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A new isoflavone from *Jacaranda obtusifolia* H.B.K. ssp. *rhombifolia* (G.F.W. Meijer) gentry

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Abstract

A new isoflavone (**1**), along with daidzein (**2**), and genistin (**3**) were isolated from the *Jacaranda obtusifolia* twig extract. Their structures were elucidated by NMR and MS spectroscopy. Compound **1** possessed significant anticancer activity against KB and NCI-H187 cell lines with the IC_{50} values of 2.52 and 7.47 μg mL^{-1}, compound **2** and **3** exhibited anticancer activity against KB and NCI-H187 cell lines with the IC_{50} values of 5.73, 8.14, 2.83 and 27.30 μg mL^{-1}, respectively. Compound **2** also showed anticancer activity against MCF-7 cell line with an IC_{50} value of 28.17 μg mL^{-1}. The isolates showed non-cytotoxic against Vero cells.

Key Words: *Jacaranda obtusifolia*, Bignoniaceae, isoflavonoids, anticancer activity, cytotoxicity

Introduction

Cancer is undoubtedly one of the major causes of death worldwide, with there being high mortality rates and the numbers of new cancer cases are expected to continue rising. It is predicted that by 2020 approximately 15 million new cancer cases diagnosed and 12 million cancer patients will die (Bray and Moller 2006, Jemal et al. 2009). So, the discovery of novel and more effective anticancer drugs is necessarily needed.

Natural products have attracted considerable attention as cancer chemopreventive agents and also as cancer therapeutics (Kinghorn 2000, Nobili et al. 2009). Therefore, we have concentrated on the potential of natural products as an anticancer activity from plant sources.

Jacaranda is one of the genera of Bignoniaceae endemic to South America and now widely distributed in the tropical and subtropical areas of the world. Some species are used in traditional medicine of difference countries to treatment of wound, rheumatism, colds and skin disease.

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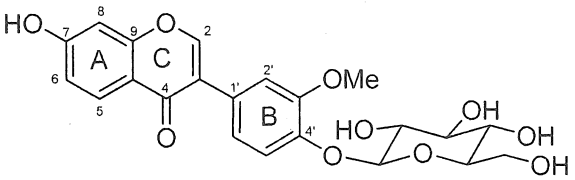
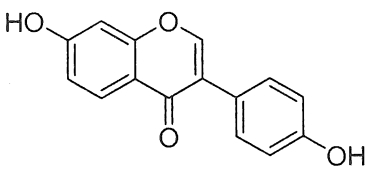
More recently, a review of the ethnobotanical and pharmacological uses of *Jacaranda* species has pointed out interesting biological activities and phytochemical constituents (Gachet and Schühly 2009). Various activities have been discovered in *Jacaranda* species such as anti-dyspeptic activity in *Jacaranda caroba* (Botion et al. 2005), cytotoxic (Ogura et al. 1976) and anti-malarial activity (Weniger et al. 2001) in *Jacaranda caucana*, lipooxygenase inhibitory in *Jacaranda filicifolia* (Ali and Houghton 1999) including cardiovascular (Nicasio and Meckes 2005) and antimicrobial activities (Binutu and Lajubutu 1994) in *Jacaranda mimosaefolia*.

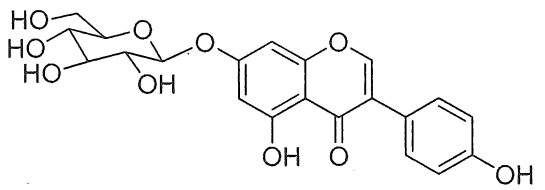
Jacaranda obtusifolia H.B.K. ssp. *rhombifolia* (G.F.W. Meijer) Gentry is now commonly cultivated in Thailand (Smitinan and Larsen 1987). According to ethnomedical investigation, its bark was used to promote wound healing and as disinfectant (Roth and Lindorf 2002). However, no chemical investigations and biological activities previously reported on this species. The aim of this work was to investigate the chemical constituents and to evaluate its biological activities. This present paper, we describe the isolation and structural elucidation of the isolated isoflavones **1-3** (Fig. 1). In addition, the cytotoxicity of the isolates is also reported.



7-hydroxy-3′-methoxy-4′-O-β-D-glucoside isoflavone **(1)**



daidzein **(2)**



genistin **(3)**

Figure 1. Structures of isoflavones isolated from the twigs of *J. obtusifolia*

Material and Method

General experimental procedures

High resolution electrospray ionization mass spectra (HR-ESIMS) and Low resolution electrospray ionization mass spectra (LR-ESIMS) were measured using a Finnigan MAT 900 XL double focusing magnetic sector mass spectrometer. Optical rotations ($[\alpha]_D$) were measured with a Perkin-Elmer 241 MC Polarimeter between 21-28°C with a path length of 1.0 dm. All NMR spectral were recorded on a Bruker Avance 400, or a Bruker Avance 500 MHz spectrometer. ^{1}H- and ^{13}C spectra were recorded relative to MeOH-d_4 (δ = 3.30 and 49.0 ppm, respectively). Infared (IR) spectra was obtained using Infared Spectrometer FT-IR Bruker model Tensor 27. Reverse phase TLC aluminium-backed were carried out on precoated silica gel plates (RP-18 F_{254s}, 20x20 cm, 0.25 mm thick, Merck). The plates were first viewed

under UV light at 254 and 365 nm then spotted using 3% cerium sulfate in 2N H_2SO_4 followed by heating. Column Chromatography (CC) was carried out using RP-C_{18} silica gel (230-400 mesh, Merk). Reverse phase high performance liquid chromatography (RP-HPLC) was performed on an Agilent 1100 system with UV detection at 254 nm using a Phenomenex Gemini C18 column (250×10 mm, 5 μm).

Plant Material

The twigs of *J. obtusifolia* were collected from Chiang Mai University in July 2008 and identified by J. F. Maxwell. A voucher specimen (S. Khamsan 1) was deposited at the Herbarium of Chiang Mai University, Chiang Mai, Thailand.

Extraction

The air-dried twig powder of *J. obtusifolia* (1 kg) was extracted successively with of *n*-hexane, $CHCl_3$ and MeOH (2.5 L, 6 h for each solvent), followed by filtration through a paper filter (Whatman No. 1). The hexane, $CHCl_3$ and MeOH extracts were concentrated under reduced pressure to obtain crude hexane extract (dark green syrup 2.8 g), crude $CHCl_3$ extract (brownish sticky solid 7.6 g) and crude MeOH extract (dark brownish sticky solid 9.3 g).

Isolation

The methanolic extract (0.88 g) was subjected to RP flash column chromatography over RP-C_{18} silica gel (1.5×15 cm, 160 g), eluted gradiently with MeOH-H_2O (25:75, 50:50, 75:25 and 100:0, v/v, each 250 mL). All fractions were collected, monitored by TLC and combined together as appropriate. The solvents were evaporated to dryness afforded 7 major fractions: **J1** (150 mg), **J2** (30 mg), **J3** (340 mg), **J4** (20 mg), **J5** (50 mg), **J6** (20 mg) and **J7** (20 mg). A portion of fraction **J3** (150 mg) was further purified by RP-HPLC with Phenomenex Gemini C_{18} column. Elution was conducted initially with 50% MeOH/H_2O up to 100% MeOH (for 40 mins, flow rate 1.5 mL min^{-1}, UV detection 254 nm) yielded 9 subfractions: **J3_1**-**J3_9**. Similarly, subfraction **J3_3** (15 mg) was subjected to RP-HPLC eluting with MeOH- H_2O (60-100%, for 30 mins, flow rate 1.5 mL min^{-1}, UV detection 254 nm) to afford compound **3** (1.2 mg, t_R = 11.4 min) and compound **2** (0.75 mg, t_R = 20.6 min), respectively. Subfraction **J3_6** (25 mg) was separated the mixture by RP-HPLC (50% MeOH/H_2O up to 100% MeOH for 30 mins, flow rate 1.5 mL min^{-1}, UV detection 254 nm) to afford compound **1** (1.2 mg, t_R = 19.9 min).

Determination of Anticancer Activity

The cytotoxic assays of crude extracts and the isolates against three cancerous human-cell lines: KB (epidermoid carcinoma of oral cavity, ATCC CCL-17), MCF-7 (breast adenocarcinoma, ATCC HTB-22) and NCI-H187 cell lines (small cell lung carcinoma, ATCC CRL-5804) were performed employing the Resazurin Microplate Assay (REMA) (Brien *et al.* 2000), and a cytotoxicity assay against Vero cells (African green monkey kidney) was performed using a Green Fluorescent Protein (GFP)-based assay (Hunt et al. 1999). Ellipticine and doxorubicine were used as positive controls which exhibited activity toward the KB, MCF-7, NCI-H187 cell lines and Vero cells with the IC_{50} ranges of 0.51-1.67 μg mL^{-1} and 0.058-1.18 μg mL^{-1}, respectively.

Results and Discussion

The methanolic extract of *J. obtusifolia* twigs showed anticancer activity against NCI-H187 cell line with an IC_{50} value of 23.15 μg mL^{-1}. Therefore, this extract was selected for further purification. Isolation and purification was carried out by reverse phase flash column chromatography on C-18 silica gel followed by reverse phase HPLC resulted in the isolation of a new isoflavone, 7-hydroxy-3′-methoxy-4′-O-β-D-glucoside isoflavone (**1**), along with two

known compounds daidzein (**2**), and genistin (**3**). The structures of the isolated compounds were completely elucidated by spectroscopic analysis on ^{1}H (Table 1), ^{13}C- NMR (Table 2), 2D NMR and MS. These data of the known compounds were also confirmed by comparison with previously reported spectral data

Table 1. ^{1}H NMR data of the isolated isoflavones from *J. obtusifolia*

Position	Chemical shift (mult, *J*)[a,b]		
	1	2	3
1			
2	7.95 (1H, s)	8.09 (1H, s)	8.13 (1H, s)
3			
4			
5	7.85 (1H, d, 9.0)	8.00 (1H, d, 8.9)	
6	6.69 (1H, dd, 2.3, 9.0)	6.88 (1H, dd, 2.2, 8.9)	6.51 (1H, d, 2.0)
7			
8	6.48 (1H, d, 2.3)	6.76 (1H, d, 2.2)	6.71 (1H, d, 2.0)
9			
10			
1′			
2′	6.84 (1H, d, 2.5)	7.35 (1H, d, 8.7)	7.35 (1H, d, 8.6)
3′		6.83 (1H, d, 8.7)	6.82 (1H, d, 8.6)
4′			
5′	7.16 (1H, d, 8.4)	6.83 (1H, d, 8.7)	6.82 (1H, d, 8.6)
6′	6.64 (1H, dd, 2.5, 8.4)	7.35 (2H, d, 8.7)	7.35 (1H, d, 8.6)
1″	4.95 (1H, d, 7.5)		5.04 (1H, d, 7.5)
2″	3.47 (1H, m)		3.49 (1H, m)
3″	3.44-3.48 (1H, m)		3.48 (1H, m)
4″	3.42 (1H, m)		3.41 (1H, m)
5″	3.44-3.48 (1H, m)		3.51 (1H, m)
6″			
6a	3.87 (1H, dd, 2.5, 12.0)		3.90 (1H, dd, 2.2, 12.2)
6b	3.65 (1H, dd, 6.0, 12.0)		3.70 (1H, dd, 5.6, 12.2)
-OCH_3	3.79 (3H, s)		

[a]500MHz, MeOH- d_4, [b]coupling constant in Hz

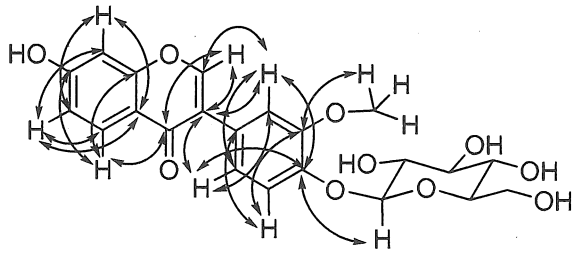
The new isoflavone **1** was isolated as an amorphous yellowish solid. The specific rotation ($[\alpha]^{21.0}_{D}$) was -50.3 (*c*=0.04/MeOH). The empirical formula was established as $C_{22}H_{22}O_{10}$ on the basis of the complete positive HR-ESIMS mass spectrum which showed the molecular ion peak at m/z 469.1102 $[M+Na]^+$ (calcd. 469.1105) that corresponded to its structure. The IR spectrum showed the hydroxyl (3600-3375 cm^{-1}), a carbonyl (1625 cm^{-1}), an aromatic (1505-1480 cm^{-1}) and C-O (1250 cm^{-1}) stretchings. The ^{1}H NMR showed a sharp singlet at δ 7.95 (1H, s), typical of a proton at C-2 of an isoflavonoid skeleton, signals of the ABX-type aromatic unit at δ 7.85 (1H, d, 9.0, H-5), 6.69 (1H, dd, J= 2.3, 9.0 Hz, H-6) and 6.48 (1H, d, 2.3, H-8) due to the A ring, there was also an aromatic unit at δ 6.84 (1H, d, 2.5 Hz, H-2′), 7.16 (1H, d, 8.4 Hz,H-5′) and 6.64 (1H, dd, 2.5, 8.4 Hz, H-6′) due to the B ring, together with an aromatic methoxy at δ 3.79 (3H, s). The doublet ascribable to the anomeric proton was also observed at δ 4.95 (1H, d, 7.5 Hz), indicating the β-configuration. An analysis of the sugar unit was performed using DQF-COSY, 1D-TOCSY, HSQC and HMBC data that identified as β-glucose. Doublet of doublets at δ 3.87 (dd, 2.5, 12.0 Hz) and 3.65 (dd, 6.0, 12.0 Hz), each integrating for one proton was the characteristic of the H-6″ protons of the sugar and these signals correlated by HSQC to the carbon at δ 61.1. In the DQF-COSY experiment, a correlation between proton H-6″ at δ 3.87 and proton H-6″ at 3.65 was also observed. The linkage position of the aromatic methoxy and β-

glucose was established by an HMBC experiment. The HMBC spectrum showed the cross-peak between the anomeric proton signal at δ 4.95 and C-4′ at δ 157.0 and the correlation of the aromatic methoxy δ 3.79 linked to C-3′ at δ 161.4 (Fig. 2).

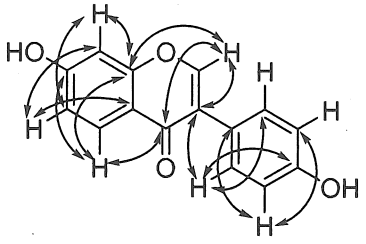
Table 2. ^{13}C NMR data of the isolated isoflavones from *J. obtusifolia*

Position	Chemical shift[a]		
	1	2	3
1			
2	153.7	152.7	155.1
3	114.9	122.4	125.2
4	177.4	176.9	182.6
5	125.6	126.8	163.5
6	120.1	116.2	101.0
7	174.1	166.1	164.6
8	102.9	102.0	95.8
9	160.2	158.9	159.2
10	112.7	115.8	108.1
1′	121.7	123.0	122.6
2′	101.7	130.1	131.2
3′	161.4	115.1	116.6
4′	157.0	157.4	159.6
5′	131.3	115.1	116.6
6′	107.3	130.1	131.2
1″	101.9		101.6
2″	73.5		74.8
3″	76.8		78.0
4″	70.1		71.0
5″	78.4		78.3
6″			
6a	61.1		62.3
6b	61.1		62.3
-OCH_3	54.5		

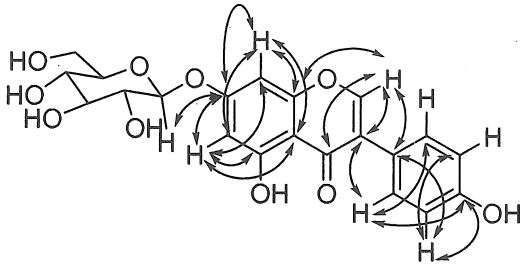
[a]125MHz, MeOH- d_4



7-hydroxy-3′-methoxy-4′-O-β-D-glucoside isoflavone **(1)**



daidzein **(2)**



genistin **(3)**

Figure 2. Significant HMBC correlations of the isolated isoflavones

The isoflavone **2** was isolated as an amorphous yellowish solid. The $[M+Na]^+$ ion in the LR-ESIMS at m/z 277 suggested a molecular formula of $C_{15}H_{10}O_4$. The ^1H NMR signals of the ABX-type aromatic unit at δ 8.00 (1H, d, J = 8.9 Hz, H-5), 6.88 (1H, dd, J = 2.2, 8.9 Hz, H-6) and 6.76 (1H, d, J = 2.2 Hz, H-8) due to the A ring; there was also an AA′BB′-type aromatic unit at δ 7.35 (2H, d, J = 8.7 Hz, H-2′,6′) and 6.83 (2H, d, J = 8.7 Hz, H-3′,5′) due to the B ring; the olefinic proton signal at δ 8.09 (1H, s) is attributed to a proton of ring C. A full analysis was completed using HSQC and HMBC and confirmed by comparison with the literature suggested that compound **2** was daidzein (Venkateswarlu et al. 2005), a compound which found mostly in soybeans, legumes and has been isolated previously from the root of *Pueraria labata* (Keung et al. 1998).

The isoflavone **3** had a molecular formula of $C_{21}H_{20}O_{10}$ as determined by LR-ESIMS at m/z 455.1 $[M+Na]^+$. The ^1H NMR data of compound **3** suggested the structure similar to that of compound **2**, differing only the sugar moiety. The ^1H NMR data of compound **3** showed a doublet at δ 5.04 (1H, d, J = 7.5 Hz), which was typical for the anomeric proton of a glucose moiety, and the *J* value suggested that the glucose moiety had a β configuration. An analysis of the sugar unit was completed using DQF-COSY, 1D-TOCSY, HSQC and HMBC data that identified as β-glucose. The location of the β-glucose on rings B was deduced from HMBC correlation between the anomeric proton signal at δ 5.04 (1H, d, J = 7.5 Hz) with C-7 at δ 164.6. The specific rotation of compound **3** ($[\alpha]^{21.0}_D$ -53.9 (*c*=0.03/MeOH) was comparable to the reported value $[\alpha]_D$ -28 which corresponded to genistin (Lee et al. 2002). The isoflavone genistin (**3**) was isolated from the MeOH extract of the fruit of *Chaenomeles sinensis* (Lee et al. 2002) and other plants such as *Thermopsis fabacea* D.C. (Arisawa et al. 1980).

The anticancer activities of the crude extracts of *J. obtusifolia* were determined by the Resazurin Microplate Assay (REMA). The methanolic extract exhibited moderate anticancer activity against the NCI-H187 cell line with the IC_{50} value of 23.15 μg mL^{-1}. All the isolated flavonoids were also subjected to anti-cancer evaluation. It demonstrated that the new isoflavone (**1**), **2** and **3** possessed strong anticancer against KB cell line with the IC_{50} values of 2.52, 5.73 and 2.83 μg mL^{-1}, respectively. Compound **2** is also showed moderate anticancer against MCF-7 cell line with the IC_{50} value of 28.17 μg mL^{-1}. A new isoflavone (**1**) exhibited significant anticancer against NCI-H187 cell line with the IC_{50} value of 7.47μg mL^{-1}, and compound **2** and **3** showed moderate anticancer against this cell line with the IC_{50} values of 8.14 and 27.30 μg mL^{-1}, respectively. In addition, the isolates showed no cytotoxicity against Vero cells (African green monkey kidney). Ellipticine and doxorubicine were assayed as positive control which showed with the IC_{50} ranges of 0.51-1.67 μg mL^{-1} and 0.058-1.18 μg mL^{-1}, respectively (Table 3).

The chemical constituents from *J. obtusifolia* have not been studied previously. This is the first report we described three isolated isoflavones and their anticancer activity from this species. Therefore, the anticancer activity of the isolated isoflavones can be supported their folklore usage and might be useful for new drug development.

Table 3. Anticancer activity of the isolated isoflavones from *J. obtusifolia*

	IC_{50}[a] ($\mu g\ mL^{-1}$)			
Sample	**Vero cells**	**KB**	**MCF-7**	**NCI-H187**
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
new isoflavone (**1**)	Non-cytotoxic	2.52	Inactive[b]	7.47
daidzein (**2**)	Non-cytotoxic	5.73	28.17	8.14
genistin (**3**)	Non-cytotoxic	2.83	Inactive[b]	27.30
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 $\mu g\ mL^{-1}$; [c,d]Anticancer drugs used as positive control

Conclusions

One new isoflavone (**1**), daidzein (**2**), and genistin (**3**) were isolated from the active methanolic twig extract of *J. obtusifolia*. All isolated compounds were identified as the respective major active constituents which the new isoflavone (**1**) possessed strong anticancer activity against KB (Oral cavity cancer) and NCI-H187 (Small cell lung cancer) with the IC_{50} values of 2.52 and 7.47 $\mu g\ mL^{-1}$, compound **2** exhibited significant anticancer activity against KB, MCF-7 (Breast cancer) and NCI-H187 cell lines with the IC_{50} values of 5.73, 28.17 and 8.14 $\mu g\ mL^{-1}$, and compound **3** exhibited strong anticancer activity against KB cell line with the IC_{50} value of 2.83 $\mu g\ mL^{-1}$ and showed moderate anticancer activity against NCI-H187 cell line with the IC_{50} value of 27.30 $\mu g\ mL^{-1}$, respectively. This is the first report describes the secondary metabolites and anticancer activity from this plant.

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