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FLAVANONES FROM THE BARK OF *BAUHINIA HULLETTII* PRAIN

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ABSTRACT

Twoflavanones,5,7,3',5'-tetrahydroxyflavanone (1) dan 5,7,4'-trihydroxy-6-methylflavanone (2), were isolated from ethyl acetate fraction of Bauhinia hullettii Prain *(Leguminoceae) bark. Molecular structures were determined on UV, IR, ^{1}H NMR,^{13}C NMR, NMR 2D spectroscopy. Compound 1 is rare to find in the plants. Compound 1 and 2 were reported for the first time from this plant.*

Keywords *–Bauhinia hullettii Prain, Flavanon, 5,7,3',5'-tetrahydroxyflavanone,5,7,4'- trihydroxy-6-methylflavanone*

1. INTRODUCTION

Leguminaceae family is one of the largest flowering plant family besides *Compositae* dan *Orchidaceae* family, widely distributed in tropical Asia, especially in Malesia included Indonesia. The main genus of *Leguminaceae* family is *Bauhinia* containing approximately 300 species, 10 species found in Indonesia[1,2]. *Bauhinia* species have been used for fodder and some medicinal purposes[3]. Pharmacology studies of this species revealed anticancer[4-6], antioxidant[4,7-11], hypoglycemia[12], hyperlipidemia[13], antivirus[14], antiinflammation[15], antimicrobial[10,16-21], gastroprotective[22], antiulcer[15], and analgetic[23] properties.

Bauhinia plants were reported rich of flavonoids and stilbenoids. Moreover, it contained coumarins, tannins, terpenoids[19], cyanoglycosides[15], steroids[18], quinones[5], and phenolic[5,20,24]. *Bauhinia* species found in Sumatera is *Bauhinia hullettii* Prain, known as akartapakkuda. Literature review shows the phytochemical and biological activities of this species have never been reported yet. In this research, it will be reported isolation and structure elucidation of the flavanones from ethyl acetate fraction from the bark of *Bauhinia hullettii* Prain.

2. MATERIALS AND METHODS

2.1 General Experimental Procedures

Melting point was determined on Fisher John, UV and IR spectra were recorded on UV-Vis Shimadzu dan Perkin Elmer spectrophotometer.^{1}H dan ^{13}C NMR spectra were recorded on JEOL JNM ECA-500 spectrofotometer, 500 MHz for ^{1}H and 125 MHz for ^{13}C. Liquid vacuum chromatography (LVC) was performed on silica gel Merck 60 GF_{254}. Flash chromatography was performed on silica gel Merck 60 G (230-400 Mesh). Thin layer chromatography (TLC) was performed on silica gel Merck 60 GF_{254}, 0,25 mm thickness. The solvents used in this study were distilled solvents.

2.2 Plant material

The bark of *B. hullettii* Prain was collected in Januari 2015 from protected forest PT. Bukit Asam, Tanjung Enim, South Sumatra. Identification was held in Herbarium AndalasUniversity(ANDA), Padang, Indonesia.

2.3 Extraction and Isolation

The bark of *B.hulllettii* Prain (3 kg) was dried, grounded and macerated successively with n-hexane, dichloromethane, and ethyl acetate. This maceration process produced n-hexane extract 30.739 g, dichloromethane extract 24.065 g, and ethyl acetate extract 27.385 g. Ethyl acetate fraction was then fractionated with LVC (Si gel, 70 g), eluted with n-hexane, n-hexane- ethyl acetate (20%-80%), ethyl acetate and ethyl acetate-methanol (20%), produced 7 main fractions (A-G). Fraction E (2,83 g) was subjected to column chromatography (CC), eluted with n-hexane and ethyl acetate with increasing polarity. This fractionation process gave 4 fractions (E_1 – E_4). Fraction E_3(215 mg) was separated with CC using n-hexane-dichloromethane and dichloromethane- ethyl acetate as the eluents to afford four fractions ($E_{3.1}$ – $E_{3.4}$). Fraction $E_{3.3}$ (146.7 mg) was then fractionated with flash chromatography, eluted with dichloromethane- ethyl acetate with increasing polarity to produce 3 fractions ($E_{3.3.1}$-$E_{3.3.3}$). Fraction $E_{3.3.2}$ (125 mg) was further fractionated with flash chromatography to yield compound 1. With the same ways, compound 2 was obtained and isolated from fraction C.

2.4 Spektral Data

5,7,3′,5′-Tetrahydroxyflavanone **(I)**. M.p. 263–265 °C; $FeCl_3$ test: (+); UV (MeOH) λ_{max} nm: 328 (bh), 288 and203; UV (MeOH+ NaOH) λ_{max} nm: 325, 265, 212; UV (MeOH+$AlCl_3$) λ_{max} nm: 368 (bh), 347,305, 264, 205; UV (MeOH+$AlCl_3$+HCl) λ_{max} nm: 368 (bh), 348, 307, 265, 206; IR (KBr) ν_{maks} cm^{-1}: 3365, 2920, 1635, 1602, 1450, 1257, 1161;^{1}H NMR (500 MHz, Metanol-*d3)δ ppm*: 2.69 (1H, dd, *J*=17 Hz, 3.0 Hz, H-3a), 3.07 (1H, dd, *J*=17 Hz, 13 Hz, H-3b); 5.28 (1H, dd, *J*=13 Hz, 3.0 Hz, H-2), 5.94 (1H, d, J= 2 Hz, H-8), 5.95 (1H, d, J= 2 Hz, H-6), 6.78 (2H, s, H-2′ and H-6′), 6.91 (1H, s, H-4′); ^{13}C NMR (125 MHz, Metanol-*d3δ ppm*: 197.84 (C4), 168.61 (C7), 164.95 (C9), 165.57 (C5), 147.00 (C3′), 146.61 (C5′), 131.86 (C1′), 119.33 (C4′), 116.32 (C2′), 114.78 (C6′), 103.40 (C10), 97.11 (C6), 96.26 (C8), 80.59 (C2) and 44.19 (C3). HMQC and HMBC: can be seen in Table 1.

5,7,4'- trihydroxy-6-methylflavanone (2). M.p. **263–265** °C; $FeCl_3$ test: (+); UV (MeOH) λ_{max} nm: 365 (bh), 291, and 212; IR ν_{maks} cm^{-1}: 3336, 2916, 1614, 1601, 1462, 1262, 1148;^{1}H NMR (500 MHz, Aseton,-*d6)δ ppm*: 1.98 (3H, s, CH_3), 2.81 (1H, dd, *J*=17 Hz, 3.0 Hz, H-3a), 3.08 (1H, dd, *J*=17 Hz, 13 Hz, H-3b); 5.76 (1H, dd, *J*=13 Hz, 3.0 Hz, H-2), 6.09 (1H, d, s, H-8), 6.93 (1H, d, 7.8 Hz, H-3′), 6.95 (1H, d, 7.8 Hz, H-5'), 7.21 (1H, dd, 7.8 Hz, 1.3 Hz, H-6′), 7.52 (1H, dd, 7.8 Hz, 1.3 Hz, H-2′); ^{13}C NMR (125 MHz, Metanol-*d3δ ppm*: 197.46 (C4), 165.00 (C7), 162.29 (C5), 162.13 (C9), 154.81 (C4′), 130.22 (C6′), 127.75 (C2′), 126.53 (C1′), 120.74 (C5′), 116.29 (C3′), 104.73 (C6), 103.05 (C10), 95.20 (C8), 75.39 (C2),42.76 (C3)and 7.12 (CH_3).HMQC and HMBC: can be seen in Tabel 2.

3. RESULTS AND DISCUSSION

Compound 1 was isolated as yellow solid. UV spectrum of compound 1 showed maximum absorption at λ_{maks}203, 288 and 328 (bh), specific absorptions for flavanones. The adding of NaOH, $AlCl_3$ and $AlCl_3$ + HCl indicated the existence of OH in C_5 and no OH in *ortho*position. IR spectrum of compound 1 showed a hydroxyl group in the skeleton (ν_{maks} 3365 cm^{-1}),C-H aliphatic (2920 cm^{-1}), carbonyl (1635, 1602 cm^{-1}) andaromatic (1450 cm^{-1}). These data indicated the signs of flavanone compounds. ^{1}H NMR spectrum (methanol-D3, 500 MHz) showed the existence of 8 protons, consisted of 5 aromatic protons and 3 aliphatic protons. The doublet signals at δ 2.69 ppm (1H, dd, *J* = 17 Hzdan 3 Hz, H-3a), δ_H3.07 ppm (1H, dd, *J* =13 Hz and 17 Hz, H-3b) and δ_H5.28 ppm (1H, dd, *J* =13 Hz and 3 Hz, H-2) were identified as spesific absorptions for protons C-2 and C-3 in flavanone compounds (coupling with ABX system). Five aromatic protons were showed at δH 5.87 ppm (1H,d, J= 2.6 Hz, H-8); 5.89 ppm (1H,d,J=2.6 Hz, H6); 6.78 ppm (2H, s, H-2' dan H-6') and 6.91 ppm (1H, s, H-4'). Proton H-8 coupled with proton H-6 in ring A showed a *meta* coupling with J=2.6 Hz.

^{13}C NMR spectrum supported with DEPT showed the presence of 15 carbon signals, were CH_2 at δ_C 48.76 ppm (C-3), 6 CH carbons at δ_C 80.61 ppm (C-2); 96.26 ppm (C-6); 97.11 ppm (C-8); 114.78 ppm (C-2'); 116.32 ppm (C-6'); 119.34 ppm (C-4'); and 8 quaternary carbons at δ_C 103.41 ppm (C-10); 131.86 ppm (C-1'); 146.61 ppm (C-5'); 146.99 ppm (C-3'); 164.95 ppm (C-9); 165.56 ppm (C-5) dan 168.52 ppm (C-7) and 197.86 ppm (C-4) (Table 1). The presence of a flavanone ring in compound 1 was more supported with one aliphatic methylene at δ_C 42.76 (C-2) and one oxycarbonatδ_C 75.39 (C-3). Furthermore, HMBC showed a three bond correlation between proton signals at δ_H5.28 ppm (H-2) with carbon at δ_C197.85 ppm (C-4), 116.32 ppm (C-2') dan 119.33 (C-6') (Figure 1). Two aliphatic protons at δ_H2.69 ppm and 3.07 ppm (H-3) had two bond correlation with δ_C197.85 ppm (C-4). By analysis of ^{1}H dan ^{13}C NMR spectra of isolated compound and comparison with the literature, compound 1 is assigned as 5,7,3',5'-tetrahydroxyflavanone .

Compound 2 showed similar UV and IR spectra as shown in compound 1. ^{1}H NMR of compound 2 showed 3 aliphatic proton signals coupling with ABX system at δ_H2.81 (1H, dd, *J*=17 Hz, 3.0 Hz, H-3a), 3.08 (1H, dd, *J*=17 Hz, 13 Hz, H-3b); 5.76 (1H, dd, *J*=13 Hz, 3.0 Hz, H-2). Aromatic signals were observed in ring A at δ_H 6.09 (1H, s, H-8) and 4 aromatic protons in ring B at δ_H6.93 (1H, d, 7.8 Hz, H-3'), 6.95 (1H, d, 7.8 Hz, H-5'), 7.21 (1H, dd, 7.8 Hz, 1.3 Hz, H-6'), 7.52 (1H, dd, 7.8 Hz, 1.3 Hz, H-2') (Table 2). Based on ^{1}H NMR, ^{13}C NMR of compound 2 designated the presence of one aliphatic methylene at δ_C 42.76 (C-2) andone oxycarbon atδ_C 75.39 (C-3). Four oxyaryl signals at δ_C 165.00 (C7), 162.29 (C5), 162.13 (C9), 154.81 (C4') and the presence of a carbonyl group signal at δ_C 197.46, were the specific carbon signals of narinengin compounds. HMBC spectrum analysis of compound 2 showed a proton correlation at δ_H 1.98 with 3 quaternary carbon signals at δ_C 104.73 (C-6), 165.00 (C-5) dan 162.29 (C-5) (Figure 2). This supported a methyl substitent in C-6. By analysis these data, compound 2 was identified as 5,7,4'- trihydroxy-6-methylflavanone.

Table 1: NMR spectral data of 5,7,3',5'-tetrahydroxyflavanone (in methanol-d_3) against a known compound

No C	HMQC*				DEPT*	HMBC*
	δ_H (ppm), multiplisit as, *J* (Hz)*	δ_H (ppm), multiplisit as, *J* (Hz)**	δ_C (ppm)*	δ_C (ppm)**		
2	5.28 (*dd*, 13 ; 3)	5.27 (*dd*, 12,6 ; 3)	80.61	805	CH	C-4, C-2', C-6'
3	2.69 (*dd,* 17 ; 3) 3.07 (*dd*, 17 ; 13)	2.69 (*dd,* 17,4 ; 3) 3.07 (*dd*, 17,4 ; 12.6)	44.16	44.1	CH_2	C-4 C-4
4	-	-	197.85	197.8	C	
5	-	-	165.56	165.5	C	
6	5.95 (*d*, 2)	5.88(*d*, 2,4)	96.26	96.2	CH	C-7, C-8, C-9
7	-	-	168.52	168.4	C	
8	5.94 (*d*, 2)	5.87 (*d*,2,4)	97.11	97.0	CH	C-6, C-10
9	-	-	164.95	164.9	C	
10	-	-	103.41	103.4	C	
1'	-	-	131.86	131.8	C	
2'	6.78 (s)	6.78 (s)	116.32	116.3	CH	C-1', C-4'
3'	-	-	146.98	146.9	C	
4'	6.91 (s)	6.91 (s)	114.77	114.7	CH	C-3',C-5', C-6'
5'	-	-	146.61	146.5	C	
6'	6.78 (s)	6.77 (s)	119.33	119.3	CH	C-1', C-4'

*Compound 1

** a known compound (in CD_3OD)[24]

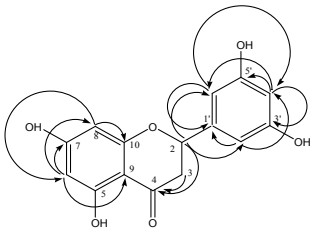

Figure 1:HMBC correlation of 5,7,3',5'-tetrahydroxyflavanone

Table 2: NMR spectral data of compound 5,7,4'-trihydroxy-6-methylflavanone(in methanol-D3)

No C	HMQC		DEPT	HMBC
	δ_H (ppm), integrasi, multiplisitas, *J* (Hz)	δ_C (ppm)		
2	5.76 (*dd*, 13 ; 3)	75.39	CH	
3	2.81 (*dd,* 17 ; 3) 3.08 (*dd*, 17 ; 13)	42.76	CH_2	C2, C4
4	-	197.46	C	
5	-	162.29	C	
6	-	104.73	C	
7	-	165.00	C	
8	6.09 (s)	95.20	CH	C9, C7
9	-	162.13	C	
10	-	103.05	C	
1'	-	126.54	C	
2'	7.52(dd, 7.8; 1.3)	127.75	CH	C2, C6', C4'
3'	6.93 (d, 7.8)	116.29	CH	C1', C5'
4'	-	154.81	C	
5'	6.95 (d, 7.8; 1.3)	127.75	CH	C2, C6', C4'
6'	7.21 (d. 7.8)	116.29	CH	C1', C5'
CH_3	1.98 (s)	154.81	C	C6, C5, C7

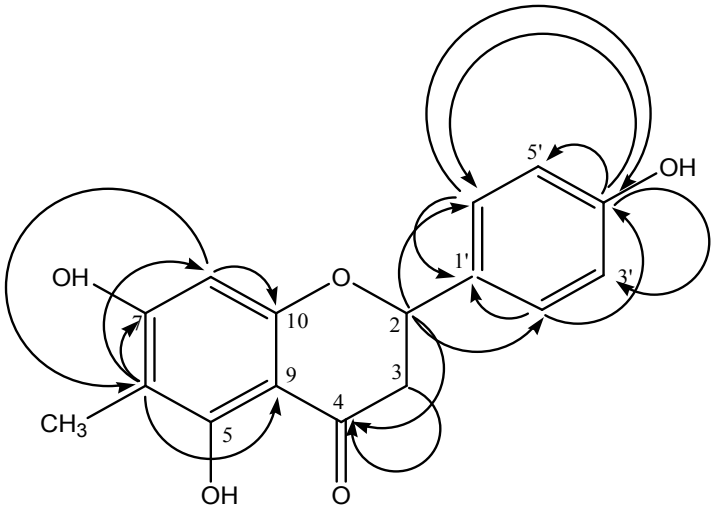

Figure 2: HMBC correlation of 5,7,4'-trihydroxy-6-methylflavanone

4. CONCLUSION

Two flavanone compounds, 5,7,3',5'-tetrahydroxyflavanone and 5,7,4'- trihydroxy-6-methylflavanone, were isolated for the first time from the bark of *Bauhinia hullettii* Prain. 5,7,4'- trihydroxy-6-methylflavanone is very rare found in other plants. Further studies of this plant are highly recommended.

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