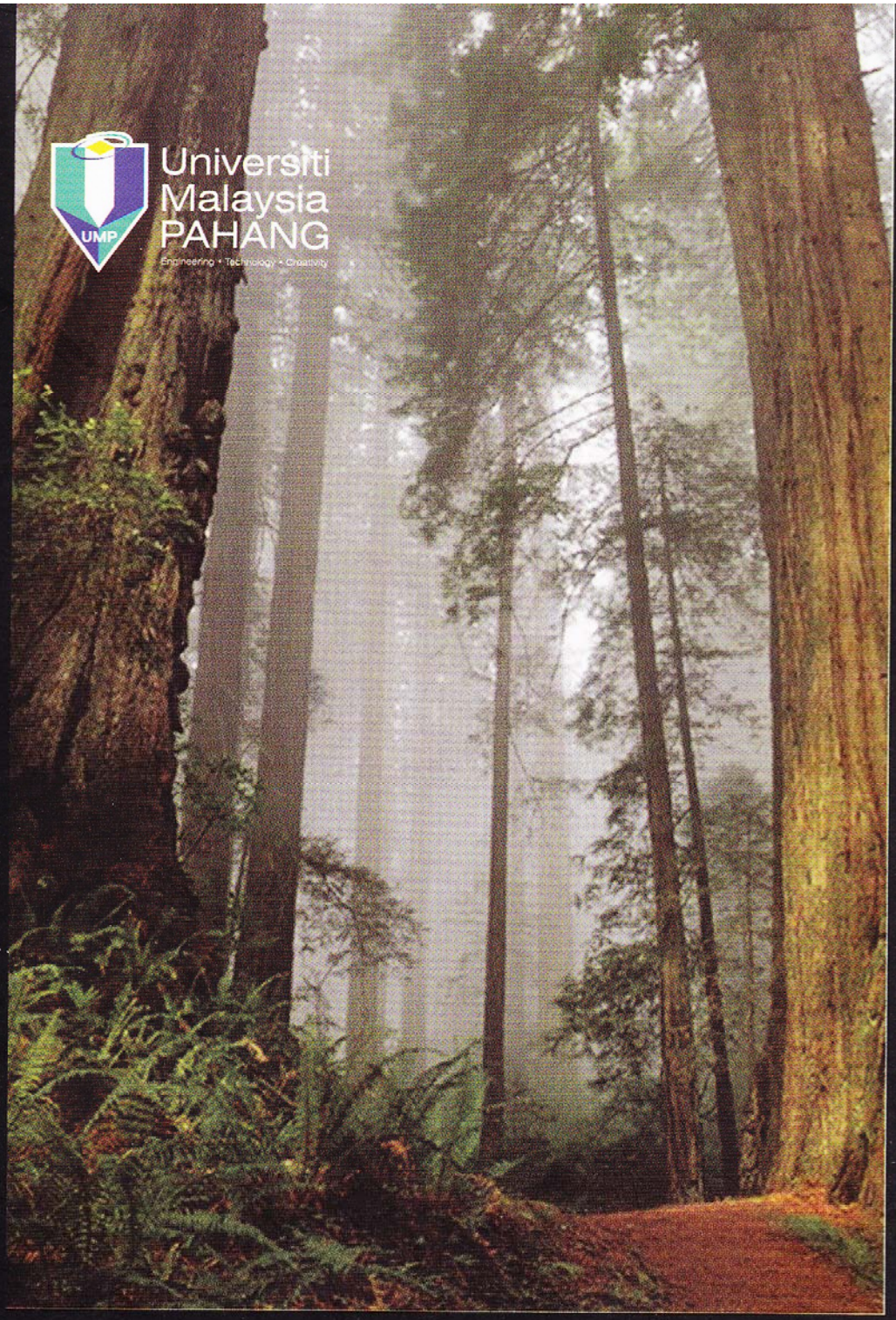

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Leads from Nature

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THE SECONDARY METABOLITE COMPOUND FROM *SESBANIA GRANDIFLORA* PLANT

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Sesbania grandiflora (L.) Pers. (family: *Leguminosae*; local name: turi) is a small erect, fast-growing, and sparsely branched tree which is native to tropical Asia and is widespread in India, Malaysia, Indonesia, and the Philippines. All parts of *Sesbania grandiflora* (L.) Pers. are utilized for medicine in Southeastern Asia and India. The root is applied as a poultice for application to inflammation and fever. The bark is considered astringent and is utilized for the treatment of smallpox, ulcers in the mouth, and the pounded bark is applied to scabies. The juice of the leaves is considered anthelmintic and tonic and is used to treat worms, biliousness, fever, gout, and itchiness. Previous study in *Sesbania*'s leaves revealed that *Sesbania* could afford a significant protective effect against erythromycin estolate-induced hepatotoxicity. Due to the medicinal value of this plant, it was selected for the isolation of its active constituents and also for the evaluation of its biological activity. A phenolic compound was isolated from the ethyl acetate extract of the root of *Sesbania grandiflora* (L.) Pers. The isolation and separation of this compound has been done by using partition and chromatography technique. Purification and characterization of the compound isolated was done by FT-IR, GC-MS, 1D and 2D NMR.

Keywords: Secondary metabolite, *Sesbania grandiflora* (L.) Pers., *Leguminosae*, phenolic compound

INTRODUCTION

The *Leguminosae* is one of the largest of flowering plants which consist of 18,000 species classified into around 650 genera (1). It is distributed throughout most of the world. Most of the *Leguminosae* family have been frequently used worldwide as traditional herbal medicine in treatment of illnesses such as diabetes, caugh, urinary disease, eye disease, lung disease, toothache, fever, dysentery, and different kinds of infections as well as inflammation of skin and mucous membranes (2, 3, 4, 5). Some genus of this family are also used against cancer, e.g. stomach cancer, and breast cancer (6. 7, 8). Since this family has been a valuable source for maintaining the human health for a long period of time, therefore more studies are still needed to further clarify the potential of this plant as a useful source of chemicals.

Literature survey of *Leguminosae* plant reveals that no extensive work has been done to investigate the chemical components of *Sesbania grandiflora* (L.) Pers. especially from the root part of the plant.

MATERIALS AND METHODS

General

Optical rotation was determined on a Digital Polarimeter (JASCO, DIP-370) with a 0.5 cm microcell. A Bruker Avance 300 spectrometer, operating at 300 MHz for ^{1}H and ^{13}C and at 400 MHz for 2D-experiments with tetramethylsilane as internal standard. The Fast Atomic Bombardment–Mass Spectroscopy (FAB–MS) data were recorded on a a Finnigan MAT95 instrument. IR spectra was recorded using a Perkin-Elmer system 2000 FT-IR Spectrometer. Spectra was obtained by potassium bromide (KBr) disc procedure. The range of measurement was from 4000 to 650 cm^{-1}. UV spectra was recorded using a Perkin-Elmer Lambda 25 Spectrometer. TLC was performed on pre-coated Merck plastic sheets (silica gel 60 PF254, 0.25 mm) and the plates were sprayed with $Ce(SO_4)_2.H_2O$ development. Preparative plates (PLC) [20x20 cm, Kieselgel F254 (0.5 mm)] were air dried and used without prior activation. Column chromatography was done on silica gel Merck Kieselgel 60 (230-400 mesh ASTM). Evaporations were done under reduced pressure at ambient temp. in a rotary evaporator. Mp's were recorded on a Stuart Scientific SMP1 apparatus.

Plant material

The roots of *Sesbania grandiflora (L.) Pers.* were collected on September 2008 from Sinarluas, Sidosari Village, Lampung Province in Indonesia. A voucher specimen has been deposited in Herbarium Bogoriense, Bogor, Indonesia.

Extraction

Dried and powdered roots (3.0 kg) were extracted exhaustively with methanol for 24 hours. The methanol extract was removed under reduced pressure to yield a dark brown gum (19 g). Liquid-liquid partition of methanol extract obtained by using *n*-hexane, chloroform, and ethylacetate as eluents yielded three corresponding fractions respectively, *n*-hexane fraction (1.9 g), $CHCl_3$ fraction (0.3 g),and EtOac fraction (4 g). Each fraction will be fractionated by chromatography technique. The EtOac fraction was repeatedly chromatographed over silica gel column using *n*-hexane and *n*-hexane-EtOAc by gradually increasing the polarity gradien to obtain seven fractions (E1-E7). Fraction E2 was selected to further purification using preparative TLC method which gave compound **1** (20 mg).

RESULTS AND DISCUSSION

Compound [**1**] was obtained as an orange pale powder, $[\alpha]^{20}_{D}$: -66.6 (c.0.1 MeOH). The positive ion FAB-MS exhibited an $[M+H]^+$ ion peak at m/z 273.1 corresponding to a molecular formula of $C_{16}H_{16}O_4$. The absorption band of [**1**] at 281 nm indicated the presence of a phenolic chromophore (see Figure1). The IR spectrum of [**1**] showed the absorptions due to hydroxyl (3361 cm^{-1}) group, aliphatic (2941 cm^{-1}) group, and aromatic ring (1624, 1455 cm^{-1})(see Figure2). The analysis of its NMR spectra including Heteronuclear Multiple Quantum Coherence (HMQC) and Heteronuclear Multiple Bond Correlation (HMBC) spectra, allowed structure to be determined, as well as for unambiguous assignment of all proton and carbon signals. The HMQC spectrum supplied to complete assignment of all protonated carbon, as the HMBC spectrum was used to determine all signals of quarternary carbon atom in [**1**]. The ^{1}H and ^{13}C-NMR spectral data of [**1**] showed similarity of the signals to those 7,4'-dihydroxy-2'-methoxyisoflavan. Further support for the structure was afforded from a comparison of spectral data of 1 with those isolated from *Cuban Propolis* (9) (see Table 1.)

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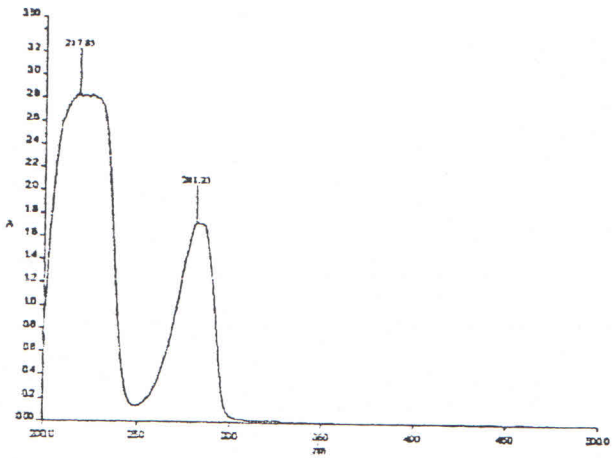

Figure 1. UV spectrum of [1]

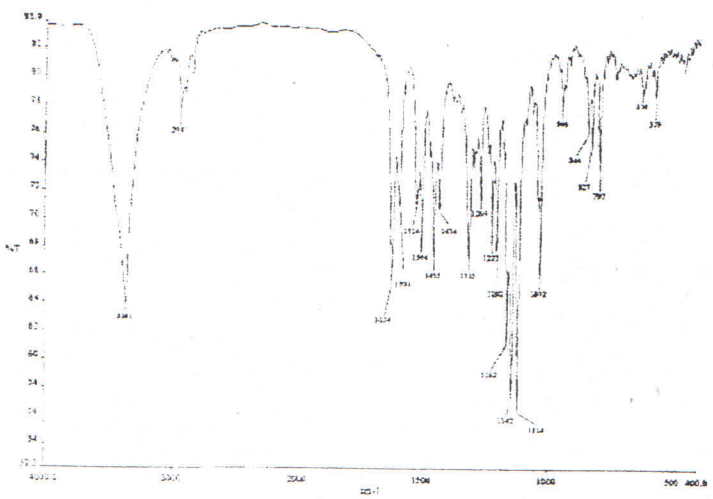

Figure 2. IR spectrum of [1]

Table 1. A comparison of ^{1}H-NMR and ^{13}C NMR spectroscopic between compound [1] and reference compound (9)

Ring	Carbon	Isolated Compound (300 MHz) (in aceton-d_6)		Reference Compound ^{1}H-NMR (599.19 MHz) and ^{13}C NMR (150.86 MHz) (in CD_3OD)	
		13C δ (ppm)	^{1}H δ (ppm), *multiplicity*, (*J*, Hz)	13C δ (ppm)	^{1}H δ (ppm), *multiplicity*, (*J*, Hz)
A	5	130.49	6.90, *d* (8.2)	131.2	6.98
	6	108.28	6.38, *dd* (8.2 & 2.4)	107.8	6.45
	7	157.03		160.4	
	8	103.99	6.29, *d* (2.4)	102.3	6.37
	9	156.23		156.5	
	10	113.83		116.1	
B	1'	120.49		120.0	
	2'	159.64		158.0	
	3'	105.24	6.43, *dd* (8.5 & 2.5)	103.6	6.34
	4'	155.64		157.3	
	5'	102.08	6.51, *d* (2.5)	107.6	6.28
	6'	128.24	7.06, *d* (8.5)	128.8	6.90
C	2	70.01	H_{α}, 3.99, *t* (10) H_{β}, 4.25, *br d* (10; 3;	71.3	H_{2ax}, 3.98, *t* (10) H_{2eq}, 4.26, *dd* (10; 3)
	3	33.69	& 2)	33.1	H_{3ax}, 3.49, *m*
	4	32.19	3.49, *m* (8; 5; & 3)	31.4	H_{4eq}, 2.82, *dd* (15.7; 5.1)
			H_{α}, 2.81, *dd* (10; 5		H_{4ax}, 3.00, *dd* (15.7;
	7-OCH_3	54.89	& 2)	55.6	10.5)
	$OH_{aromatic}$		H_{β}, 2.98, *dd* (16, 5)		3.76
	$OH_{aromatic}$		3.73, *s*		
			8.14, *br s*		
			8.59, *br s*		