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by Nanik Siti Aminah

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VLADINOL F, NEOLIGNAN COMPOUND FROM THE STEM BARK OF *Dryobalanops oblongifolia* (DIPTEROCARPACEAE) AND ANTIPLASMODIAL ACTIVITY

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ABSTRACT

Drybalanops oblongifolia known as kayu kapur is a plant member of Dipterocarpaceae family. A neolignan, vladinol F, was successfully isolated from the dissolved fraction in acetone-ether of *Dryobalanops oblongifolia* stem bark. The chemical structure's determination was done based on an analysis of UV-Vis, NMR, and MS spectra and a comparison with the reference. *In vitro* test of this compound against *Plasmodium falciparum* 3D7 showed that IC_{50} value was 3.51 µg/mL.

Keywords: *Drybalanops oblongifolia*, Neolignan, Vladinol F, Antiplasmodial Activity, *Plasmodium falciparum*
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INTRODUCTION

Various researches to find new drug candidates have been carried out.[1-3] Malaria is a parasitic infectious disease which causes millions of deaths every year. Besides that, the resistance of *P. falciparum* to nearly all available antimalarial drugs are increasing today.[4] Thus, the new antimalarial drugs are urgently needed that will be used to prevent the spread of the infection and to cure infected patients. *Dryobalanops oblongifolia* is a species of the *Dryobalanops* genus belonging to the Dipterocarpaceae family. *Dryobalanops* is called Kayu Kapur by the local community[5,6] and is recognized as a source of oligostilbene agents.[7-10] A previously isolated constituent from *D. oblongifolia* reported that dimerstilbene, (-) ampelopsin F, had a potential activity as antiplasmodium.[11]

In continuation of our research program on biologically active compounds from plants, we investigated this plant's antiplasmodial constituent. From this research, we have isolated vladinol F from the acetone extract of *D. oblongifolia* stem bark.

EXPERIMENTAL

Material and Methods

The stem bark of *D. oblongifolia* was gathered from Gunung Mali, Tempunak, Sintang, West Kalimantan in 2012. The plant was identified at the Biological Research Center, LIPI, Bogor, Indonesia, and the specimen's voucher was stored at the herbarium.

General Procedure

Using acetone, as many as 5 kg of D. *oblongifolia* stem bark powder was macerated twice at room temperature and then concentrated by vacuum evaporator. The acetone extract was divided into two fractions, that were soluble or insoluble in acetone – ether. By vacuum liquid chromatography (VLC) (*n*-hexane-EtOAc, rising polarity), 48 g dissolved part in acetone-ether was fractionated to yield fractions A-D. 1.2 g of fraction D was repeatedly GCC separated using *n*-heksane – EtOAc 5:5 – EtOAc 100% to give subfractions D1 - D3. Subfraction D3 (50 mg), on repeated separation and

purification using radial chromatography (gradient system of $CHCl_3$ – MeOH), acquired vladinol F (10 mg) (Fig.-1).

Detection Method

The elucidation structure of the vladinol F molecule using UV-Vis, ^1H and ^{13}C NMR and 2D NMR spectroscopy. ESI-MS spectrometer was used to record the molecular mass of this compound.

RESULTS AND DISCUSSION

Vladinol F, a colorless amorphous solid, mp. 105^0 - 107^0; UV λ_{max} (MeOH): 216, 230, dan 284nm. ESI-MS: *m/z* $[M-H]^-$ 359,2 (Fig.-2, supplementary data). ^1H- NMR (methanol-d4, 500 MHz) (Fig.-1, supplementary data), δ_H (ppm): 1.87 (2H, *m*, H-8a); 2.63 (2H, *t*, H-7a); 3.46 (1H, *q*, H-8); 3.57 (2H, *t*, H-9a); 3.75 (2H, *dd* & *dd*, H-9); 3.81 (3H, *s*); 3.85 (3H, *s*); 6.72 (1H, *s*, H-2a); 6.73 (1H, *s*, H-6a); 6.76 (1H, *d*, H-3); 6.82 (1H, *dd*, H-2); 6.95 (1H, *d*, H-6). ^{13}C-NMR (methanol-d4, 125 MHz) (Fig.-3, Suplementary data), δ_C (ppm): 32.9 (C-7a); 35.8 (C-8a); 55.5 (C-8); 56.4 (C5a-OCH_3); 56.8 (C5-OCH_3); 62.2 (C-9a); 65.0 (C-9); 88.9 (C-7); 110.6 (C-6); 129.9 (C-3a); 114.2 (C-6a); 115.9 (C-3); 117.9 (C-2a); 119.7 (C-2); 134.8 (C-1); 136.9 (C-1a); 145.2 (C-5a); 147.5 (C-4a); 147.6 (C-4); 149.1 (C-5).

Vladinol F was obtained as a colorless amorphous solid having a melting point of 105^0 - 107^0. Its molecular formula was determined as $C_{20}H_{24}O_6$ by ESI-MS ($[M-H]^-$ ion at m/z 359.2). The UV (216, 230, and 284 nm) spectra of this compound were typical for a phenolic chromophore. The ^{13}C-NMR (Nuclear Magnetic Resonance) (Table-1) and DEPT (Distortion less Enhancement by Polarization Transverse) (90, 135) spectra of the isolated compound revealed 20 carbon signals including two aromatic rings (δ_C 110.6-149.1), one oxygenated methine group (δ_C 88.9), one methine group (δ_C 55.5), two methylene groups ((δ_C 32.9, 35.8), two oxygenated methylene groups ((δ_C 62.2, 65.0), and two methoxy groups (δ_C 56.4, 56.8). The ^1H-NMR spectra indicated five protons of two aromatic regions, one was at δ_H 6.82 (1H, *dd*, *J*=8.1, 1.9, H-2), δ_H 6.76 (1H, *d*, *J*=8.1, H-3), δ_H 6.95 (1H, *d*, *J*=1.9, H-6), and another was δ_H 6.72 (1H, *s*, H-2a) and δ_H 6,73 (1H, *s*, H-6a). Two singlet signals of methoxy group were at δ_H 3.81(3H) and δ_H 3.85 (3H). Furthermore, in the ^1H-NMR spectra showed a structure of propanol side chain at δ_H 2.63 (2H, *t*, *J*=6.5, H-7a), δ_H 1.87 (2H, *m*, H-8a), δ_H 3.57 (2H, *t*, *J*=5.4, H-9a). Four hydrogen proton signals at δ_H 5.49 (1H, *d*, *J*= 6.3 , H-7), δ_H 3.46 (1H, *q*, H-8) δ_H 3.75 (1H, *dd*, *J*=6.0, 9.2, H-9'), and δ_H 3.83 (1H, *dd*, *J*=5.6, 9.6, H-9") were a -CH-CH-CH_2- a structural unit which formed a dihydrofuran ring. The HMBC spectrum of this compound showed correlations between H-7/C-4a, H-7/C-3a, H-7/C-2, H-7/C-6, H-2/C-7, H-6/C-1, H-6/C-7, and H-6a/C-4a. These correlations indicated that dihydrofuran ring fused at C-3a and C-4a of the aromatic ring and a phenyl group was located at C-7 of dihydrofuran ring. Long-range couplings determined the placement propanol group at position C-1a of the aromatic ring in the HMBC spectrum between H-2a/C-7a and H-8a/C-1a. Based on the spectroscopic analysis, the isolated compound was established as vladinol F (Fig.-1), 7-phenyldihydrobenzofuranpropanol-type neolignane.[12,13] We compared it with the previously reported Vladinol F (see Table-1) to validate the isolated compound.

Furthermore, the isolated compound was checked out as antiplasmodial. Methods previously described by Widyawaruyanti determined the antiplasmodial activity of the isolated compound.[14] The samples were dissolved in DMSO and kept at −20 °C until use. The parasite *P. falciparum* (3D7) clone was propagated in a 24-well culture plate in the presence of 100, 10, 1, 0.1 and 0.01 µg/ml range of concentrations of the sample. Parasitaemia was monitored over 48 hours by making a blood smear fixed with MeOH and stained with Geimsa (Merck). The antiplasmodial activity test of the vladinol F toward *P. falciparum* showed the IC_{50} value of 3.51 µg/ml. This result showed that vladinol F was good as an antiplasmodial agent.[15]

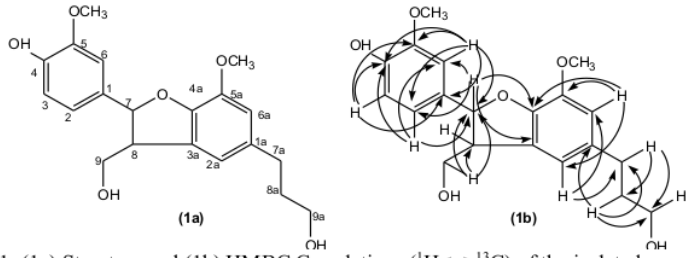

Fig.-1: (1a) Structure and (1b) HMBC Correlations (^1H<=>^{13}C) of the isolated compound

Table-1: ^{1}H-NMR (500 Mhz) and ^{13}C-NMR (125 MHz) Spectral Data of Isolated Compound in methanol-d4 (Figure 3 & 4, supplementary data) and Vladinol F (Isao Kouno, 1993)[12]

C	Isolated Compound			Vladinol F (Isao Kouno, 1993)[9]	
	δ_H (ppm) (multiplet, *J* in Hz)	δ_C (ppm)	HMBC (^{1}H<=>^{13}C)	δ_H (ppm) (multiplet, *J* in Hz)	δ_C (ppm)
1	-	134.8	-	-	137.9
2	6.82 (*dd*, 8.1 & 1.9)	119.7	C-4, C-6, C-7	6.93 (*dd*, 8.1 & 1.8)	119.4
3	6.76 (*d*, 8.1)	115.9	C-1, C-4, C-5	7.06 (*d*, 8.1)	118.9
4	-	147.6	-	-	148.5
5	-	149,1	-	-	152,1
6	6.95 (*d*, 1.9)	110.6	C-1, C-2, C-4, C-5, C-7	7.03 (*d*, 1.8)	111.3
7	5.49 (*d*, 6.3)	88.9	C-2, C-6, C-8, C-3a, C-4a	5,.5 (*d*, 5,9)	88.6
8	3.46 (*brd q*)	55.5	-	3.46 (*dd*, 12.5 & 5.5)	55.6
9	3.75 & 3.83 (*dd* & *dd*)	65.0	C-7, C-8	3.73 & 3.84 (*m*)	65.1
1a	-	136.9	-	-	137.0
2a	6.72 (*s*)	117.9	C-6a, C-7a	6.72 (*s*)	118.0
3a	-	129.9	-	-	129.7
4a	-	147.5	-	-	147.5
5a	-	145.2	-	-	145.3
6a	6.73 (*s*)	114.3	C-2a, C-4a, C-5a	6.73 (*s*)	114.3
7a	2.63 (*t*)	32.9	C-8a, C-9a	2.62 (*t*, 7.7)	32.9
8a	1.87 (*p*)	35.8	C-1a, C-7a, C-9a	1.82 (*m*)	35.8
9a	3.57 (*t*)	62.2	C-7a, C-8a	3.56 (*t*, 6.4)	62.3
5a-OCH_3	3.81 (*s*)	56.4	-	3.82 (*s*)	56.5
8-OCH_3	3.85 (*s*)	56.8	-	3.85 (*s*)	56.9

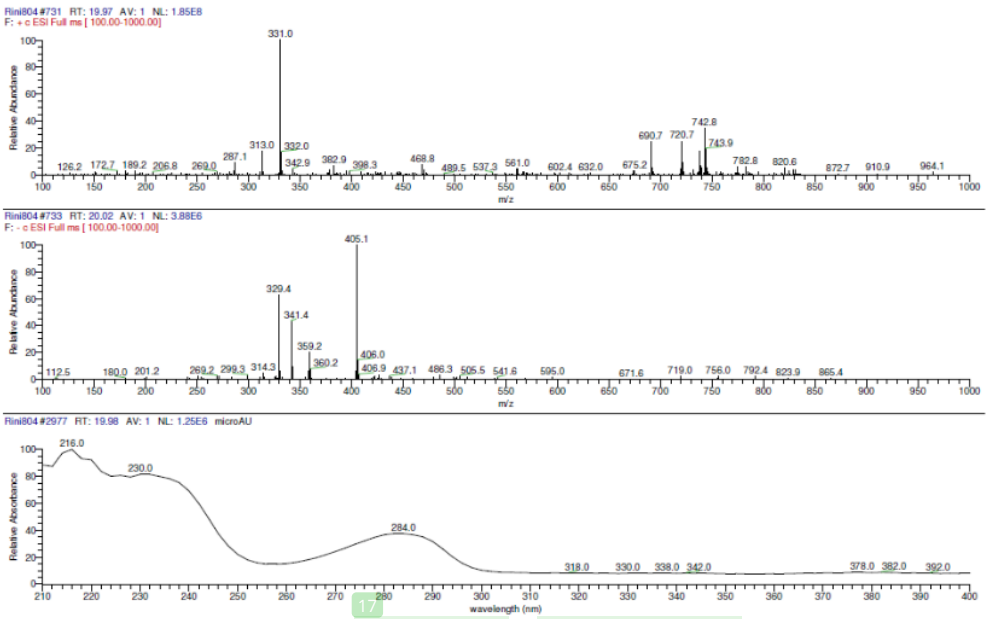

Fig.-2: ESI-MS Spectral Data of the Isolated Compound

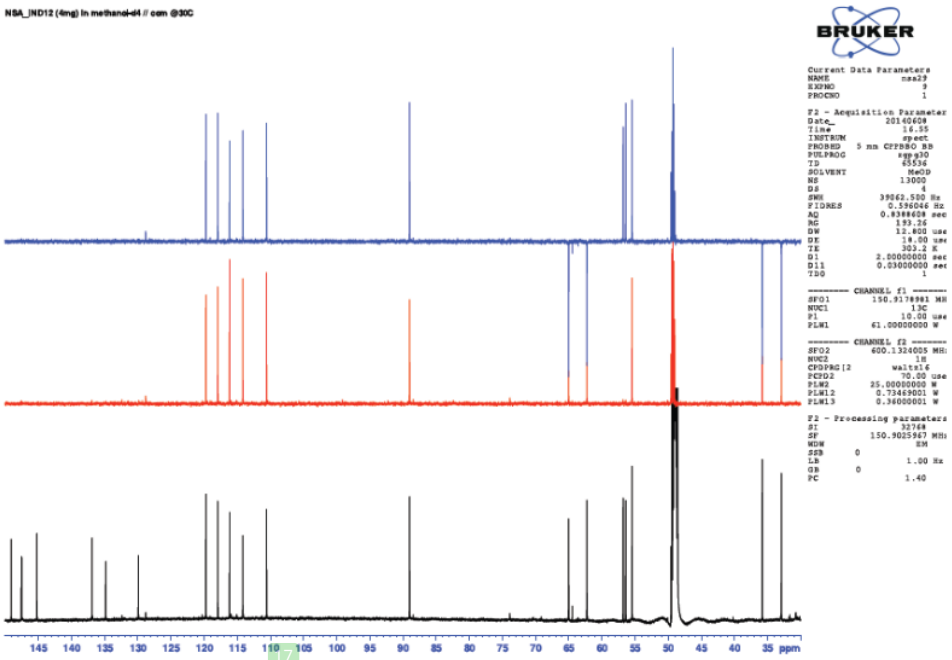

Fig.-3 ^{13}C-NMR and DEPT Spectral Data of the Isolated Compound

CONCLUSION

A neolignan, vladinol F, was successfully isolated from the dissolved fraction in acetone-ether of *Dryobalanops oblongifolia* stem bark. The antiplasmodial activity test of the vladinol F toward *P. falciparum* showed the IC_{50} value of 3.51 µg/mL. This result showed that vladinol F was good as an antiplasmodial agent.

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