

## New Prenylated Stilbenes and Antioxidant Activities of *Cajanus cajan* (L.) Millsp. (Pigeon pea)

Taslim Ersam\*, Sri Fatmawati, and Dini Nur Fauzia

Department of Chemistry, Sepuluh Nopember Institute of Technology (ITS)  
Jl. Arief Rahman Hakim, Sukolilo, Surabaya 60111, Indonesia

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### ABSTRACT

New Prenylated stilbene derivatives, 3-hydroxy-4-prenyl-5-methoxy-stilbene acid (**1**) and 5,4'-dihydroxy-3-methoxy-2-prenyl-stilbene (**2**) had been isolated from methanol extract of *Cajanus cajan* (L.) Millsp. (Pigeon pea), a member of family fabaceae. The aim of this present work was to evaluate its *in vitro* antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging. Isolation of compounds was achieved by extraction (maceration) and chromatographic technique. The structures of the compounds were determined based on UV, IR, <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra and also by comparing with the previously reported data. Antioxidant activities (IC<sub>50</sub>) of **1** and **2** were 413.68 µg/mL and 271.53 µg/mL, respectively.

**Keywords:** *Cajanus cajan* (L.) Millsp.; prenylated stilbene; antioxidant; 2,2-diphenyl-1-picrylhydrazyl

### ABSTRAK

Dua turunan stilben terprenilasi baru, Asam 3-hidroksi-4-prenil-5-metoksi-stilben (**1**) dan 5,4'-dihidroksi-3-metoksi-2-prenil-stilben (**2**) telah berhasil diisolasi dari ekstrak metanol tumbuhan kacang kayu (*Cajanus cajan* (L.) Millsp.) anggota famili fabaceae. Tujuan penelitian ini adalah untuk mengetahui potensi senyawa-senyawa tersebut sebagai antioksidan secara *in vitro* menggunakan radikal 2,2-difenil-1-pikrihidrazil (DPPH). Pemisahan senyawa dilakukan dengan ekstraksi secara maserasi dan teknik kromatografi. Penentuan struktur dilakukan menggunakan data spektroskopi UV, IR, <sup>1</sup>H-NMR dan <sup>13</sup>C-NMR dan dengan perbandingan data yang telah dilaporkan sebelumnya. Aktivitas antioksidan (IC<sub>50</sub>) senyawa **1** dan **2** masing-masing 413,68 dan 271,53 µg/mL.

**Kata Kunci:** *Cajanus cajan* (L.) Millsp.; stilben terprenilasi; antioksidan; 2,2-difenil-1-pikrihidrazil (DPPH)

### INTRODUCTION

Family *Fabaceae* is widely distributed in various parts of tropical and subtropical areas of the world produce a high diversity of secondary metabolites. Some members of these families are considered potential sources of phenolic compounds flavonoids, coumarin, tocopherol, chalcone, stilbene, xanthone and other cinnamate acid derivatives [1]. This phenolic compound has been used medicinally to treat various diseases such as antibacterial, anti-inflammatory, antiplasmodial, anti-ulcer, wound healing, hepatoprotective and anti-asthmatic ailments [2]. These applications are commonly practiced by the villagers [3].

*Cajanus cajan* (L.) Millsp. (Family *Fabaceae*, local name: *kacang kayu/kacang gude*) is a perennial member of the family *leguminosae*. It is widely cultivated in Poteran Island, Madura for its edible seeds. Chemical constituent investigations have indicated that *Cajanus cajan* leaves are rich of flavonoids such as pinostrobin, longistilins [4], cajachalcone [5], coumarin and stilbenes [6], which are considered responsible for the beneficial

efficacies of *C. cajan* leaves on human health [7-8]. The objective of the present study was to evaluate the compounds potential of methanol extract of leaves, stems and roots of the plant and their antioxidant activities.

Antioxidant has gained importance in the current scenario as it has an ability to trap free radicals which are produced during the degenerative diseases [9]. Activity Natural antioxidant is considered superior to synthetic as it is safe and produces a prominent action, especially plant phenolics, flavonoids, tannins and anthocyanidins [10]. Herein, we presented antioxidant activity of stilbene compounds from methanol extract of pigeon pea (*C. cajan* (L.) Millsp.), using DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity.

### EXPERIMENTAL SECTION

#### Materials

Leaves, stems and roots of *Cajanus cajan* (L.) Millsp. were collected from Poteran Island-Madura,

\* Corresponding author.  
Email address : taslimersam@its.ac.id

Indonesia in March, 2014. VLC was carried out using Merck Si gel 60 GF<sub>254</sub> and TLC analysis on precoated Si gel plates (Merck Kieselgel 60 F<sub>254</sub>, 0.25 mm).

### Instrumentation

Melting points were determined on Fisher John-melting point Apparatus. UV and IR spectra were measured with UV-1800 series (SHIMADZU) and FT-IR PRESTIGE 21 (SHIMADZU) spectrophotometers, respectively. <sup>1</sup>H and <sup>13</sup>C-NMR spectra were recorded with JEOL-Nuclear Magnetic Resonance (JNM ECS-400), operating at 400.0 MHz using standards from residual and deuterated solvent peaks, respectively. <sup>13</sup>C-NMR data were obtained from the indirectly detected dimension in HMQC and HMBC experiments. Spectra were referenced to residual solvent resonances at δ<sub>H/C</sub> 7.25/77.0 (CDCl<sub>3</sub>) relative to TMS.

### Procedure

#### Extraction and isolation

The dried powdered of *kacang kayu* (2.25 kg) was macerated in MeOH and the MeOH extract (80 g) was sequentially fractionated by VLC Si-gel using *n*-hexane-EtOAc in order of increasing polarity to give 10 fractions (A-J). Fraction E (6.12 g) was further subjected to VLC hexane-EtOAc in order of increasing polarity. Seven fractions (E<sub>1</sub>-E<sub>7</sub>) were ultimately obtained on combining the eluates on the basis of TLC. Fraction E<sub>5</sub> afforded, after crystallization from hexane-EtOAc, compound **1** (68.7 mg). Using the same methods, fraction E<sub>3</sub> (3.8 g) was subjected to VLC petroleum ether-dichloromethane in order of increasing polarity over Si-gel to afford 10 fractions (EE<sub>1</sub>-EE<sub>10</sub>). From the third fraction, compound **2** (332.8 mg) was obtained. The structures of the compounds were determined based on UV, IR, <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra.

#### DPPH free radical scavenging activity

The DPPH method was used to evaluate the antioxidant activity of the phenolic compounds [11-12]. The antioxidant activity of the **1** and **2** was determined in terms of hydrogen donating or radical scavenging ability, using the stable radical DPPH. Samples at various concentrations (600; 400; 200; 100; 50; 10 µg/mL) were added to 1 mL of DPPH and 2 mL methanol and allowed to stand for 30 min at 37 °C. The absorbance of the sample was measured at 517 nm. All experiments were repeated three times. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the formula [13].

$$\% \text{ inhibition} = \left[ \frac{(Abs_{control} - Abs_{test})}{Abs_{control}} \right] \times 100\% \quad (1)$$

**Table 1.** <sup>1</sup>H and <sup>13</sup>C-NMR data of compounds **1** (400 MHz, *d* -CDCl<sub>3</sub>, δ in ppm and *J* values (Hz) in parentheses)

Position	<sup>1</sup> H (δ <sub>H</sub> , multiplicity, <i>J</i> )	<sup>13</sup> C (δ <sub>C</sub> )	HMBC Correlation
1		141.8	
2		102.9	
3		162.2	
4		116.7	
5		162.4	
6	6.66 s	103.2	C-2, C-1, C=O, C-β, C-4, C-4
α	7.84 d (16)	130.4	C-2, C-β
β	6.83 d (16)	130.7	C-1, C-2', C-6', C-α
1'		137.2	
2', 6'	7.53 d (8)	126.7	C-3', C-5'
3', 5'	7.38 d (7.6)	128.7	C-1', C-2', C-6'
1"	3.37 d (6.8)	22.1	C-5", C-4, C-2", C-3", C-5
2"	5.21 t (7.6 & 6.8)	121.9	C-4", C-1", C-5"
3"		132.0	
4"	1.79 s	17.8	C-5", C-4, C-2", C-3"
5"	1.68 s	25.8	C-4", C-4, C-2", C-3"
C-OMe	3.95 s	55.7	C-5
C=O		175.4	
3-OH	11.53 s		

## RESULT AND DISCUSSION

### The Compound of 3-hydroxy-4-prenyl-5-methoxy-stilbene Acid (**1**)

The compound of 3-hydroxy-4-prenyl-5-methoxy stilbene acid (**1**) was obtained as a pale green needle crystalline, mp 177-178 °C. The UV spectrum exhibited a maximum absorption band at 307, 255, 203 nm revealed the presence of substituted benzene and a highly conjugated chromophore. It was typical of stilbene [14]. Inspection of the IR spectrum of compound **1** showed strong absorption bands that clearly indicated the presence of hydroxyl groups (3080 cm<sup>-1</sup>), carbonyl groups (1631 cm<sup>-1</sup>) and aromatic rings (1600, 1562, and 1440 cm<sup>-1</sup>). The <sup>1</sup>H-NMR spectrum of **1** (Table 1) showed the presence of resonances for a double bond in the *E* configuration at δ<sub>H</sub> 6.83 (1H, d, *J* = 16 Hz, H-β) and 7.84 (1H, d, *J* = 16 Hz, H-α). Long-range <sup>3</sup>*J* (<sup>1</sup>H-<sup>13</sup>C) correlations observed in HMBC data (Table 1) between the double-bond protons and carbon

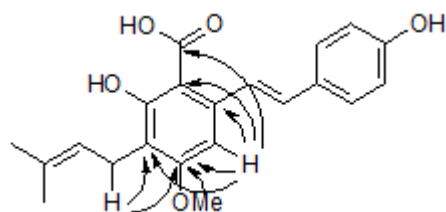


Fig 1. HMBC Correlation of 1

**Table 2.**  $^1\text{H}$  and  $^{13}\text{C}$ -NMR data of compounds **2** (400 MHz,  $d$   $-\text{CDCl}_3$ ,  $\delta$  in ppm and  $J$  values (Hz) in parentheses)

Position	$^1\text{H}$ ( $\delta_{\text{H}}$ , multiplicity, $J$ )	$^{13}\text{C}$ ( $\delta_{\text{C}}$ )	HMBC Correlation
1		137.5	
2		120.9	
3		158.5	
4	6.37 d (2)	98.4	C-6, C-2, C-5, C-3
5		154.3	
6	6.68 d (2)	104.0	C-4, C-2, C-5
$\alpha$	7.32 d (16)	127.6	C-1, C-1', C-6, C-2
$\beta$	6.92 d (16)	130.5	C-1, C-1', C-6, C-2
1'		137.5	
2', 6'	7.48 d (8)	126.4	C-1', C- $\alpha$ , C- $\beta$
3', 5'	7.36 d (8)	128.6	C-1', C-2', C-6'
1''	3.41 d (6.4)	24.4	C-5'', C-2, C-2'', C- $\beta$ , C-3
2''	5.10 d (6.4 & 6.8)	123.4	C-4'', C-5''
3''		130.5	
4''	1.80 s	17.9	C-5'', C-2'', C- $\beta$
5''	1.67 s	25.7	C-4'', C-2'', C- $\beta$
C-OMe	3.80 s	55.7	C-3

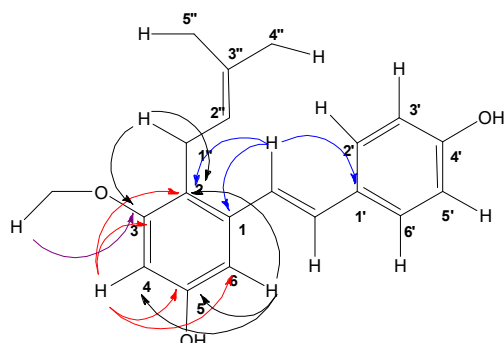


Fig 2. HMBC Correlation of 2

atoms of both phenyl rings confirmed that **1** was a stilbene.

The spectrum its also shows an AA'BB' system which clearly identify a para-substituted benzene ring at

$\delta_{\text{H}}$  7.53 (2H, d,  $J = 8$  Hz, H-2', H-6') and 7.38 (2H, d,  $J = 7.6$  Hz, H-3', H-5'). A single aromatic proton at  $\delta_{\text{H}}$  6.66 (1H, s, H-6) indicating the presence of a pentasubstituted phenyl ring and the low field shift of hydroxyl group at  $\delta_{\text{H}}$  11.53 (1H, s, 3-OH) indicates that it is adjacent to a carbonyl function. In addition, the  $^1\text{H}$ -NMR spectrum also indicated the presence of a methoxyl groups at  $\delta_{\text{H}}$  3.95 (3H, s). A group of signals corresponding to prenyl skeleton at  $\delta_{\text{H}}$  1.79 (3H, s, H-4''), 1.68 (3H, s, H-5''), 5.21 (1H, t,  $J = 6.8$  Hz and 7.6 Hz, H-2''), 3.37 (2H, d,  $J = 6.8$  Hz, H-1'') with proved signal two methyl carbon at  $\delta_{\text{C}}$  17.8 (C-4'') and 25.8 (C-5''), a methylene carbon 22.1(C-1''), a methyne carbon 121.9 (C-2'') and quaternary carbon 132.0 (C-3'').  $^1\text{H}$ -NMR data were obtained on compound **1** has similarities with the data cajanin acid stilbene found on pods of *Cajanus cajan* (L.) Millsp. from India [15].

Further evidence was provided by HMQC and HMBC spectra. The HMBC spectrum of **1** showed long-range correlation between the proton of  $\delta_{\text{H}}$  3.37 to C-4 (116.7) and C-5 (162.4) indicated that the prenyl moiety is attached in C-4. The HMBC experiments also showed the connectivities between the methoxyl protons at  $\delta_{\text{H}}$  3.95 and a quaternary carbon C-5 (162.4), also between the aromatic proton at  $\delta_{\text{H}}$  6.38 correlation to C-5 (162.4); C-1 (141.8); C-2 (102.9) and C-4 (116.7).

### The Compound of 5,4'-dihydroxy-3-methoxy-2-prenyl-stilbene (2)

The compound of 5,4'-dihydroxy-3-methoxy-2-prenyl-stilbene (**2**) was obtained as a white needle crystalline, mp 94-95 °C. Compound **2** has a resemble proton spectrum compared to compound **1**. From the  $^1\text{H}$  NMR data (Table 2), we can see clearly the same pattern of coupling at ring B as in compound **2** which indicates an AA'BB' system which clearly identify a para-substituted benzene ring.

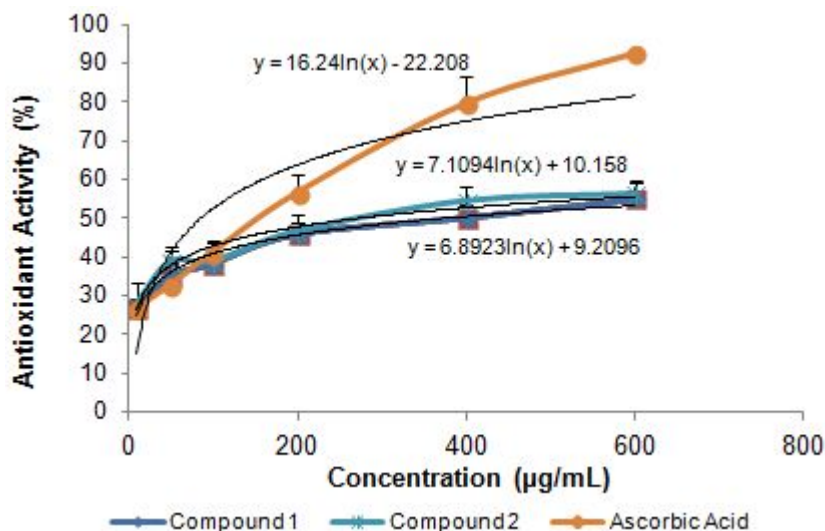
Ring A showed two aromatic protons at  $\delta_{\text{H}}$  6.68 and 6.37 with coupling constant  $J = 2$  Hz which observed meta position at C-4 and C-6 [11]. Also signals for a prenyl skeleton have been identified at  $\delta_{\text{H}}$  1.80 (3H, s, H-4''); 1.67 (3H, s, H-5''); 3.41 (2H, d,  $J = 6.4$  Hz, H-1''); 5.10 (2H, t,  $J = 6.4, 6.8$  Hz, H-2''), with proved signal two methyl carbon at  $\delta_{\text{C}}$  17.9 (C-4'') and 25.7 (C-5''), a methylene carbon at 24.4 (C-1''); a methyne carbon at 123.4 (C-2'') and quaternary carbon 130.5 (C-3'').

HMBC experiment (Table 2) revealed a long distance coupling between the methoxy group protons  $\delta_{\text{H}}$  3.80 (3H, s) and C-3. Also, there was a long distance correlation between the methylene protons of the prenyl group to C-3 and C-2.

**Table 3.** DPPH Radical scavenging activity of isolated compounds and their IC<sub>50</sub> values

Compound	DPPH Scavenging Activity (%) <sup>a</sup>	IC <sub>50</sub> (µg/mL)
1	55.1±4.20	413.68
2	56.5±3.09	271.53
Ascorbic Acid	92.5±0.89	2.21

<sup>a</sup>Value are the mean±SD for three parallel assessment (n=3)



**Fig 3.** DPPH Radical scavenging activity of isolated compounds from *C. cajan*. Value are the mean±SD for three parallel assessment (n=3)

### DPPH Free Radical Scavenging Activity

Radical scavenging activity is very important due to the deleterious role of free radicals in foods and in biological systems. DPPH has been widely used to evaluate the free radical scavenging effectiveness of various antioxidant substances [13]. In the DPPH assay, the antioxidants were able to reduce the stable radical DPPH to the yellow colored diphenyl-picrylhydrazine. The method is based on the reduction of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH-H by the reaction [13,16]. In the current investigation (Table 3), the DPPH scavenging activities of compounds **1** and **2** were detected. The order of the percent radical scavenging activities for the investigated compound **2** (IC<sub>50</sub> 271.53 µg/mL) is higher than **1** (IC<sub>50</sub> 413.67 µg/mL). These compounds exhibited weak antioxidant activity.

### CONCLUSION

Stilbene cajanus from Poteran Island in Madura-Indonesia is a unique and interesting type of *C. cajan* distinctly different from other types of *C. cajan* reported. It contains a para-substituted benzene ring (B) hydroxy substituted. Prenylated stilbene derivatives had been isolated from methanol extract namely 3-hydroxy-4-

prenyl-5-methoxy-stilbene acid (**1**) and 5,4'-dihydroxy-3-methoxy-2-prenyl-stilbene (**2**). These compounds were evaluated for its *in vitro* antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging had IC<sub>50</sub> 413.68 and 271.53 µg/mL, respectively.

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