MACRONONE, A NOVEL DIEPOXYLIGNAN FROM BARK OF *MAHKOTA DEWA* (*Phaleria macrocarpa* (*Scheff.*) Boerl.) AND ITS ANTIOXIDANT ACTIVITY

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ABSTRACT

Mahkota dewa (Phaleria macrocarpa (Scheff.) Boerl.) which is belong to family of Thymelaeaceae is one of Indonesian traditional medicines. Chemical constituent has been isolated from bark of mahkota dewa. Sample was extracted with methanol. Concentrated methanol extract was extracted by n-hexane, chloroform and ethyl acetate. A Compound that separated and purified by column chromatography from ethyl acetate extract is a red spherical crystal (m.p. 94-95 °C). Its spot gave yellow fluorescence at TLC plate (UV_{366}) and has optical rotation of -9.3° (c. 2 mg/mL, methanol). Structure elucidation by UV, IR, ^{1}H-NMR, ^{13}C-NMR and NMR 2 dimension (HMQC, COSY, HMBC and DEPT-135) spectroscopy show that the compound gives a name macronone. Computational chemistry calculation using Hyperchem on the level of semiempirical method PM3 was confirmed the conformation of macronone. DPPH method shows that macronone has lower antioxidant activity compare to the ethyl acetate extract.

Keywords: *macronone; diepoxylignan; mahkota dewa; Phaleria macrocarpa (Scheff.) Boerl.; DPPH*

ABSTRAK

Mahkota dewa (Phaleria macrocarpa (Scheff.) Boerl.) yang termasuk kedalam famili Thymelaeaceae merupakan salah satu obat tradisional Indonesia. Konstituen kimia telah diisolasi dari ekstrak etil asetat kulit batang mahkota dewa. Sampel diekstrak dengan metanol, dipekatkan kemudian diekstrak dengan n-heksana, khloroform dan etil asetat. Satu senyawa dipisahkan dan dimurnikan dengan kromatografi kolom dari ekstrak etil asetat sebagai kristal bulat anggur berwarna merah (Tl 94-95 °C). Noda pada TLC memberikan fluorisensi gelap (UV_{366}) dan mempunyai putaran optik -9,3° (2 mg/mL, metanol). Elusidasi struktur dengan spektroskopi UV, IR, ^{1}H-NMR, ^{13}C-NMR dan NMR 2 dimensi (HMQC, COSY, HMBC dan DEPT-135) menunjukkan senyawa yang diberi nama makronon. Perhitungan kimia komputasi menggunakan Hyperchem pada metode semiempirik PM3 mengkonfirmasi konformasi dari makronon. Metode DPPH menunjukkan bahwa makronon mempunyai aktivitas antioksidan yang lebih rendah dibandingkan dengan ekstrak etil asetat.

Kata Kunci: *makronon; diepoksilignan; mahkota dewa; Phaleria macrocarpa (Scheff.) Boerl.; DPPH*

INTRODUCTION

Mahkota dewa plant (*Phaleria macrocarpa* (*Scheff*.) Boerl.) a Thymelaeaceae, is widely found in Indonesia. This plant has synonym of *Phaleria papuana var warb wichnanmi* (val) Back. In English, it is known as crown of God. In Sumatra (Malay) and Depok it is known as *simalakama*. In Java, it is also called as *makutadewa*, *makuto rajo*, *makuto ratu* or *makuto mewo* [1].

Mahkota dewa fruit is frequently and empirically utilized by Indonesian to treat various diseases with satisfactory results [1]. *Mahkota dewa* is one of Indonesian traditional medicines. Information about the bioactivity test (antimicrobial, brine shrimp, cytotoxic, pharmacological and antioxidant activity) of the extract or fraction of seed and fruits had been studied intensively. However, isolation and purification of active compound in *mahkota dewa* was rarely performed.

The isolated chemical constituents of *mahkota dewa* fruit were isolated as icariside C3, benzophenone derivative, mangiferin [2] and lignan [3]. From its seed, 29-norcurcubitacin and its derivatives [4], mahkoside A, mangiferin and kaempferol 3-O-β-D-glucoside [5] had been isolated. In addition, phalerin was isolated from the methanol extract of its leave. [6]. Aglucon benzophenone and isophalerin were obtained from ethyl acetate extract of its leave [7]. Ethyl acetate extract

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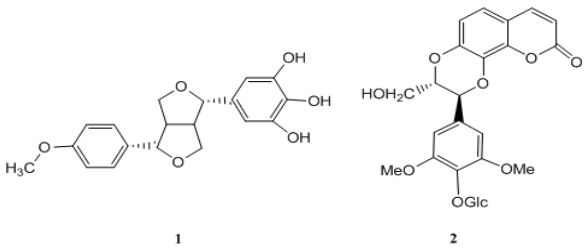

Fig 1. Structure of lignan, compounds isolated from Thymelaeaceae

of its bark gave one compound of benzophenone glucoside [8]

Lignans of culture of *mahkota dewa* had been extracted and identified [9]. Lignan **(1)** which was similar with syringaresinol (Lirioresinol B) was 5-[4{4-methoxy-phenyl)-tetrahydrofuro[3,4-c]furan-1-yl]-benzene-1,2,3-triol with molecular formula of $C_{19}H_{20}O_6$. It had been isolated from ethyl acetate extract of *mahkota dewa* fruit and separated with column chromatography by step Gradient polarity (SGP) method. This compound had a tendency as a sitostatica compound based on taxonomic approach/chemotaxonomy [4]. While lignan conjugated with coumarin **(2)** in the form of glucosides had been obtained from *Daphne oleoides* (family Thymelaeaceae) [10] (Fig. 1).

This paper reported the isolation and structure elucidation of phenolic compound from the ethyl acetate extract of bark of *mahkota dewa* and its antioxidant activity. Structure elucidation of isolated compound was performed by means of spectroscopy analyses (UV, IR, ^{1}H-NMR, ^{13}C-NMR, HMBC, HMQC, COSY and DEPT-135). The measurement of antioxidant activity was performed by DPPH method.

EXPERIMENTAL SECTION

Materials

Bark of *mahkota dewa* was collected from Universitas Gadjah Mada (UGM), Yogyakarta, Indonesia in January 2009. The plant was identified by Plant Taxonomy Laboratory, Faculty of Biology, UGM. The used chemicals were methanol (technical, distilled) and p.a. (Merck), n-hexane (technical, distilled), ethyl acetate (technical, distilled), chloroform p.a. (Merck), acetone p.a. (Merck), ethanol p.a. (Merck), $FeCl_3$ (Merck), DPPH (Merck).

Instrumentation

Melting point apparatus (Electrothermal 9100), polarimeter (Atago Polax-D), UV-vis spectrophotometer (Spectronic 3000, Genesis 10), Fourier Transformation-Infra Red (FT-IR) spectrophotometer (Shimadzu IRPrestige-21). Nuclear Magnetic Resonance, ^{1}H-NMR and ^{13}C-NMR (JEOL JNM ECA-500 spectrometer), operating at 500 MHz (^{1}H-NMR) and 125 MHz (^{13}C-NMR), using Tetramethyl silane (TMS) as an internal standard. Mass Spectrophotometer (MS-QP2010S Shimadzu), Column chromatography was conducted using Merck silica gel 60 (70-230 mesh ASTM). Thin Layer Chromatography (TLC) analysis was conducted on precoated Silica gel plates (Merck silica gel GF 254). TLC glass preparative. Software of computational chemistry of HyperChem for Windows version 7.0 (Hypercube).

Procedure

Extraction, Isolation and Identification

One kg of dried bark of *mahkota dewa* was extracted using macerator (drip pan) with methanol by heating (60 °C) for 7 h then allowed to cool at room temperature for up to 24 h. The residue was macerated for 3 times and all the filtrates were combined (11.5 L) and concentrated using vacuum distillation and rotary evaporator. The methanol extract was partitioned with n-hexane-water. Into the residue of methanol extract, chloroform was added to give chloroform extract and the residue was added with ethyl acetate to produce ethyl acetate extract. Ethyl acetate extract (7 g) was fractionated by column chromatography on silica gel using isocratic elution by n-hexane : ethyl acetate (3:7) to give 7 fractions. The column was eluted by ethyl acetate until give the orange eluate. Having dried by rotary evaporator, orange eluate was obtained in 3.28 g.

The orange eluate (3.28 g) was refractioned by column chromatography using gradient elution by eluents of n-hexane : ethyl acetate (3:7), ethyl acetate, ethyl acetate : methanol (1:1) and methanol. The 5th fraction (+ phenolic with $FeCl_3$) consisted of two spots in the plate. TLC preparative was then performed with eluents of ethyl acetate : methanol (9:1). The spot with yellow fluorescence was scraped, dissolved in methanol for overnight and filtered. The filtrate was formed crystal at room temperature. It was purified by recrystallization using methanol : water (3:1) and give red spherical crystal after washed by chloroform. Structure elucidation of the crystal was performed using UV, IR, H-NMR, C-NMR, HMQC, COSY, HMBC, DEPT and MS.

Computational Method

Based on spectroscopy analyses, the isolated compound has a lot of C chiral, so that it has stereoisomers. ^{1}H-NMR and ^{13}C-NMR spectra data were compared with the estimated spectra using a series of computational calculation with HyperChem

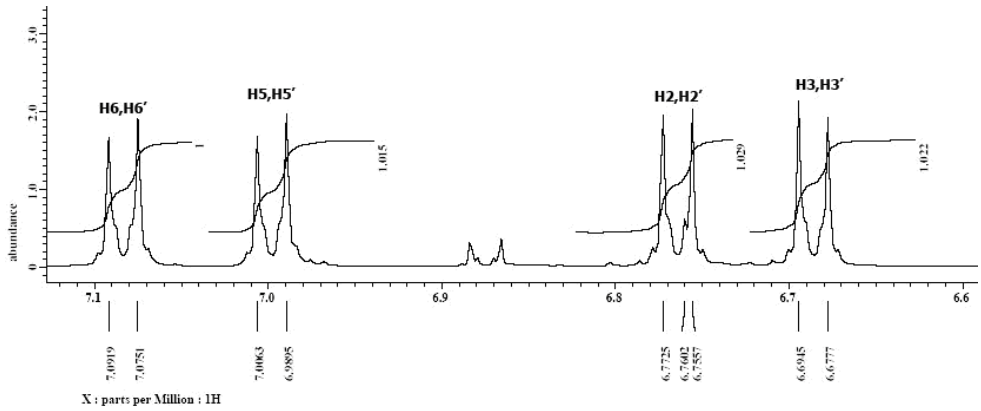

Fig 2. ^{1}H-NMR spectrum of isolated compound (acetone d_6 500 MHz) at 6.6 - 7.1 ppm

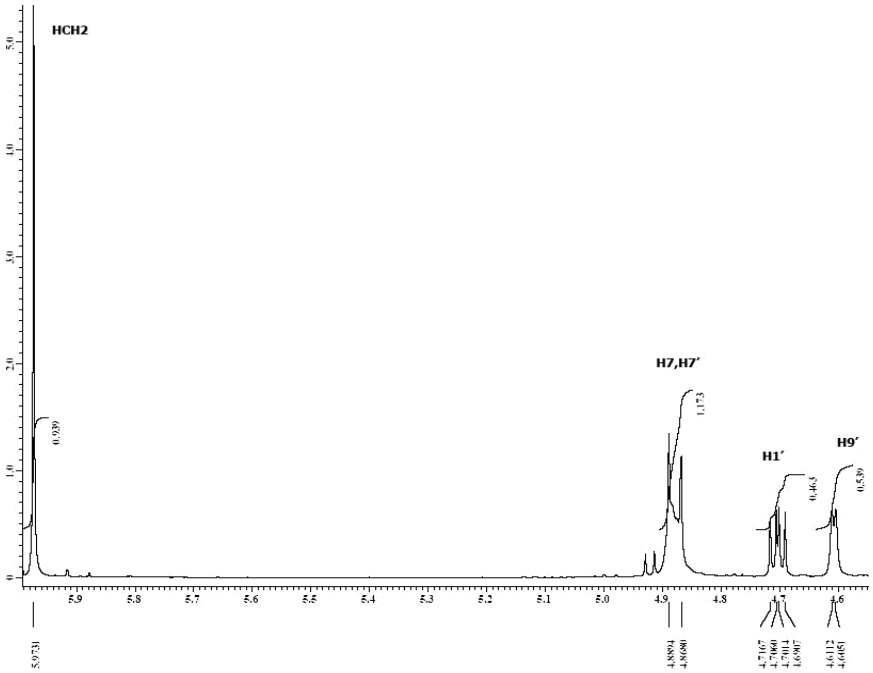

Fig 3. ^{1}H-NMR spectrum of isolated compound (acetone d_6 500 MHz) at 4.6 - 6.0 ppm

version 7.0 for Windows using semiempirical, AM1 and PM3 methods.

Method which gave the results of geometry optimization calculations that closest to the experimental data was considered as the most representative semiempirical method for computing the next calculation step. Based on the optimized geometry of isolated compound, all of the possible geometries were studied and the most stable geometry with the lowest energy was obtained to give estimation of UV, IR and NMR spectra.

Antioxidant Activity Test

The measurement of antioxidant activity was performed according to a procedure described previously [11]. Quercetin was used as the standard antioxidant. DPPH and MeOH were used as the stable free radical reagent and reference, respectively. The sample (isolated compound) was dissolved in MeOH. It was then diluted to give concentrations of 40, 100, 140, 200 and 400 µg/mL. As much as 250 µL of each concentration was transferred to different vials, and add 1 mL DPPH (0.4 mM) and methanol until 5 mL. The absorbance at wavelength of 515 nm was determined after 30 min. The antioxidant activity was measured as the decrease in the absorbance of DPPH and expressed as percentage of the absorbance of control DPPH solution without sample. Color of solution would change from violet to yellow if solution has activity of antioxidant. The IC_{50} value was defined as the amount of antioxidant needed to decrease the initial concentration of DPPH by 50%. Total antioxidant activity

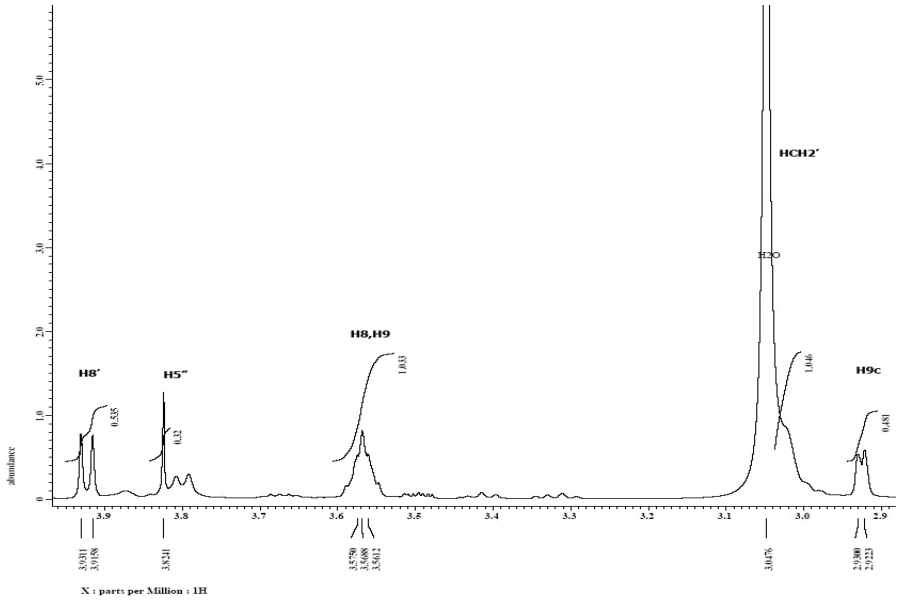

Fig 4. ^{1}H-NMR spectrum of isolated compound (acetone d_6 500 MHz) at 2.9 - 3.9 ppm

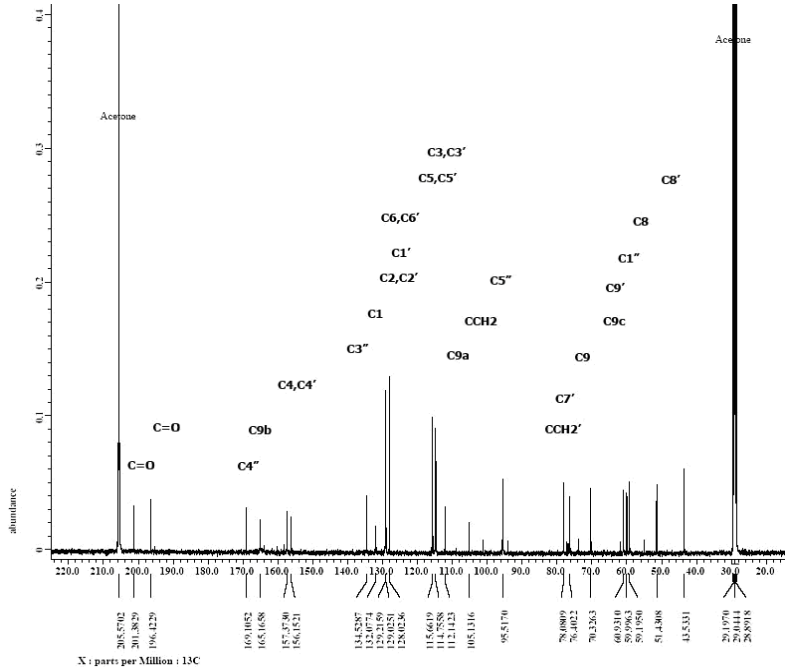

Fig 5. ^{13}C-NMR spectrum of isolated compound (acetone d_6, 125 MHz)

was expressed as the inhibition percentage of the DPPH radical and was determined by the following equation:

$$\% \text{ TAA} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100\%$$

where TAA was Total antioxidant activity and A was the absorbance.

The data of the activity percentage on concentration variation were then used to determine the value of IC_{50} of sample using a linear regression equation.

RESULT AND DISCUSSION

The ethyl acetate extract of the bark of *mahkota dewa* was fractionated by column chromatography. The fifth fraction was purified using TLC preparative and recrystallization to give red spherical crystal (m.p. 94-95 °C) in 72.5 mg from one kg of *mahkota dewa* bark.

Table 1. Data of ^{13}C-NMR (acetone d_6, 125 MHz), ^{1}H-NMR (acetone d_6 500 MHz) and correlation HMQC, COSY and HMBC of isolated compound

C atoms	^{13}C-NMR δ (ppm)	^{1}H-NMR δ (ppm), *J* (Hz)	HMQC	COSY	HMBC
1	132	-	-	-	-
1'	129	-	-	-	-
2/2'	129.2	7.00 (d, 2H, 8.4 Hz)	C2/2'	H5/5' H3/3' H6/6'	C1',C4, C7
3/3'	114.7	6.69 (d, 2H, 8.4 Hz)	C3/3'	H5/5' H2/2' H6/6'	C4, C6/6', C3/3'
4	157.3	-	-	-	-
4'	156.1	-	-	-	-
5/5'	115.6	6.76 (d, 2H, 8.4 Hz)	C5/5'	H3/3' H2/2' H6/6'	C4', C3"
6/6'	128	7.09 (d, 2H, 8.4 Hz)	C6/6'	H5/5'/ H3/3'	C6/6', C4', C8'
7	77.8	4.87 (d, 1H)	C7	H9c, H8, H9	C2/2',C9
8	51.4	3.57 (t, 1H)	C8	H9', H1",H7	-
9	70.3	3.57 (t, 1H)	C9	H9', H1",H7	-
9a	112.1	-	-	-	-
9b	165.1	-	-	-	-
9c	60.9	2.93 (d, 1H)	C9c	H7	-
CH2	105	5.97 (s, 2H)	C_{CH2}	-	C9b, C9a, C5"
CH2'	78	3.05 (s, 2H)	-	-	-
7'	76.4	4.87 (d, 1H)	C7'	H9c, H8, H9	C2/2', C9
8'	43.5	3.92 (d, 1H)	C8'	-	CC=O (2), C3", C1', C7', C9'
9'	59.9	4.61 (d, 1H)	C9'	H8, H9	-
1"	59.1	4.70 (dd/k, 1H)	C1"	H8, H9, H8'	CC=O, C4", C3", C8
C=O	196.4	-	-	-	-
3"	134.5	-	-	-	-
4"	169.1	-	-	-	-
5"	95.5	3.82 (s, 1H)	-	-	-
C=O	201.3	-	-	-	-
H_2O	-	3.05 (s, 2H)	-	-	-
Acetone d6	205.6	-	-	-	$C_{acetone\ d6}$
	29 (sept)	2.05 (k)	$C_{aceton\ d6}$	-	-

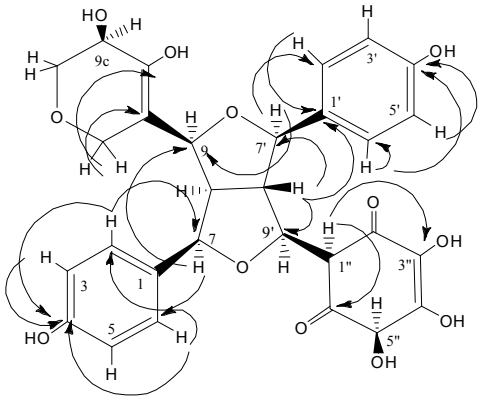

Fig 6. Correlation HMBC of macronone

Its spot gave yellow fluorescence at TLC plate (UV_{366}) with Rf of 0.2 at TLC chromatogram with eluent of n-hexane : ethyl acetate (3:7); 0.4 with n-hexane : ethyl acetate (1:9) ; 0.6 with ethyl acetate and 0.8 with chloroform : methanol (8:2). This compound was dissolved in methanol and acetone. The compound was tested with $FeCl_3$ in order to show the presence of phenolic compound. The optical rotation was -9.3° (c. 2 mg/mL, methanol).

The UV spectrum (MeOH) of isolated compound showed maximum absorbance at λ 205, 227 and 298 nm. Absorption at 298 nm described the substituted aromatic ketones. Absorbances of this UV spectrum were similar with lignan from ethyl acetate extract of fruit of *mahkota dewa* which were at 220, 240 and 290 nm. Characteristic absorbances of lignan compound from literature were at 210, 230 and 280 nm [3].

The IR spectrum (KBr) of isolated compound showed absorption bands at 3402 cm^{-1} indicating the presence of OH group. Absorption band at 2924 cm^{-1} indicated the presence of saturated C-H group. Absorption bands at 1512, 1442, and 825 cm^{-1} indicated the present of aromatic ring. Characteristic absorption band at 1604 cm^{-1} strongly correlated with -C=O group. Absorption band at 1095 cm^{-1} came from the vibration of ether.

Table 2. Analysis of ^{13}C-NMR spectrum with computational chemistry by Hyperchem for lignan 1-16 stereoisomers

C	C-NMR of	^{13}C –NMR, δ (ppm) (ΔPM3) of Lignan stereoisomers															
atom	Compound	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	132	9.2	9.2	8.7	9.6	9.2	9.1	9.1	9.2	8.2	9.3	9.4	9.3	9.2	**8.6**	8.9	9
1'	129	12.3	12.3	12.3	12	**10.8**	**10.8**	12	12.3	12	12.6	11.4	11.4	12.3	11.4	12.2	12
2/2'	129.2	5	5	4.8	5.2	4.8	**4**	5.2	5	4.6	5.2	4.5	4.8	4.6	**4**	4.6	5
3/3'	114.7	15.1	15.1	15.1	15.3	14.9	15	15.5	15.2	15.2	15.3	**12.9**	15	14.9	14.7	15.1	15.1
4	157.3	8.2	8.2	8.1	8.1	8.4	8.2	8.3	8.2	8.2	8.1	8.1	8.1	8.3	**7.2**	8.1	8.3
4'	156.1	9.9	9.9	9.4	10.1	**8.7**	9.1	10.1	9.9	9.8	9.5	9.4	9.6	9.2	9.5	9.9	9.9
5/5'	115.6	14.2	14.4	14	14.4	14	14.1	14.2	13.8	14.4	14.4	**12.6**	**12.6**	14.4	13.8	14.1	14.2
6/6'	128	7.4	7.4	6.8	8.1	6.8	6.8	7.5	7.4	7.8	7.1	6.1	6.3	7.4	**6**	7.2	7.5
7	77.8	5.27	5.27	5	5.3	5.3	7.6	6.7	5.2	5.1	10	5.1	6.9	7.7	**2.9**	4.6	7.1
8	51.4	18.6	18.6	18.1	13.4	17.6	14.6	14.1	18.6	13.6	16.6	18.3	13.7	21.1	**13.3**	18.7	14
9	70.3	4.1	4.1	4.8	7.1	4.4	7.3	7.8	4.1	4.1	17.9	4.6	7.8	3.9	8.4	**3.6**	5.7
9a	112.1	24.7	24.7	**24.5**	25.3	25.4	25.3	25.7	24.7	24.9	26.5	24.6	25.2	24.8	25.2	24.8	24.9
9b	165.1	6.5	6.5	6.6	**4.1**	5.2	4.2	4.4	6.5	6.2	6.6	6.7	4.5	6.5	**4.1**	8.5	6
9c	60.9	4.1	4.1	4.2	2.8	4.4	2.8	2.9	4.1	3.8	4.4	4.2	3	4.2	2.8	**0**	1.1
CH2	105	34.8	31.2	34.3	30.9	32.4	34.5	**21.2**	34.2	33.3	32.3	34.1	31.2	34.2	35	35.8	33.6
CH2'	78	7.2	7.9	7.7	8	7.6	7.9	7.9	7.8	7.5	8.7	7.8	8.1	7.6	8	**5.2**	5.5
7'	76.4	5.2	5.2	3.3	5.8	1.9	4.5	5.2	5.2	5.5	13.3	3.7	3.5	**0.4**	3.1	5.3	6.2
8'	43.5	28.1	28.1	24.3	30.5	21	26.8	31.8	38.1	27.4	26.5	23.5	27.3	**20.8**	23.5	28	32.1
9'	59.9	21.3	21.3	**20.9**	23.1	26.9	22.8	22.3	21.2	24.9	35.3	23.3	23.6	26.9	25.6	21.2	21.8
1"	59.1	0.4	0.4	5.6	2.6	3.3	0.5	0.4	0.4	2.2	1.4	2.8	3	0.8	6.6	0.3	**0.2**
C=O	196.4	10.6	10.6	9.1	10.1	8.4	8.8	11	10.6	10.9	9.6	**7.3**	7.4	10.3	7.8	10.7	10.8
3"	134.5	13.6	13.6	12	13.2	12.6	13.1	13.6	13.6	14.7	13.3	**11**	**11**	14.2	11.5	13.5	13.5
4"	169.1	8	8	10.5	10	7.2	7.7	8	8.1	**6.3**	7.8	13.5	13.7	8.4	8.4	8.1	8.2
5"	95.5	31.6	31.3	46	47.5	30.5	32	31.3	31.6	37.4	31.6	32.5	32.9	28.8	**26.9**	31.7	32
C=O	201.3	10.3	10.3	**2.7**	6.5	9.1	9.3	10.6	10.3	7.2	8.7	9	8.6	8.5	6.9	10.4	10.4
Total		0	0	3	1	2	2	1	0	1	0	4	2	2	**8**	3	1

Note: numeral in bold: ΔPM3 smallest, closest to the experimental

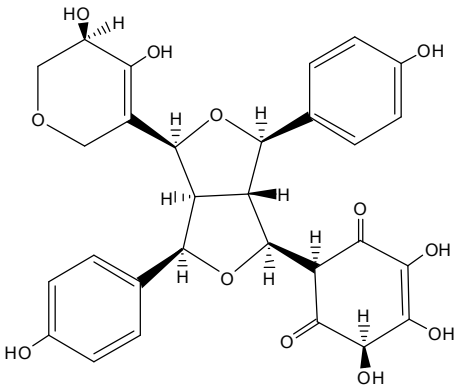

Fig 7. Structure of macronone

Analyses using ^{1}H-NMR and ^{13}C-NMR were performed to elucidate for the structure of isolated compound (Table 1). The ^{1}H-NMR (acetone d6, 500 MHz) data (Fig. 2-4) indicated the presence of 21 H atoms (7 H of hydroxyl were absent). There were signals at δ 7.00, 6.69, 6.76 and 7.09 ppm (doublet, 2H, 8.4 Hz) respectively derived from H2/H2', H3/H3', H5/H5' and H6/H6' of 2 symmetric aromatic rings.

According to COSY Spectrum, the target compound was presumably of 7,9' : 7',9 diepoxylignan as observed that the presence of eight aromatic protons was correlated each other. This is only possible with the deployment of eight protons in 2 symmetrical aromatic should be the same chemical environment. Based on the value of coupling constant, *J*, eighth proton have the same *J* of 8.4 Hz indicated *ortho* coupling, so in *ortho* position each other.

Based on ^{13}C-NMR (aceton-d6, 125 MHz) (Fig. 5) and DEPT analyses, isolated compound contained 29 carbon atoms which consisted of 2 ketone C atom at δ = 201.3 and 196.4 ppm, 8 quarternary C at δ 169.1, 165.2, 157.4, 156.2, 134.5, 132, 129, and 112.1 ppm. Seventeen methine carbons (tertiary carbon) at δ 129.2 (2), 128.0 (2), 115.6 (2), 114.7 (2), 95.5, 77.8, 76.4, 70.3, 60.9, 59.9, 59.1, 51.4, and 43.5 ppm. Methylene carbons (secondary carbon) at δ 105.1 and 78.1 ppm. Primary carbon was not detected in DEPT-135.

Data of ^{1}H-^{13}C HMQC spectrum could provide correlation between protons with the carbon in which it was attached. H2/2' (7.00 ppm) correlated with C2/2' (129.2 ppm), H7/7' (4.87 ppm) with C7/7' (77.8 and 76.4 ppm) and H9' (4.61 ppm) with C9' (59.9 ppm). The

Table 3. Antioxidant activities of ethyl acetate extract, macronone and quercetin using the free radical-scavenging assay (DPPH)

Compounds	Concentrations (µg/mL)	Antioxidant activities (%)	Linear Regression equation	IC_{50} (µg/mL)
Ethyl acetate extract*	50	52.77	-	47.38
Macronone	40	24.38		
	100	30.08	Y = 0. 121 X + 20.815	240.14 ± 4.19
	140	38.29		
	200	51.20		
	400	67.08		
Quercetin	1.2	1.95		
	1.8	17.29		
	2.4	36.79	Y = 19.657 X + 6.9034	2.93 ± 2.12
	3.0	54.21		
	3.6	66.52		

* The measurement of antioxidant activity from ethyl acetate extract was performed only one concentration

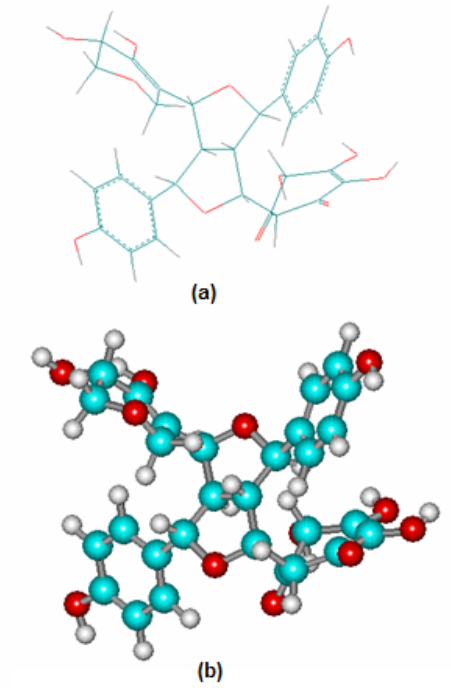

Fig 8. Structure of macronone (Lignan 14 stereoisomer) using PM3 calculation (a) sticks, (b) balls and cylinders

spectrum of ^{1}H-^{1}H COSY represents autocorrelation spectra by connecting the dots signals from protons contained in the spectrum. H2/2' (7.00 ppm) coupled each other with H3/3' (6.69 ppm) and H5/H5' (6.76 ppm). The spectrum of HMBC provided information about the correlation between each proton with the neighboring carbon atom up to 2 bond (^{1}H-C-^{13}C) or 3 bond (^{1}H-CC-^{13}C). H2/2' (7.00 ppm) correlated with C1' (129 ppm), C4 (157.3 ppm) and C7 (77.8 ppm). Like that also H7/7' (4.87 ppm) correlated with the C2/2' (129.2 ppm) and C9 (70.3 ppm). H8' (3.92 ppm) correlated with C7' (76.4 ppm) and C9'(59.9 ppm). (Table 1, Fig. 6).

Data of MS confirmed the existence of core diepoxylignan at m/z of 260, where value of m/z of 77 was the peak of benzene cation. From the spectroscopy analyses UV, IR, H-NMR, C-NMR, NMR 2 dimension and MS, isolated compound was predicted as derivative of diepoxylignan i.e. macronone (2-(4-(4,5-dihydroxy-5,6-dihydro-2H-pyran-3-yl)-3,6-bis(4-hydroxyphenyl)hexahydrofuro[3,4-c]furan-1-yl)-4,5,6-trihydroxycyclohex-4-ene-1,3-dione) with molecular formula of $C_{29}H_{28}O_{12}$ (Fig. 7).

Macronone has nine chiral C atoms so it had many stereoisomers. Analysis using computational chemistry is needed to support the spectroscopy analysis. This compound was calculated with Hyperchem using semiempirical method (AM1 and PM3). Having applied these methods to this compound, semiempirical method of PM3 analysis was closer with experimental data. The possible conformations of macronone were studied and the closest ^{13}C-NMR to experimental data is lignan 14 (Fig. 8, Table 2).

In this research, antioxidant assay using DPPH method showed IC_{50} values of macronone and ethyl acetate extract were 47.38 and 240.14 µg/mL, respectively (Table 3). The IC_{50} value of macronone is higher, 5 times than ethyl acetate extract; it means that antioxidant activity of macronone is lower, 1/5 times than ethyl acetate extract. So, macronone is not a contributor of prominent to antioxidant activity of ethyl acetate extract from bark of *mahkota dewa*.

CONCLUSION

Based on spectroscopy data and supported with computational analysis, a novel compound obtained from ethyl acetate extract of bark of *mahkota dewa* was identified as diepoxylignan derivative and named macronone in the form of red spherical crystal. This compound showed weak antioxidant activity on DPPH with IC_{50} value of 240.14 ± 4.19 µg/mL.

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