

## UGONIN J FLAVONOID FROM TUNJUK LANGIT (*Helminthostachys zeylanica* Linn.) ROOT EXTRACT

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### ABSTRACT

A flavonoid compound was isolated from dried rhizomes of tunjuk langit (*Helminthostachys zeylanica* Lin), a traditional medicine from South Sumatera, Indonesia. Extraction was done by maceration method and separation of isolated compound was conducted by chromatographic technique. The structure of this compound was determined based on spectroscopic data such as including UV, IR, 1-D, 2-D NMR, and comparison with the reported data. Based on spectral data analysis, concluded that isolated compound was Ugonin J (5,7,3',4'-tetrahydroxy-6-(6,6-dimethyl-2-methylenecyclo-hexylmethyl)flavone).

**Keywords:** Flavonoid, Ugonin J, *Helminthostachys zeylanica*

### INTRODUCTION

Tunjuk langit (*Helminthostachys zeylanica* Linn.) (Ophioglossaceae) is pteridophyte show medicinal utility. The rhizome of tunjuk langit traditionally used as cytotoxic, antiinflammatory and pulmonary disease. The rhizome of tunjuk langit is Chinese herbal medicine used as antypiretic and antiphlogistic agent. In India, the rhizome of tunjuk langit used for curing impotency [1]. In Malaysia, the rhizome used as antidiarrheal agent and chewed with areca for whooping cough relief [2]. Suja reported that ethanol extract of rhizome of *H. zeylanica* showed aphrodisiac properties [4]. Study of ethanol extract of rhizome also showed significant hepatoprotective effect [5]. Huang yielded eight flavonoids, ugonin E-L from the rhizome of tunjuk langit Ugonin J, K and L showed significant antioxidant activity [6]. Yi Chen reported that ugonin K, a flavonoid from rhizome of tunjuk langit has neuroprotective activity in neuroblastoma SH-SY5Y cells [7] and ugonin L showed antiinflammatory effect [8].

*H. zeylanica* is pteridophytes, widely distributed in tropical Asia and Australia. This plant is locally named, paku payung, pancar bumi (South Sumatra), tapak jalak (Sunda), bute-bute (Makasar), pakis urang, pakis kaler, ceker ayam (Java) [9]. Three new cyclized stilbenes, ugonstilben A, B and C and 3-hidroxy asetophenon compound were isolated from the rhizome of *Helminthostachys zeylanica* [10]. Four flavonoid compounds ugonin A-D were isolated too [11].

This plant widely growth in Lahat, South Sumatra. In Indralaya, traditionally used as cytotoxic, antiinflammatory and pulmonary disease treatment. In this paper we described the isolation and structure

flavonoid compound from ethylacetate extract from rhizome of *H. zeylanica*.

### EXPERIMENTAL SECTION

#### Materials

The rhizome of *H. zeylanica* were collected from Jati village and Kuba village, Kecamatan Pulau Pinang Kabupaten Lahat South Sumatra. The plant was also identified by comparison with a voucher specimen already deposited at the Herbarium ANDA, Andalas University. Vacuum liquid chromatography (VLC) was carried out using Merck Si gel 60 GF<sub>254</sub> (230-400 Mesh) and column chromatography using Si gel Merck G 60 (70-230 Mesh), thin layer chromatography (TLC) analysis was performed on precoated Si Gel plates (Merck Kiesel gel 60 GF<sub>254</sub>, 0.25 mm 20 x 20 cm).

#### Instrumentation

UV and IR spectra were measured with spektrofotometer Beckman DU-700 and Shimadzu FTIR 8400. <sup>1</sup>H and <sup>13</sup>C NMR spectra was recorded JEOL JNM ECA-500 500 MHz (<sup>1</sup>H) and 125 MHz (<sup>13</sup>C) using internal standard TMS.

#### Procedure

##### *Extraction and isolation*

The powdered of rhizome of *H. zeylanica* (5 kg) was extracted with n-hexane, ethylacetate and methanol respectively (8 L x 3). The extract was filtered

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and the filtrate concentrated under reduced pressure. The EtOAc extract was chromatographed on column.

EtOAc extract (50 g), was subjected to vacuum liquid chromatography eluted with a gradient system (n-hexana, n-hexane: EtOAc = 9:1; 8:2; 7:3; 6:4 and EtOAc) to afford 5 fractions F1–F5. Fraction F3 was further purified column chromatography (eluted with MTC-MeOH 9.5:0.5) to afford 4 subfraction F3.1-F3.4. Subfraction F3.3 was repeatedly separated over silica gel columns to afford F3.3.1-F3.3.1. F3.3.4 after purification with recrystallization gave a pure compound as yellow powder (50 mg).

### Structure elucidation

The structure was elucidating using UV, IR, 1D and 2D NMR spectroscopic data. The  $^{13}\text{C}$  NMR signals were assigned from DEPT, HMQC, HMBC and COSY spectra. The  $^1\text{H}$  and  $^{13}\text{C}$  spectra data also compared with those reported in the literature. In the UV spectra, we can obtain information about chromophore, information from IR spectra was specific functional group.  $^1\text{H}$  NMR we were obtain information about quantity of proton, chemical shift, coupling constant, and  $^{13}\text{C}$  NMR used to implied total carbon and DEPT (Distortionless Enhancement by Polarization Transfer) spectrum showed kinds of C, CH,  $\text{CH}_2$  and  $\text{CH}_3$ . HMQC (Heteronuclear Multiple Quantum Coherence) used to identified signals of carbon which attached proton, HMBC (Heteronuclear Multiple Bond Connectivity) showed long range correlation  $^2\text{J}$  and  $^3\text{J}$  of proton to carbon, and COSY (Correlated Spectroscopy) showed proton adjacent. [12-13].

## RESULT AND DISCUSSION

### Extraction and Isolation

Dried rhizome of *H. zeylanica* (5 kg) extracted with n-hexane, EtOAc and MeOH respectively and resulted n-hexane extract (30 g), EtOAc extract (51 g) and MeOH extract (30 g). EtOAc extract separated over silica gel column to afford a pure compound.

### Structure Elucidation

The pure compound as yellow powder has melting point 229-230 °C and  $[\alpha]_{\text{D}}^{20}$  -49° (c 1.0, MeOH). The structure was elucidating using 1D and 2D NMR spectroscopic data. The  $^{13}\text{C}$  NMR signals were assigned from DEPT, HMQC, HMBC and COSY spectra. The  $^1\text{H}$  and  $^{13}\text{C}$  spectra data also compared with those reported in the literature. The UV spectrum (MeOH) showed  $\lambda_{\text{max}}$  absorption at 347(4.27), 275 (4.16) and 215 (4.5) nm characteristic to flavone nucleus [14]. The UV spectrum in the presence of NaOH showed bathochromic shift  $\lambda_{\text{max}}$

(nm) 405, 278 and 215 indicated there was phenolic chromophore. The IR spectrum (KBr) showed absorptions typical of hydroxyl ( $3440\text{ cm}^{-1}$ ), C-H aliphatic ( $2920, 2962\text{ cm}^{-1}$ ), carbonyl ( $1651$ ), aromatic ( $1651, 1612$  and  $1465\text{ cm}^{-1}$ ) and alcohol ( $1172\text{ cm}^{-1}$ ).

The  $^1\text{H}$  NMR and  $^{13}\text{C}$  HNMR (methanol-*d*4) spectra of pure compound (Table 1) showed characteristic signals for flavone.  $^1\text{H}$  NMR spectrum showed signal at 7.36 (1H, *dd*,  $J = 1.8$  and  $8.5$  Hz), can be attribute to aromatic proton which *orto* and *meta* coupling with 6.89 (1H, *d*,  $J = 8.5$  Hz) and  $\delta_{\text{H}}$  7.35 (1H, *d*,  $J = 1.8$  Hz) proton from trisubstituted benzene ring. Signals at  $\delta_{\text{H}}$  6.51 (1H, *s*) and 6.42 (1H, *s*) assigned two aromatic which uncoupled. Five specific protons indicated that this compound was flavone.

Furthermore, signals at  $\delta_{\text{H}}$  2.73 (H-9a) (1H, *dd*,  $J = 3.7$  and  $12.9$  Hz), and 2.94 (H-9b) (1H, *t*,  $J = 12.9$ ) attribute to methylene group which *orto*-coupling with proton at  $\delta_{\text{H}}$  2.41 (1H, *dd*,  $J = 3.7$ ) and coupling gemynal. Signal at  $\delta_{\text{H}}$  4.42 and 4.19 (1H, *brs*) was for methine proton from C-18, suggesting gemynal coupling.  $^1\text{H}$  NMR spectrum showed three signals for methylene SP3 at 2.56 (H-12a), 1.95 (12b), 1.59 (13-a), 1.48 (13-b), 1.70 (14a), and 1.28 (14-b) respectively (1H, *m*). Signals at  $\delta_{\text{H}}$  1.06 (3H, *s*) and 0.94 (3H, *s*) attributed to methyl proton from prenyl unit.  $^1\text{H}$  NMR spectrum showed at Fig. 1. Based on signals at under 3 ppm suggested that the pure compound contain cyclic geranyl unit. That was flavone which cyclic geranyl substituted.

$^{13}\text{C}$  NMR spectrum showed 25 signals and 15 of these were SP2 signals for aromatic carbon and one of these for carbonyl carbon at 184.0 ppm, can see at Fig. 2. These signals specific for carbon or flavonoid typical flavone. Nine remaining signals were signals typical resulted from geranyl unit which has one carbon SP2. That was strengthen two signals for  $\text{CH}_3$  only, revealed the presence two signals  $\text{CH}_3$ . Therefore, these data supporting isolated pure compound to be a flavone which geranyl substituted.

At DEPT Spectrum (Fig. 2) showed two signals methyl at  $\delta_{\text{C}}$  28.6 (C-16) and 28.8 (C-17), five methylene signals ( $\text{CH}_2$ ) at  $\delta_{\text{C}}$  22.4 (C-9); 24.6 (C-13); 32.3 (C-12); 35.9 (C-14); and 109.8 (C-18), six methine signals (CH) at  $\delta_{\text{C}}$  53.6 (C-10); 93.9 (C-8); 103.8 (C-3); 114.2 (C-2'); 116.5 (C-5') and 120.3 (C-6'), and twelve signals kuartener carbon at  $\delta_{\text{C}}$  35.5 (C-15); 104.9 (C-C-4<sup>a</sup>); 113.5 (C-6'); 123.9 (C-1'); 147.1 (C-3); 150.9 (C-11); 151.2 (C-4); and 157.2. Furthermore, isolated compound structure predicted supporting by NMR 2-D data analysis. HMQC spectrum showed correlation between proton at  $\delta_{\text{H}}$  6.51 (H-3) with carbon at  $\delta_{\text{C}}$  103.8 and HMBC spectrum showed there were long range correlation with C-4a at  $\delta_{\text{C}}$  104.9; 123.9 (C-1'); and displayed correlation ( $^2\text{J}$ ) with carbon at  $\delta_{\text{C}}$  165.9 (C-2)

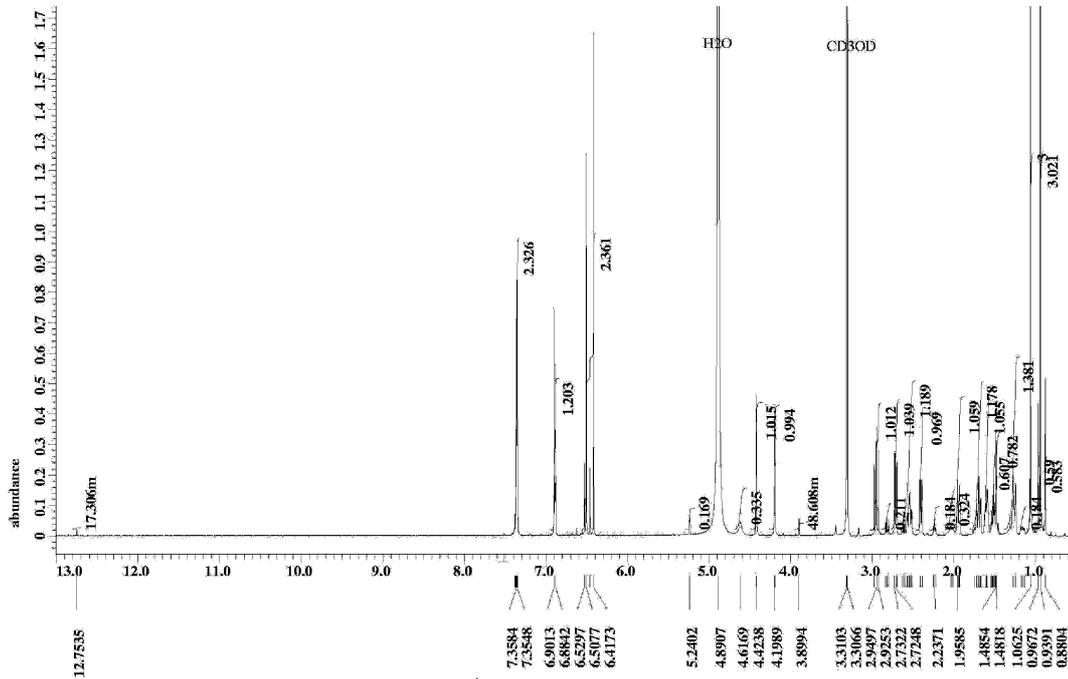


Fig 1. <sup>1</sup>H NMR spectrum

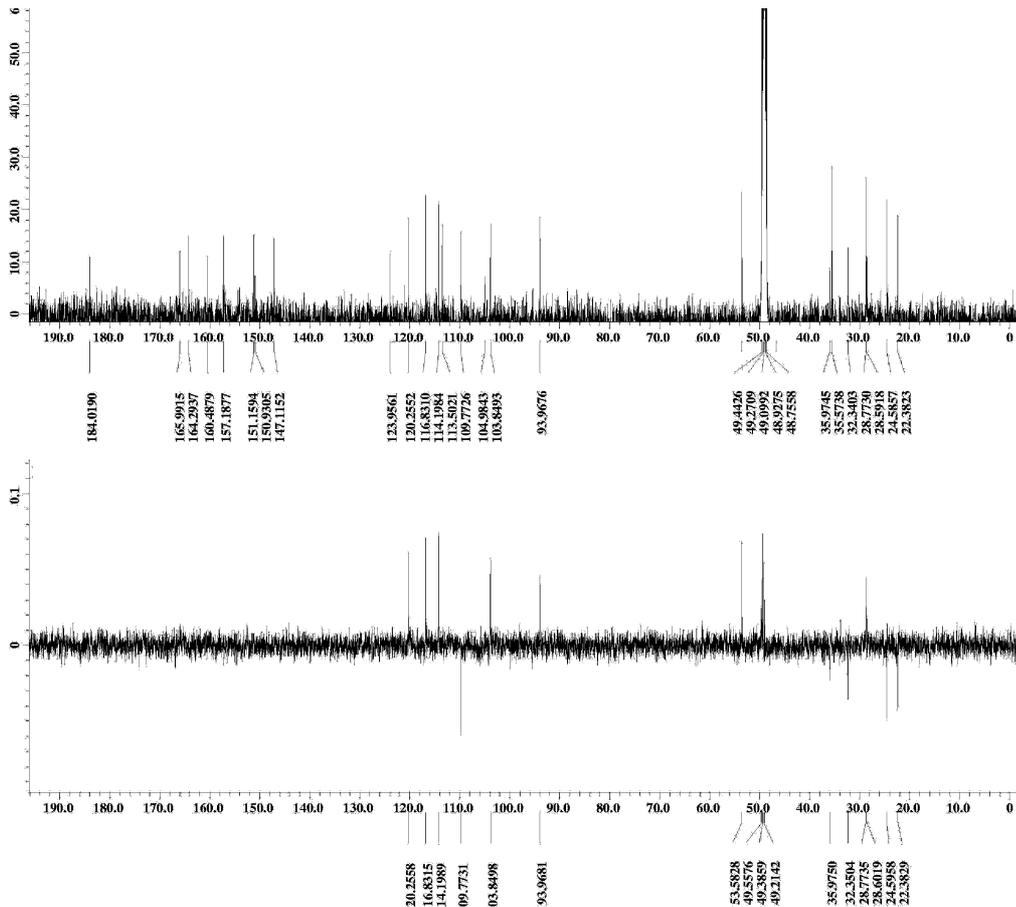


Fig 2. <sup>13</sup>C NMR and DEPT spectrum

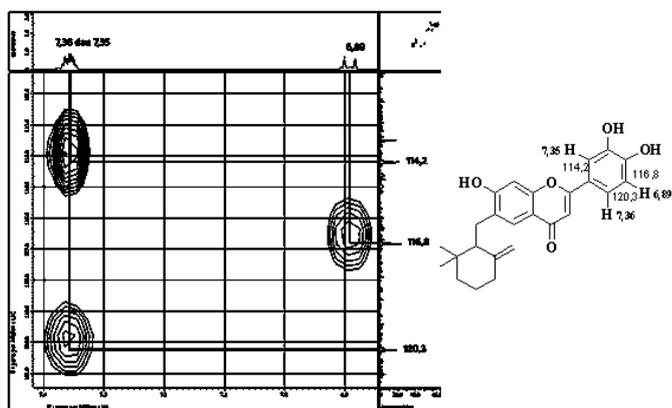
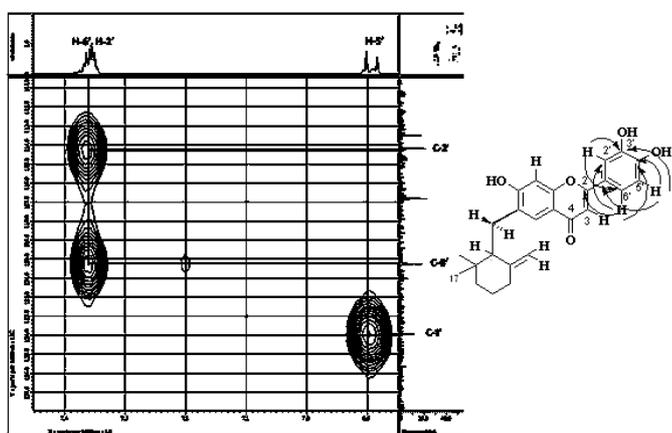
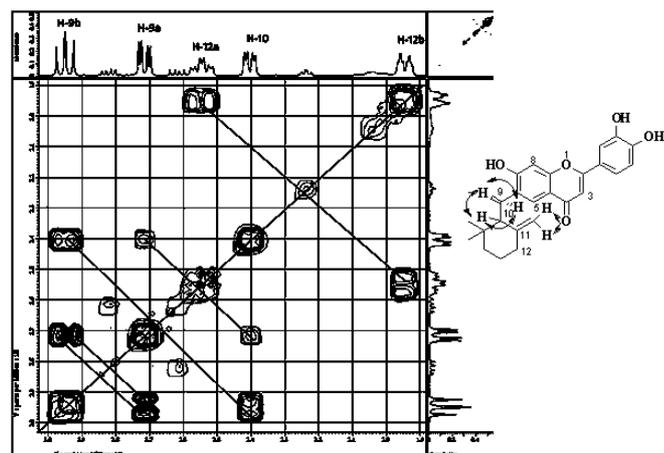
Fig 3. HMQC at  $\delta_C$  6.8 – 7.4 ppm spectrumFig 4. HMBC at  $\delta_C$  6.8 – 7.4 ppm spectrum

Fig 5. COSY spectrum

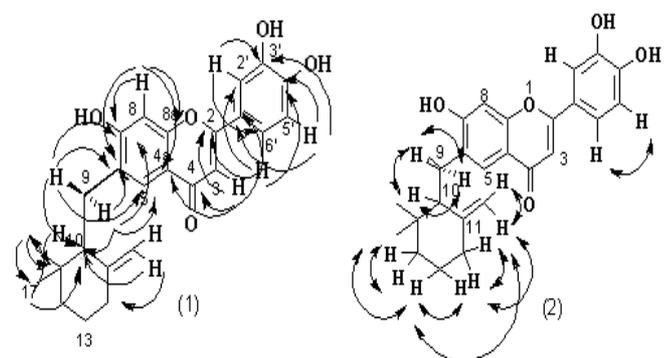


Fig 6. HMBC and COSY correlation of Ugonin J (A)

and 184.0 (C-4), so this proton deduced attached to C-2. The HMBC spectrum also displayed long range correlation  $^3J$  between proton at  $\delta_H$  6.42 (H-8) with carbon at  $\delta_C$  104.9 (C-4a); 113.5 (C-6) and  $^2J$  coupling with carbon at  $\delta_C$  157.2 (C-8a) and 164.3 (C-7) so this proton attributed to C-8. The HMQC spectrum displayed this proton correlated to carbon at  $\delta_C$  93.9.

Furthermore, the HMQC spectrum (Fig. 3) showed proton at 6.89 attached at  $\delta_C$  116.8 ppm (C-5'), and HMBC spectrum at Fig. 4 showed correlation with  $\delta_C$  147.1 (C-3') and 123.9 (C-1') so this proton attributed at C-5'. Proton at 7.35 (H-2') and 7.36 (C-6') at spectrum HMQC, attached to carbon at  $\delta_C$  114.2 and 120.3 showed  $^3J$  coupling with carbon at  $\delta_C$  120.3 (C-6') and 114.2 (C-2') and  $^2J$  with carbon at  $\delta_C$  147.1 (C-3'), so deduced this proton attached at C-2' and C-6'.

HMQC spectrum also showed proton at  $\delta_H$  2.73 (H-9a) and 2.94 (H-9b) attached to carbon at 22.4 ppm and HMBC spectrum displayed correlation with carbon at 53.6 (C-10), and 113.5 (C-6) attributed to C-9. In the COSY spectrum (Fig. 5) displayed correlation between proton at  $\delta_H$  2.73 (H-9a) and  $\delta_H$  2.94 (H-9b) with proton

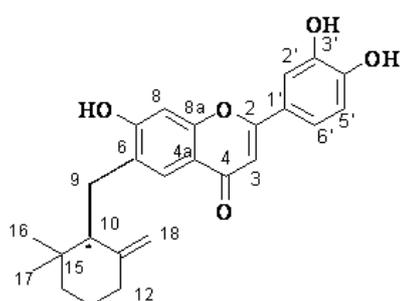
at 2.41 (H-10), in HMBC spectrum displayed correlation with carbon at 35.5 (C-15) so this signal appeared as doublet-doublet. The COSY spectrum also displayed correlation between proton at  $\delta_H$  2.56 (H-12a) with proton at  $\delta_H$  1.59 (H-12b), proton at 1.59 (C-13a) and 1.48 (C-13b), and proton at  $\delta_H$  1.70 (H-14a) and 1.28 (H-14b) so this proton appeared as multiplets.

Correlation long range coupling  $^2J$  and  $^3J$  between proton with carbon from HMBC and correlation between protons with proton at COSY showed at Fig. 6.

The  $^{13}C$  NMR data of isolated compound (A) showed same with Ugonin J (A\*) as reported data (Table 1), but there was difference because isolated compound measured in methanol-*d*4 whereas (-)-Ugonin J measured in DMSO-*d*6. There was also same of melting point and optical density isolated compound 229-230 °C and  $[\alpha]_D -49^\circ$  (c 1.0; MeOH) respectively whereas Ugonin J as reported data was  $[\alpha]_D^{20} -50^\circ$  (MeOH). Based on above data concluded the isolated compound was Ugonin J showed at Fig. 7.

**Table 1.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR 1-D and 2-D data (methanol- $d_4$ ) for isolated compound (A) and Ugonin K (A\*) as comparing data [6].

Position	$\delta_{\text{H}}$ (ppm), integration, mult, J (Hz)	$\delta_{\text{C}}$ (ppm)		DEPT	HMBC	COSY
		A	A*			
2		165.9	164.7	C		
3	6.51 (1H, s)	103.8	104.2	CH	C-4a, C-1', C2, C4	
4		184.0	183.0	C		
4a		104.9	104.9	C		
5		160.5	160.3	C		
6		113.5	112.4	C		
7		164.3	162.7	C		
8	6.42 (1H,s)	93.9	93.7	CH	C-4a, C-6, C8a, C7	
8 <sup>a</sup>		157.2	156.3	C		
9	2.73 (1H,dd, 3,7; 12,9) 2.94 (1H, t, 7,9; 12,9)	22.4	21.9	CH <sub>2</sub>	C-5, C-6, C-7, C-10	H-10
10	2.41 (1H, dd, 3,7; 7,9)	53.6	53.0	CH	C-15	H-9a, H-9b
11		150.9	150.5	C		
12	2.56 (1H, m) 1.95 (1H, m)	32.3	32.0	CH <sub>2</sub>		H-12b H-12a
13	1.59 (1H, m) 1.48 (1H, m)	24.6	24.1	CH <sub>2</sub>		H-12 <sup>a</sup> H-12b, H-14 <sup>a</sup>
14	1.70 (1H, m) 1.28 (1H,m)	35.9	35.2	CH <sub>2</sub>		H-14b H-14a
15		35.5	35.2	C		
16	1.06 (3H, s)	28.6	28.1	CH <sub>3</sub>	C-10, C-15, C-17	
17	0.94 (3H, s)	28.8	28.5	CH <sub>3</sub>	C-10, C-16	
18	4.42 (1H, brs) 4.19 (1H,brs)	109.8	109.4	CH <sub>2</sub>		H-18b H-18a
1'		123.9	123.9	C		
2'	7.35 (1H, m, 1,8)	114.2	113.9	CH	C-3', C-6'	
3'		147.1	146.3	C		
4'		151.2	149.8	C		
5'	6.89 (1H, d)	116.8	116.5	CH	C-3', C-1', C-4'	H-6'
6'	7.40 (1H, dd, 1,8))	120.3	120.0	CH	C-4', C-2	H-5'

**Fig 7.** Structure of ugonin J

## CONCLUSION

Ugonin J or 5,7,3',4'-tetrahidoksi-6-(6,6-di-metil-2-metilen-sikloheksilmetil) flavone) had been isolated from ethylacetate extract of rhizome of *Helmynthostachys zeylanica*

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