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SHORT COMMUNICATION

Flavonoids from the Borneo Plant Species: *Eusideroxylon zwageri* Teijsm. & Binn.

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ABSTRACT

A new flavonoid, 7,3′-dihydroxy-3,5,4′-trimethoxyflavone, along with two known flavonoids, 7-hydroxy-5,4′-dimethoxyflavone and 7-hydroxy-3,5,4′-trimethoxyflavone were isolated from the leaves ethyl acetate extract of *Eusideroxylon zwageri* Teijsm. & Binn. (Lauraceae). Structures were elucidated by spectroscopic techniques such as NMR, IR, and Orbitrap Mass Spectrometry.

Keywords: *Eusideroxylon zwageri*, Lauraceae, flavonoid

1. INTRODUCTION

E. zwageri Teijsm. & Binn. (Lauraceae), locally known as Belian, Bulian, Ulin, and Borneo ironwood, is the only species that belongs to the genus of *Eusideroxylon*. It is an indigenous plant in Sabah, Malaysia. Traditionally, this plant is used by the natives as a remedy to treat toothache, jaundice, postpartum, and diabetes mellitus (Maharani and Fernandes, 2021). It was reported to contain lignans, flavonoids, and alkaloids, which possess antimicrobial and antioxidant activities (Timotius and Rahayu, 2021; Salim et al., 2022). In this study, a new flavonoid, 7,3′-dihydroxy-3,5,4′-trimethoxyflavone **3** along with two known flavonoids, 7-hydroxy-5,4′-dimethoxyflavone **1** and 7-hydroxy-3,5,4′-trimethoxyflavone **2** are reported from the ethyl acetate extract of *E. zwageri* leaves. To the best of our knowledge, it is the first report on the phytochemistry of Malaysian *E. zwageri* plant species.

2. MATERIALS AND METHODS

2.1. General experimental procedures

The 1D- and 2D-NMR data were recorded in chloroform-*d* on Bruker Ascend^{TM} 600 NMR Spectrometer (600 MHz for ^{1}H- and 150 MHz for ^{13}C-, respectively) at 27°C. Chemical

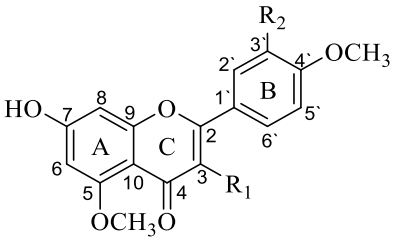
shifts were reported in δ ppm scale and coupling constants were given in Hz. The infrared (IR) spectra were recorded on PerkinElmer Spectrum 100 Fourier Transform Infrared (FT-IR) Spectrometer using a KBr pellet. The masses of isolated compounds were measured by the Thermo Scientific Orbitrap Fusion Tribrid System.

2.2. Plant material

The leaves of *E. zwageri* (BORH 5583) were collected at Imbak Canyon Conservation Area (ICCA), Sabah, Malaysia, December 2019. It was identified by Mr Alasri Anis, an experienced ethno-ranger from the Imbak Canyon Study Centre (ICSC), Sabah, Malaysia. It was deposited in the BORNEENSIS Herbarium (BORH), Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, Malaysia.

2.3. Extraction and purification

The dried leaf powder (1.16 kg) of *E. zwageri* was successively extracted by maceration at room temperature with increasing polarity of solvents, starting from hexane, ethyl acetate and methanol. Extracts were concentrated using a rotary evaporator to produce 47.67 g of ethyl acetate extract. About 500 mg of ethyl acetate extract was subjected to an open column of size exclusion chromatography (Sephadex LH-20) using an isocratic elution of dichloromethane and methanol (1:1 ratio) for fractionation. The sample was loaded by the wet packing method. The fractionation was repeated 12 times and afforded five fractions, EZEF1 to EZEF5. Fraction EZEF5 was selected for isolation. Isolation and purification were performed by Preparative High-Performance Chromatography (PHPLC) and Preparative Thin Layer Chromatography (PTLC). Isolation was carried out on the WATERS PHPLC system using a reversed phase of Eclipse XDB-C18 (21.2 × 250 mm, 7 um) column at 15 mL/min under gradient elution of deionised water and acetonitrile. The sample was injected into the column at 20 µL (concentration 50 mg/L) and observed at 210 nm wavelength. This step yielded four subfractions, EZEF5-P1 to EZEF5-P4. Subfractions EZEF5-P1, EZEF5-P3, and EZEF5-P4 were purified by normal phase PTLC in a 9:1 ratio of chloroform and hexane to give compounds **1** (7.9 mg), **2** (7.1 mg) and **3** (11.3 mg). The chemical structures of the isolated compounds are shown in Figure 1.



	R_1	R_2
1	H	H
2	OCH_3	H
3	OCH_3	OH

Figure 1. Chemical structures of isolated compounds from *E. zwageri*

3. RESULTS AND DISCUSSION

Compound **1** was isolated as a white powder, with the molecular formula of $C_{17}H_{15}O_5$, determined by the pseudomolecular ion peak $[M+H]^+$ at *m/z* 299.0914 (calculated for 299.0914). The IR spectrum showed an adsorption band at 3445, 2921, 1665, and 1243 cm^{-1} for the OH, sp^3 C-H, C=O, and C-O functionality. The ^{1}H-NMR (Table 1) indicated the presence of two *meta*-coupled aromatic protons at δ_H 6.39 and 6.51 (*d*, *J* = 2.0 Hz) assigned to H-6 and H-8 of the ring A, while an AA´XX´ spin system at δ_H 7.87 and 7.05 (*d*, *J* = 9.0 Hz) with the integration of two protons for each was assigned to H-2´, H-6´, H-3´, and H-5´, respectively, at an

oxygenated C-4′ in ring B. The isolated signal at δ_H 6.61 (*s*) was assigned to H-3 of ring C. A singlet of methoxy at δ_H 3.90 (3H, *s*) was assigned at C-5, while hydroxyl attachment was established at C-7 due to the absence of a chelated hydroxyl proton in the ^{1}H-NMR spectrum. The ^{13}C-NMR spectrum shows the presence of fifteen carbon signals. There was one carbonyl (δ_C 182.4), five oxyaryl (δ_C 157.7, 162.2 (2 carbons), 164.0 and 165.5), two methoxy (δ_C 55.5 and 55.8), two quaternary (δ_C 105.5 and 123.6) and five methine sp^2 carbons (δ_C 92.6, 98.0, 104.3, 114.5 and 128.0). The ^{1}H- and ^{13}C-NMR spectra of **1** have a strong resemblance to the aglycone part of 5,4′-dimethoxyflavone-7-*O*-glucoxyloside (Ramsewak et al., 1999) with C-7 substituted with a hydroxyl group. The attachment of the methoxy group at C-4′ in **1** was confirmed by HMBC correlation of its protons with the carbon signal of C-4′ (δ_C 162.2). The assignment was also supported through the NOESY correlation when the methoxy proton showed in-space correlation with H-3′ and H-5′. The remaining quaternary carbons at C-2, C-4, C-5, C-7, C-9, C-1′, and C-4′ were established through their long-range HMBC correlations. Based on the spectroscopic data, the structure of **1** was established as 7-hydroxy-5,4′-dimethoxyflavone, a flavone compound first isolated naturally (Nguyen et al., 2017).

Compound **2** was obtained as a yellow amorphous solid and the molecular formula $C_{18}H_{17}O_6$ was determined based on the pseudomolecular ion peak $[M+H]^+$ at *m/z* 329.1022 (calculated for 329.1020). The ^{1}H-NMR spectrum of **2** is similar to **1**, except for C-3(δ_C 138.9) is an oxymethine proton attachment. The attachment also was supported by the long-range correlation observed between methoxy proton and C-3 in HMBC experiments. Based on the data presented, **2** was concluded as a derivative of **1**, named 7-hydroxy-3,5,4′-trimethoxyflavone (Mei et al., 2015).

Compound **3** was obtained as a white powder and the molecular formula $C_{18}H_{17}O_7$ was determined based on the pseudomolecular ion peak $[M+H]^+$ at *m/z* 345.0968 (calculated for 345.0968). The sixteen-addition mass unit of this compound suggested that it is the hydroxyl derivative of **2**. The assignment of the hydroxy group at C-3′ was in agreement with the proton signals observed at δ_H 6.95 (*d*, *J* = 8.5 Hz), 7.67 (*d*, *J* = 2.0 Hz), and 7.70 (*dd*, *J* = 2.0 and 8.5 Hz) in the ^{1}H-NMR spectrum. Thus, ring B is established to have an ABX spin system. Based on the spectroscopic evidence, **3** is a new flavone derivative named 7,3′-dihydroxy-3,5,4′-trimethoxyflavone.

Table 1. ^{1}H-NMR and ^{13}C-NMR data of **1**, **2**, and **3** in $CDCl_3$ (500 MHz)

Position	**1**		**2**		**3**	
	δ_C	**δ_H (*J* in Hz)**	**δ_C**	**δ_H (*J* in Hz)**	**δ_C**	**δ_H (*J* in Hz)**
2	164.0	-	156.0	-	145.5	-
3	104.3	6.61 (s)	138.9	-	139.2	-
4	182.4	-	178.8	-	178.8	-
5	165.5	-	165.4	-	165.5	-
6	98.0	6.39 (1H, *d*, 2.0)	97.8	6.34 (1H, *d*, 2.0)	97.9	6.33 (1H, *d*, 2.5)
7	162.2	-	162.0	-	162.0	-
8	92.6	6.51 (1H, *d*, 2.0)	92.2	6.43 (1H, *d*, 2.0)	92.1	6.43 (1H, *d*, 2.5)
9	157.7	-	156.8	-	156.8	-
10	105.5	-	106.1	-	106.1	-
1′	123.6	-	122.8	-	123.7	-
2′	128.0	7.87 (1H, *d*, 9.0)	130.2	8.07 (1H, *d*, 9.0)	114.4	7.67 (1H, *d*, 2.0)
3′	114.5	7.05 (1H, *d*, 9.0)	114.0	7.01 (1H, *d*, 9.0)	155.6	-
4′	162.2	-	161.7	-	148.8	-
5′	114.5	7.05 (1H, *d*, 9.0)	114.1	7.01 (1H, *d*, 9.0)	110.4	6.95 (1H, *d*, 8.5)
6′	128.0	7.87 (1H, *d*, 9.0)	130.2	8.07 (1H, *d*, 9.0)	121.6	7.70 (1H, *dd*, 2.0, 8.5)
3-OMe	-	-	60.1	3.84 (*s*)	60.1	3.85 (*s*)
5-OMe	55.5	3.90 (*s*)	55.8	3.85 (*s*)	55.8	3.85 (*s*)
4′-OMe	55.8	3.91 (*s*)	55.4	3.88 (*s*)	56.0	3.97 (*s*)

4. CONCLUSION

The isolation and characterisation of the isolated compounds from the extract of ethyl acetate leaves of *E. zwageri* revealed one new and two known flavonoids named 7,3´-dihydroxy-3,5,4´-trimethoxyflavone, 7-hydroxy-5,4´-dimethoxyflavone and 7-hydroxy-3,5,4´-trimethoxyflavone.

Declaration of Interest
The authors declare that there is no conflict of interest.

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