inactive at 50 μg mL⁻¹.

^{c,d} Anticancer drugs used as positive controls.

isoliquiritigenin. The LR-ESIMS mass spectrum showed the molecular ion peak at m/z 279.1 [M+Na⁺], which corresponded to isoliquiritigenin.

Compound 4 showed the molecular ion peak at m/z 441.0 [M+Na⁺] in its LR-ESIMS. Compound 4 had a specific rotation of $[\alpha]_D^{21} -53$ (γ 0.04 g mL⁻¹, MeOH). The ¹H NMR data (Table I) of compound 4 suggested a structure similar to that of compound 3, differing only in the presence of a β -glucose moiety. HMBC experiment showed the linkage position of the sugar (Fig. 2), indicating the correlation of an anomeric proton signal at δ 5.02 ppm (1H, d, $J = 7.5$ Hz) with C-4 at δ 163.5 ppm. A comprehensive analysis of DQF-COSY, HSQC and HMBC spectra of compound 4 allowed complete assignment of its proton and carbon signals. On the basis of this finding and from a comparison with literature NMR data (12), compound 4 was identified as isoliquiritin.

Isoflavone 5 showed ¹H NMR signals for the ABX-type aromatic unit at δ (ppm) of 7.91 (1H, d, $J = 8.9$ Hz, H-5), 6.75 (1H, dd, $J = 2.2, 8.9$ Hz, H-6) and 6.58 (1H, d, $J = 2.2$ Hz, H-8) due to the A ring; AA'BB'-type aromatic unit at δ (ppm) of 7.44 (2H, d, $J = 8.7$ Hz, H-2', 6') and 6.96 (2H, d, $J = 8.7$ Hz, H-3', 5') due to the B ring; the δ of 8.02 ppm (1H, s) signal was attributed to a proton of ring C and there was also a methoxy substituent at δ 3.82 ppm (3H, s, –OCH₃) (Table I). The linkage position of –OCH₃ was obtained from an HMBC experiment (Fig. 2), which showed the correlation of the proton signal at δ 3.82 ppm (3H, s) with C-4' at δ 159.7 ppm. Compound 5 exhibited the molecular ion peak at m/z 291.0 [M+Na⁺] in its LR-ESIMS. Comparison with the previously reported data (13) indicated that compound 5 was formononetin.

Anticancer activity

The methanolic extract exhibited anticancer activity against the NCI-H187 cancer cell line with an IC_{50} value of $23.2 \mu\text{g mL}^{-1}$. The IC_{50} values of isolated compounds are presented in Table III. Isoliquiritigenin (**3**) exhibited the most potent anticancer activity against NCI-H187 with an IC_{50} of $16.6 \mu\text{g mL}^{-1}$, while (–)-liquiritigenin (**1**) showed moderate anticancer activity against NCI-H187 ($IC_{50} = 30.1 \mu\text{g mL}^{-1}$). In addition, isolated compounds **1**–**5** showed no cytotoxicity against Vero cells (African green monkey kidney). Ellipticine and doxorubicin were used as positive controls. Ellipticine showed anticancer activity against KB and NCI-H 187 cell lines with the IC_{50} values of 0.5 and $1.2 \mu\text{g mL}^{-1}$, respectively. Doxorubicin exhibited activity toward KB, MCF-7 and NCI-H 187 cell lines with the IC_{50} in the range of 0.06– $0.34 \mu\text{g mL}^{-1}$.

CONCLUSIONS

Bioactivity-guided fractionation of the active methanolic extract of the *J. obtusifolia* twigs led to the isolation of (–)-liquiritigenin (**1**), (–)-neoliquiritin (**2**), isoliquiritigenin (**3**), isoliquiritin (**4**) and formononetin (**5**). (–)-Liquiritigenin (**1**) and isoliquiritigenin (**3**) were demonstrated to be active constituents against NCI-H187 (small cell lung cancer) with IC_{50} values of 30.1 and $16.6 \mu\text{g mL}^{-1}$, respectively, but were non-cytotoxic to Vero cells (African green monkey kidney).

Therefore, the anticancer activity of the isolated flavonoids supports the documented folklore usage of the extracts of this plant to treat cancers.

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REFERENCES

1. R. M. Ali and P. J. Houghton, A new phenolic fatty acid ester with lipoxygenase inhibitory activity from *Jacaranda filicifolia*, *Planta Med.* **65** (1999) 455–457; DOI: 10.1055/s-2006-960811.
2. M. S. Gachet and W. Schühly, *Jacaranda* – An ethnopharmacological and phytochemical review, *J. Ethnopharmacol.* **121** (2009) 14–27; DOI: 10.1016/j.jep.2008. 10.015.
3. T. Smitinan and K. Larsen, *Flora of Thailand*, The Chutima Press, Bangkok 1987.
4. S. Khamsan, S. Liawruangrath, A. Teerawutkulrag, S. G. Pyne, M. J. Garson and B. Liawruangrath, A new isoflavone from *Jacaranda obtusifolia* H.B.K. ssp. *rhombifolia* (G.F.W. Meijer) Gentry, *Acta Pharm. Sci.* **53** (2011) 181–188.
5. J. O. Brien, I. Wilson, T. Orton and F. Pognan, Investigation of the alamar blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity, *Eur. J. Biochem.* **267** (2000) 5421–5426; DOI: 10.1046/j.1432-1327.2000.01606.x.
6. L. Hunt, M. Jordan, M. De Jesus and F. M. Wurm, GFP expressing mammalian cells for fast, sensitive, noninvasive cell growth assessment in a kinetic mode, *Biotechnol. Bioeng.* **65** (1999) 201–205; DOI: 10.1002/(SICI)1097-0290(19991020)65:2< 201::AID-BIT10>3.0.CO;2-H.

7. C.-J. Ma, G.-S. Li, D.-L. Zhang, K. Liu and X. Fan, One step isolation and purification of liquiritigenin and isoliquiritigenin from *Glycyrrhiza uralensis* Risch. using high-speed counter-current chromatography, *J. Chromatogr. A* **1078** (2005) 188–192; DOI: 10.1016/j.chroma.2005.01.053.
8. N. I. Kulesh, N. A. Vasilevskaya, M. V. Veselova, V. A. Denisenko and S. A. Fedoreev, Minor polyphenols from *Maackia amurensis* wood, *Chem. Nat. Compds.* **44** (2008) 712–714; DOI: 10.1007/s10600-009-9195-3.
9. E. Garo, J.-L. Wolfender, K. Hostettmann, W. Hiller, S. Antus and S. Mavi, Prenylated flavanones from *Monotes engleri*. online structure elucidation by LC/UV/NMR, *Helv. Chim. Acta* **81** (1998) 754–763; DOI: 10.1002/hlca.19980810325.
10. J. Li, S. Kadota, Y. Kawata, M. Hattori, G. Xu and T. Namba, Constituents of the roots of *Cynanchum bungei* Decne. Isolation and structures of four new glucosides, bungeiside A, B, C, and D, *Chem. Pharm. Bull.* **40** (1992) 3133–3137.
11. W. Fan, Y. Tezuka, K. M. Ni and S. Kadota, Prolyl endopeptidase inhibitors from the underground part of *Rhodiola sachalinensis*, *Chem. Pharm. Bull.* **49** (2001) 396–401.
12. B. Fu, H. Li, X. Wang, F. S. C Lee and S. Cui, Isolation and identification of flavonoids in licorice and a study of their inhibitory effects on tyrosinase, *J. Ag. Food Chem.* **53** (2005) 7408–7414; DOI: 10.1021/jf051258h.
13. T. Han, H. Li, Q. Zhang, H. Zheng and L. Qin, New thiazinediones and other components from *Xanthium strumarium*, *Chem. Nat. Compds.* **42** (2006) 567–570; DOI: 10.1007/s10600-006-0215-2.

S A Ž E T A K

Izolacija bioaktivnih flavonoida iz biljke *Jacaranda obtusifolia* H. B. K. ssp. *rhombifolia* (G. F. W. Meijer) Gentry

SORACHAI KHAMSAN, SAISUNEE LIAWRUANGRATH, APHIWAT TEERAWUTKULRAG,
STEPHEN G. PYNE, MARY J. GARSON i BOONSOM LIAWRUANGRATH

U radu je opisana izolacija, određivanje strukture i antitumorsko djelovanje pet flavonoida: (–)-likviritigenina (1), (–)-neolikviritina (2), izolikviritigenina (3), izolikviritina (4) i formononetina (5) iz plodova biljke *Jacaranda obtusifolia* H. B. K. ssp. *rhombifolia* (G. F. W. Meijer) Gentry. Strukture su određene na temelju ¹H, ¹³C NMR, 2D NMR, MS i usporedbom s ranije objavljenim spektroskopskim podacima. Spojevi 1 i 3 imaju inhibični učinak *in vitro* na tumorsku staničnu liniju raka pluća NCI-H187 (IC₅₀ vrijednost 30,1, odnosno 16,6 μg mL⁻¹). Izolirani flavonoidi nisu citotoksični za Vero stanice (bubrežne stanice afričkog zelenog majmuna).

Ključne riječi: *Jacaranda obtusifolia* (Bignoniaceae), flavonoidi, antitumorsko djelovanje

Faculty of Pharmacy, Department of Pharmaceutical Science, Chiang Mai University, Chiang Mai, 50200 Thailand

Faculty of Science, Department of Chemistry and Center for Innovation in Chemistry, Chiang Mai University, Chiang Mai, 50200 Thailand

School of Chemistry, University of Wollongong, Wollongong, NSW, 2522 Australia

School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, QLD, 4072 Australia