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Short communication

Cytotoxic constituents from *Butea superba* Roxb.

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Abstract

A carpin (3-hydroxy-9-methoxypterocarpan) (Medicarpin) (**1**) and four isoflavones, 7-hydroxy-4′-methoxy-isoflavone (Formononetin) (**2**); 7,4′-dimethoxyisoflavone (**3**); 5,4′-dihydroxy-7-methoxy-isoflavone (Prunetin) (**4**) and 7-hydroxy-6,4′-dimethoxyisoflavone (**5**) were isolated from the tuber roots of *Butea superba* Roxb. Compounds **2** and **4** showed moderate cytotoxic activity on KB cell lines with IC_{50} (μM) values of 37.3 ± 2.5 and 71.1 ± 0.8 and on BC cell lines with IC_{50} (μM) values of 32.7 ± 1.5 and 47.3 ± 0.3, respectively.

Keywords: *Butea superba*; Isoflavones; Carpin; Cytotoxicity

1. Introduction

With emphasis to the plants in the genus *Butea*, information of the isolated chemicals and/or bioactivities derived mostly from *Butea frondosa* Koen. ex Roxb. (synonym; *Butea monosperma*). The plant contained lectin in seeds and exhibited estrogenic and postcoital anti-conceptive activity in rats (Bhargava, 1986; Wongkham et al., 1994). Glucosides were also found in the plant extract (Gupta et al., 1970). The plant leaves exhibited anti-inflammatory effect (Mengi and Deshpande, 1999) and effect on stress, anxiety, and cognition in rats (Soman et al., 2004). The plant extract exhibited various bioactivities, including aphrodisiac activity in male rats (Ramachandran et al., 2004), protective role against thioacetamide-mediated hepatic alterations in Wistar rats (Sehrawat et al., 2006), *in vivo* anthelmintic activity against *Trichostrongylid nematodes* in sheep (Iqbal et al., 2006), anti-diabetic in rats (Somani et al., 2006), anti-diarrhoeal activity in experimental animals (Gunakkunru et al., 2005), dermal wound healing in rats (Sumitra et al., 2005), anti-convulsive activity in laboratory animals (Kasture et al., 2000; Veena et al., 2002), anthelmintic activity (Prashanth et al., 2001) and anti-fungal (Bandara et al., 1989).

Butea superba Roxb. is a plant in the Family Leguminosae with the common name of "red Kwao Krua" in Thailand. The plant is abundantly distributed in the Thai deciduous forests in many parts of the country. This indigenous herb has been traditionally consumed among Thai males for the purposes of rejuvenate as well as maintain sexual performance or prevent erectile dysfunction (Suntara, 1931). The Thai *Butea superba* Roxb. was found to contain flavonoid and flavonoid glycosides with potent cAMP phosphodiesterase inhibitory activity (Roengsumran et al., 2000). The plant crude extract exhibited significant inhibitory activity on acetylcholinesterase (Ingkaninan et al., 2003). The clinical trial of the plant powder as crude drug showed effective treatment of erectile dysfunction with a comparable method with sildenafil treatment (Cherdshewasart and Nimsakul, 2003; Cappelleri and Rosen, 2005). Besides, *Butea superba* Roxb. exhibited anti-proliferation effect on the growth of MCF-7 and Hela cells in relation with a possible anti-estrogen mechanism or a potent cytotoxic effect (Cherdshewasart et al., 2004a,b). The Indian *Butea superba* Roxb. stems contained flavone glycoside and flavonol glycoside (Yadava and Reddy,

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1998a,b). This suggests that the chemical constituents of this plant might partially show cytotoxic activity.

Therefore, this work describes the isolation and structural determination of the chemical constituents of this plant and their cytotoxic activity.

2. Materials and methods

2.1. General procedures

Melting points were determined on Electrothermal 9100. The optical rotations were determined on a Perkin-Elmer 341. Measurements of UV spectra were carried out on the Perkin-Elmer Lambda 25 UV–vis spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded at 400.00 and 100.00 MHz, respectively, on a Varian Mercury-400 plus NMR spectrometer. MS spectra were measured with high-resolution electron spray/time of flight (HRES/TOF) mass spectrometer (Micromass UK Limited). Column chromatography was performed on silica gel 230–400 mesh for flash column chromatography (Isco CombiFlash^{TM} Graduate^{TM}).

2.2. Plant materials

The tuber roots of *Butea superba* Roxb. (Leguminosae) were collected from Lampang Province, Thailand in April 2002. The plant was authenticated by comparison with the herbarium collection number No. BCU 11046 in the Department of Botany, Faculty of Science, Chulalongkorn University, Bangkok, Thailand.

2.3. Extraction and isolation

The dried tuber roots powder of *Butea superba* (5.5 kg) was extracted with MeOH (3× 15 L) and the methanolic extract (522 g) was re-extracted with hexane (2× 700 mL) and then $CHCl_3$ (2× 700 mL). The hexane and $CHCl_3$ extracts were evaporated under reduced pressure to give a crude hexane extract (1.2 g; 0.03%, w/w) and crude $CHCl_3$ extract (40.0 g; 0.73%, w/w). The crude $CHCl_3$ extract was separated by flash column chromatography and eluted with 100% of CH_2Cl_2, CH_2Cl_2–MeOH mixtures; the gradual concentration has increase in a stepwise of 1% MeOH. Compounds **1**–**5** (Fig. 1) were obtained from 1, 1.5 and 2% MeOH in CH_2Cl_2 and identified by comparison of their spectral data with reported values in the literatures.

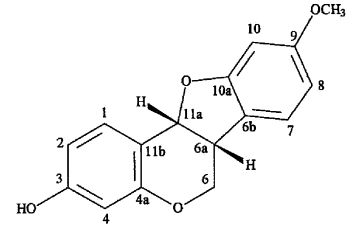
2.4. Cell lines

KB, human epidermoid carcinoma of cavity (ATCC CCL-17); BC, breast cancer cell line; NCI-H 187, human small cell lung carcinoma (ATCC CRL-5804).

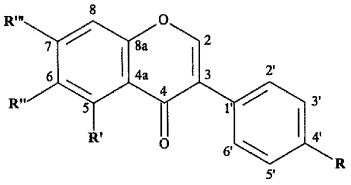
2.5. Cytotoxicity assay

KB and BC cell lines were determined by colorimetric cytotoxicity assay that measured cell growth from cellular protein content according to Skehan et al. (1990). Briefly, cells at a logarithimic growth phase were harvested and diluted to 10^5 cells/mL with fresh medium and gently mixed. Test compounds were dissolved with few drops of DMSO and diluted with water to make the concentration of DMSO less than 0.1%, added into 96-well plates in total volume 200 μL and incubated at 37 °C for 72 h. After incubation period, cells were fixed by 50% trichloroacetic acid, incubated at 4 °C for 30 min, washed with distilled water, dried at room temperature and stained with 0.05% (w/v) sulforhodamine B (SRB) dissolved in 1% acetic for 30 min. Unbound dye was removed with 1% acetic acid, and protein-bound dye was extracted with 10 mM Tris base [tris(hydroxymethyl)aminomethane] for determination of optical density (OD) at 510 nm.

NCI-H 187 were determined by MTT (3-[4,5-dimethylhiazol-2-yl]-2,5-diphenyl tetrazolium bromide) assay previously described in detail by Plumb et al. (1989). Briefly, cells were diluted to 10^5 cells/mL. Test compounds were dissolved with few drops of DMSO and diluted with water to make the concentration of DMSO less than 0.1%, added into 96-well plates and incubated at 37 °C for 5 days. MTT solution (2 mg/mL) was added into each well and then incubated at 37 °C for 4 h. The MTT formazan crystals were dissolved in 200 μL for 100%



3-Hydroxy-9-methoxypterocarpan (1)



R	R'	R''	R'''	
OCH_3	H	H	OH	7-Hydroxy-4'-methoxy-isoflavone (2)
OCH_3	H	H	OCH_3	7,4'-Dimethoxyisoflavone (3)
OH	OH	H	OCH_3	5,4'-Dihydroxy-7-methoxy-isoflavone (4)
OCH_3	H	OCH_3	OH	7-Hydroxy-6,4'-dimethoxyisoflavone (5)

Fig. 1. Compounds isolated from *Butea superba* Roxb.

DMSO and 25 μL of Sorensen's glycine buffer. After 20 min, the optical density (OD) was measured with microtiter plate reader at wavelength of 510 nm.

Doxorubincin hydrochloride was used as a positive control and DMSO was used as a negative control.

2.6. Data analysis

Cell lines growth and growth inhibition were expressed in terms of mean (±1 of S.D.) absorbance units and/or percentage of control absorbance (±1 of S.D.%) following subtraction of mean "background" absorbance. In addition, the IC_{50} was expressed as the sample concentration in micromolar that caused a 50% inhibition of growth compared with controls. The analysis was assisted with SPSS program.

3. Results and discussion

In the present study, the isolation of the chemical constituents with cytotoxicity from the tuber roots of *Butea superba* Roxb. were carried out. The dried tuber roots were extracted with methanol and re-extracted with hexane and chloroform at room temperature. The extracts were concentrated under reduce pressure, and then chloroform crude extract was chromatographed on flash column by eluting with gradients of dichloromethane and methanol to give compounds **1**–**5**.

3-Hydroxy-9-methoxypterocarpan (Medicarpin) (**1**) (5 mg, 0.00009% yields from dried powder) was obtained from 1% MeOH in CH_2C1_2 as white solid mp 127–129 °C, UV($CHC1_3$) 236.06 and 265.93 nm (log ε 4.14 and 4.34) at 27 °C; $[\alpha]_D^{265}$ −223.60 (*c* 0.01 g/100 mL, CH_2Cl_2). The structure of compound **1** was also confirmed by 2D-NMR, which is in agreement to those previously reported (Stadler et al., 1994; Chan et al., 1998; Chang et al., 1997; Konoshima et al., 1997).

7-Hydroxy-4′-methoxy-isoflavone (Formononetin) (**2**) (6.0 mg, 0.00013% yields from dried powder) was obtained from 2% MeOH in CH_2Cl_2 as orange solid mp 261–262 °C, UV($CHC1_3$) λ_{max} 273.00 and 295.01 nm (log ε 3.19 and 3.34) at 27 °C; $[\alpha]_D^{265}$ −32.85 (*c* 0.01 g/100 mL, $CHCl_3$). The structure of compound **2** was also confirmed by 2D-NMR, which is in agreement to those previously reported (Lopes et al., 1999; Khan et al., 2000).

7,4′-Dimethoxyisoflavone (**3**) (1.2 mg, 0.000009% yields from dried powder) was obtained from 1.5% MeOH in CH_2C1_2 as yellow solid mp 162–163 °C, UV($CHC1_3$) λ_{max} 239.05 and 300.97 nm (log ε 5.24 and 5.20) at 27 °C; $[\alpha]_D^{265}$ −19.68 (*c* 0.05 g/100 mL, $CHCl_3$). The structure of compound **3** was also confirmed by 2D-NMR, which is in agreement to those previously reported (Ingham et al., 1981; Veitch et al., 2003).

5,4′-Dihydroxy-7-methoxy-isoflavone (Prunetin) (**4**) (6.0 mg, 0.00013% yields from dried powder) was obtained from 1% MeOH in CH_2C1_2 as brown solid mp 239–240 °C, UV ($CHCl_3$) λ_{max} 287.94 nm (log ε 3.21) at 27 °C; $[\alpha]_D^{265}$ −19.90 (*c* 0.04 g/100 mL, $CHCl_3$). The structure of compound **4** was also confirmed by 2D-NMR, which is in agreement to those previously reported (Baker et al., 1953; Tanaka et al., 1998; Talukdar et al., 2000).

7-Hydroxy-6,4′-dimethoxyisoflavone (**5**) (4.0 mg, 0.00007% yields from dried powder) was obtained from 1.5% MeOH in CH_2C1_2 as yellow solid mp 228–229 °C, UV($CHC1_3$) λ_{max} 240.93, 270.97 and 315.07 nm (log ε 5.04, 4.99 and 5.34) at 27 °C; $[\alpha]_D^{265}$ −5.65 (*c* 0.08 g/100 mL, $CHCl_3$). The structure of compound **5** was also confirmed by 2D-NMR, which is in agreement to those previously reported (McMurry and Theng, 1960; Herath et al., 1998).

The cytotoxicity of compounds **1**–**5** was assessed by SRB (for KB and BC cell lines) and MTT assay (for NCI-H187 cell line). Compounds **2** and **4** showed moderate cytotoxic activity on KB cell lines with IC_{50} (μM) values of 37.3 ± 2.5 and 71.1 ± 0.8 and on BC cell lines with IC_{50} (μM) values of 32.7 ± 1.5 and 47.3 ± 0.3, respectively. Compounds **1**, **3** and **5** were considered to be inactive (IC_{50} > 100 μM). Under the same test condition, doxorubicin hydrochloride exhibited cytotoxic activity against KB cell lines, BC cell lines and NCI-H187 cell line at IC_{50} (μM) values of 0.3, 0.4 and 0.5, respectively (Table 1).

Compounds **1**–**5** have been reported in many plant species and they exhibit various biological activities. Compound **1** showed anti-microbial activity (Stadler et al., 1994), anti-tumor promoting activity (Konoshima et al., 1997) and apoptosis-inducing effects on human PBMCs (Zhen-Li et al., 2002). Compound **2** showed anti-fungal activity against *Cladosporium cladosporioides* at 10-fold higher than the positive control Nystatin (Lopes et al., 1999). Compound **3** showed anti-giardial activity (Khan et al., 2000) and anti-plasmodial activity against *Plas-*

Table 1
Cytotoxicity of compounds **1**–**5** on KB, BC and NCI-H 187 cells[a]

Compound	IC_{50}(μM)		
	KB	BC	NCI-H 187
Medicarpin (**1**)	>100[b]	>100[b]	>100[b]
Formononetin (**2**)	37.3 ± 2.5	32.7 ± 1.5	>100[b]
7,4′-Dimethoxyisoflavone (**3**)	>100[b]	>100[b]	>100[b]
Prunetin (**4**)	71.1 ± 0.8	47.3 ± 0.3	>100[b]
7-Hydroxy-6,4′-dimethoxyisoflavone (**5**)	>100[b]	>100[b]	>100[b]
Doxorubicin hydrochloride[c]	0.3 ± 0.2	0.4 ± 0.1	0.5 ± 0.3

[a] Results are the mean of three replications.
[b] Considered inactive.
[c] Cytotoxic reference compound.

modium falciparum (Kraft et al., 2001). Compound **4** showed only moderate anti-plasmodial activity against *Plasmodium falciparum* (Carola et al., 2000). Therefore, this work reveals the chemical constituents and their moderate to weak cytotoxicity of *Butea superba* Roxb. for the first time. Moreover, other biological activities could be realized from its chemical constituents.

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