ANTIOXIDANT ACTIVITY OF 2,6,4'-TRIHYDROXY-4-METHOXY BENZOPHENONE FROM ETHYL ACETATE EXTRACT OF LEAVES OF MAHKOTA DEWA (*Phaleria macrocarpa* (Scheff.) Boerl.)

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ABSTRACT

Mahkota dewa plant (Phaleria macrocarpa (Scheff.) Boerl.) which is included into family of Thymelaeaceae is one of Indonesia's traditional medicines. Chemical constituent has been isolated from ethyl acetate extract of leaves of mahkota dewa. Sample was extracted with methanol, concentrated then extracted by n-hexane, chloroform and ethyl acetate. The ethyl acetate extract was separated and fractionated by column chromatography. The first fraction was purified by TLC preparative and recrystalization. Compound was isolated as red-brown spherical crystal in 8 mg (m.p. 129-131 °C). Its spot gave dark fluoroscence at TLC plate (UV_{366}) with Rf of 0.3 at TLC chromatogram with eluent of n-hexane : ethyl acetate (7:3); 0.6 with n-hexane : ethyl acetate (1:1); 0.9 with –hexane : ethyl acetate (4:6). This compound was dissolved in methanol. Compound was identified by UV, IR, ^{1}H NMR, ^{13}C NMR and NMR 2 dimension (HMQC, COSY, HMBC and DEPT-135) spectroscopic as 2,6,4'-trihydroxy-4-methoxybenzophenon. This compound as well as the ethyl acetate extract showed antioxidant activity on DPPH with IC_{50} was 10.57 and 101.06 µg/mL, respectively. This compound showed strong antioxidant activity on DPPH, almost to the standard antioxidant activity of quercetin (IC_{50} of 2.93 µg/mL)

Keywords: *Mahkota dewa, Phaleria macrocarpa (Scheff.) Boerl., benzophenone aglucon 2,6,4 '-trihydroxy-4-methoxybenzophenon, DPPH*

INTRODUCTION

Mahkota dewa plant (*Phaleria macrocarpa* (Scheff.) Boerl.*)* a Thymelaeaceae, is much found in Indonesia. Mahkota dewa is classified as plant capable of living in various conditions, from lowland to highland. This plant can be grown in the garden and also in the pot. Its cultivation is easily done in either vegetative or generative processes. Its productivity was able to reach tens of year. This plant has synonym of *Phaleria papuana var warb wichnanmi* (val) Back. Its trade name in English is crown of God. The name of this plant in Sumatra (Malay) and Depok (West Java) is *simalakama*. In Java, it is also called as *makutadewa*, *makuto rajo*, *makuto ratu* or *makuto mewo* [1].

Mahkota dewa fruit is most frequently and empirically utilized by Indonesian various diseases treatment with satisfactory results [2]. Mahkota dewa is one of Indonesia's traditional medicines which have not had complete reference information yet in order to be applied optimally. Information about chemical content of mahkota dewa were limited, mostly only about the bioactivity test (antimicrobial, cytotoxic, pharmacological and antioxidant activity) of the extract or fraction of seeds and fruits that were raw materials of drugs [3]

Chemical contents of mahkota dewa fruit were already known from the literatures. They were icariside C_3 (sesquiterpene glucoside) **(1)**, benzophenone derivative **(2)** from chloroform extract, mangiferin (xanthone glycoside) **(3)** from methanol extract of ripe fruit of mahkota dewa. All compounds were separated with column chromatography silica gel by *Step Gradient Polarity (*SGP) method and continued with purification using HPLC [4]. In addition, it has been isolated as a benzophenone glucoside **(2)** from ethyl acetate extract of red fruit of mahkota dewa separated with column chromatography by SGP method and continued with radial chromatography. The benzophenone glucoside has bioactivity with α, α-diphenyl-β-picrylhydrazyl (DPPH) and P-388 murine cells [5]. Benzophenone glucoside has been also isolated from n-butanol extract of the fruit of mahkota dewa, separated with column chromatography by SGP method and then continued with isocratic method [6]. Lignan **(4)** has been isolated from the ethyl acetate fraction of mahkota dewa fruit and separated with column chromatography by SGP method [7]. Structure

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of isolated compounds from mahkota dewa is given on Fig. 1.

The ethyl acetate extract of mahkota dewa bark which was isolated by silica gel column chromatography and HPLC semipreparative gave benzophenone glucoside of 6,4'-dihydroxy-4-methoxybenzophenone-2-O-β-D-glucopiranoside **(2)** that showed activity as inhibitor of L1210 leukemia cell line [8]. Phalerin (4,5-dihydroxy-4'-methoxybenzophenone-3-O-β-D-glucopirano side) **(5)** from the methanol extract of leaves of mahkota dewa was obtained after separation by chromatographic column with the SGP method and purified by preparative TLC. These compounds had LC_{50} values of small brine shrimp test. Phalerin displayed also cytotoxic activity in uterine cells NS-1 [9]. From the seed of mahkota dewa, mahkoside A (phenolic glycosides new) **(6)**, mangiferin (xanthone glycosides) **(3)** and kaempferol 3-O-β-D-glucoside (flavonoid) **(7)** have been isolated [10]. Benzophenone glucoside has been isolated from *Gnidia invoclurata* which also included into the family Thymelaeaceae **(2)** [11].

This paper reported the isolation and structure elucidation of phenolic compound from the ethyl acetate extract of mahkota dewa leaves 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.

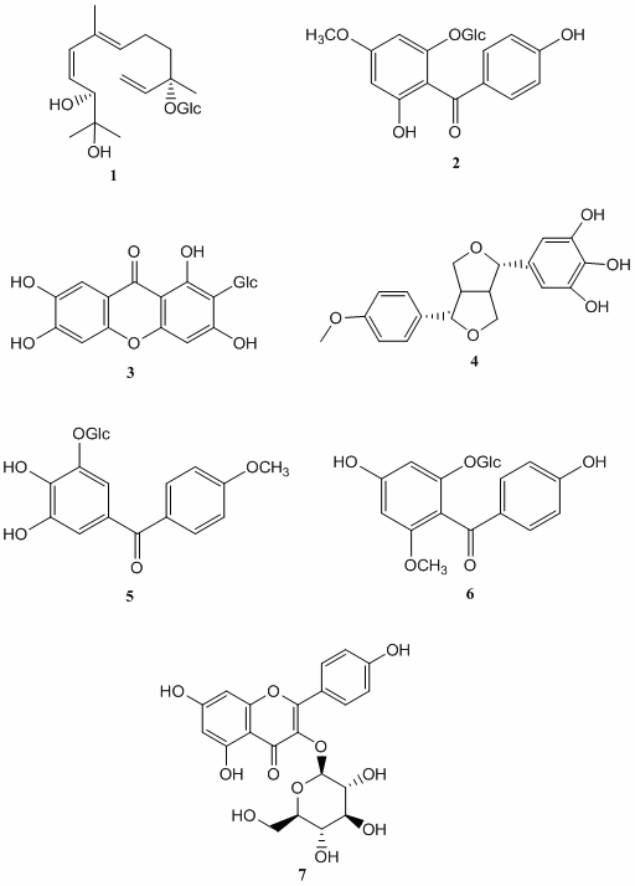

Fig 1. Structure of compounds isolated from mahkota dewa

EXPERIMENTAL SECTION

Materials

Leaves of mahkota dewa were collected from campus of Universitas Gadjah Mada (UGM), Yogyakarta Indonesia in January 2009. The plant was identified by Plant Taxonomy Laboratory, Faculty of Biology, UGM. Chemicals used consisted of 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) $FeCl_3$, Mg powder, HCl, KOH, NH_4OH, dragendorff, acetic anhydride, sulphuric acid, DPPH.

Instrumentation

Melting point apparatus (Electrothermal 9100), UV-vis spectrophotometer UV-vis (Spectronic 3000, Genesis 10), Fourier Transform-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), were using Tetramethyl silane (TMS) as an internal standard. Column chromatography was carried out 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.

Procedure

Extraction, Isolation and Identification

The dried leaves (530 g) of mahkota dewa were extracted using macerator (drip pan) with methanol by heating (60 °C) for 7 h then allowed at room temperature for up to 24 h. The residue was macerated for 3 times and all the filtrates were combined (22 L) and concentrated using vacuum distillation and rotary evaporator. The methanol extract was partitioned with n-hexane-water. Into residue of methanol extract, chloroform was added to give chloroform extract and the residue was added with ethyl acetate to give ethyl acetate extract (25 g). Ethyl acetate extract (18 g) was then fractionated by column chromatography on silica gel using gradient elution (n-hexane, ethyl acetate and methanol) to give 5 fractions. The first fraction consisted of two spots in the plate. TLC preparative

was then performed with eluents of n-hexane : ethyl acetate (3:7) to produce three spots. The spots with the highest Rf value which showed dark fluorescence was scraped, dissolved in chloroform : methanol (1:1), left overnight and filtered. The treatment was repeated once again. The filtrate was formed dirty crystal. It was purified by recrystallization with methanol : water (3:1), washed with chloroform to give red-brown spherical crystal, and washed again with chloroform to obtain pure crystal.

Antioxidant Activity Test

The measurement of antioxidant activity was performed according to a procedure described previously [12]. Quercetin was used as the standard antioxidant sample. DPPH and MeOH were used as the stable free radical reagent and blank, respectively. The sample (isolated compound) was dissolved in MeOH. It was then diluted to achieve concentrations of 18, 16, 14, 12 and 10 µg/mL. As much as 250 µL of each concentration was transferred to different vials, 1 mL DPPH (0.4 mM) and methanol until 5 mL were added to these vials. The absorbance at wavelength 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. At solution of compounds that have antioxidant activity, color solution would turn from violet to yellow. The IC_{50} value was defined as the amount of antioxidant needed to decrease the initial concentration of DPPH by 50%, was calculated from the results and used for comparison. Total antioxidant activity (TAA) was expressed as the percentage inhibition of the DPPH radical and was determined by the following equation:

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

where Abs was the absorbance.

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

RESULT AND DISCUSSION

The ethyl acetate extract of the leaves of mahkota dewa was fractionated by column chromatography. The first fraction was purified by TLC preparative and recrystallization to give benzophenone with molecular formula of $C_{14}H_{12}O_5$, as red-brown spherical crystal in 8 mg (m.p. 129–131 °C). Its spot gave dark fluorescence at TLC plate (UV_{366}) with Rf of 0.3 at TLC chromatogram with eluent of n-hexane : ethyl acetate (7:3); 0.6 with n-hexane : ethyl acetate (1:1); 0.9 with n–hexane : ethyl acetate (4:6). This compound was dissolved in methanol.

The isolated compound was tested with some phytochemical reagents ($FeCl_3$ for phenolic, Shinode (Mg and HCl) and NH_4OH for flavonoid, Lieberman Burchard (acetic anhydride and sulfuric acid) for triterpenoid and steroid, KOH in ethanol for coumarin). The results were positive for $FeCl_3$ and negative for other reagents. This indicated isolated compound was classified to the phenolic compound.

IR spectrum (KBr) of isolated compound showed absorption bands at 3425 and 3232 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 1604, 1512, 1442 and 833 cm^{-1} represented the aromatic system. Characteristic absorption band at 1604 cm^{-1} strongly correlated with C=O functional group. Absorption band at 1064 cm^{-1} came from vibration of aromatic ether [14].

Analyses to elucidate the structure of isolated compound were done by ^{1}H-NMR and ^{13}C-NMR spectrometers (Table 1). The ^{1}H-NMR (CD_3OD 500 MHz) data indicated the presence of 9 H atoms where signals at δ 5.98 ppm (singlet, 2H, *J* = 1.95 Hz) derived from H_3/H_5 of the first aromatic ring. The second ring had proton aromatics symmetrically at δ = 6.7 (doublet-doublet, 2H, *J* = 1.95 Hz) derived from H_3' and H_5'. The signal at δ 7.6 ppm (doublet-doublet, 2H, *J* = 1.95 Hz) derived from H_2' dan H_6' which had the same chemical environment. The signal at δ 3.77 ppm (singlet, 3H) was derived from OCH_3 protons which has the same chemical environment. Proton signals of OH were not appear in the ^{1}H-NMR spectrum. Signal at δ 3.3 ppm (multiplet, 1H) came from the proton it CD_3OD-d4 solvent. The signal at δ 4.87 ppm (doublet, 2H) came from dissolved water in deuterated solvent (CD_3OD-d4).

Based on ^{13}C-NMR (CD_3OD-d4 125 MHz) and Distortionless Enhancement Polarization Transfer (DEPT) analysis, isolated compound contained 14 carbon atoms which consisted of one C=O (C_7), six quarternery C, six CH (methine carbon or tertiary carbon), one CH_3 (methyl carbon/primary carbon) and no methylene carbon (secondary C). The existing of C=O (C_7) at δ 198.64 ppm was supported by UV absorbance at 303 nm indicating the C=O from substituted aromatic ketone.

Chemical shifts at 165.78, 163.13, 161.36 (2), 132.89, and 108.8 ppm were specific for 6 quartenary carbon atoms. Chemical Shifts at 133.09 (2), δ 115.62 (2), δ and 94.40 (2) were specific for 6 methine atom C (tertiary C). Chemical shift at 55.86 ppm was specific for 1 methyl carbon atom (C primary). From the chemical shift of proton and carbon, the CH_3 existed as

Table 1. Data ^{13}C NMR (CD_3OD-d4, 125 MHz) and ^{1}H-NMR (CD_3OD-d4, 500 MHz) of isolated compound and 2,6,4'-trihydroxy-4-methoxybenzophenone (d-Py) [6]

NO. atom C	Compound (CD3OD-d4)		2,6,4'-trihydroxy-4-methoxybenzophenone (d-Py) [6]	
	^{13}C –NMR δ (ppm)	^{1}H –NMR δ (ppm), *J* (Hz)	^{13}C –NMR δ (ppm)	^{1}H –NMR δ (ppm), *J* (Hz)
C=O	198.64	-	198.59	-
1	108.10	-	108.01	-
2/6	161.36	-	161.26	-
3/5	94.40	5.98 (s, 2H)	94.35	5.98 (s)
4	165.78	-	165.69	-
1'	132.89	-	132.79	-
2'/6'	133.09	7.6 (dd, 2H, 1.95)	133.01	7.62 (dd, 2.2)
3'/5'	115.62	6.7 (dd, 2H, 1.95)	115.56	6.78 (dd, 2.2)
4'	163.13	-	163.04	-
OCH_3	55.86	3.77 (s, 3H)	55.79	3.77 (s)
H_2O	-	4.87		-
CD_3OD-d_4	48.9-49.4 (k)	3.33 (m=5)		-

Table 2. Data ^{13}C-NMR (CD_3OD-d_4, 125 MHz) and ^{1}H-NMR (CD_3OD-d_4 500 MHz) and correlation HMQC, COSY and HMBC of isolated compound

No Atom C	^{13}C-NMR δ (ppm)	^{1}H-NMR δ (ppm), *J* (Hz)	HMQC	COSY	HMBC
C=O	198.64	-	-	-	-
1	108.10	-	-	-	-
2/6	161.36	-	-	-	-
3/5	94.40	5.98 (s, 2H)	C3/5	-	C3/5, C1, C2/6, C4 -
4	165.78	-	-	-	-
1'	132.89	-	-	-	C3'/5', C2'/6', C4'
2'/6'	133.09	7.6 (dd, 2H, 1,95)	C2'/6'	H3'/5'	C3'/C5', C2'/C6',C4'C=O
3'/5'	115.62	6.7 (dd, 2H, 1,95)	C3'/5'	H2'/6'	-
4'	163.13	-	-	-	C4
OCH3	55.86	3.77 (s, 3H)	C_{OCH3}	-	C_{CD3OD}
H_2O	-	4.87	-	-	-
CD_3OD-d_4	48.9-49.4 (k)	3.33 (m=5)	-	-	

OCH_3. Solvent of CD_3OD d4 appeared as multiplet from δ 48.93 until 49.43 ppm.

Data ^{1}H-^{13}C HMQC spectrum could provide correlation between protons with the carbon in which it was attached. For example: H_{OCH3} (3.77 ppm) correlated with C_{OCH3} (55.86 ppm), H3/5 (5.98 ppm) correlated with C3/5 (94.40 ppm), H3'/5' (6.7 ppm) correlated with C3'/5' (115.6 ppm) and H2'/6' (7.6 ppm) correlated with C2'/6' (133.09 ppm) (Table 2).

The spectra of ^{1}H-^{1}H COSY represents autocorrelation spectra by connecting the dots signals from protons contained in the spectrum. As in H2'/6' (7.6 ppm) coupled each other with H3'/H5' (6.7 ppm).

HMBC spectrum 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). Example: H3/5 (5.98 ppm) correlated with C3/5 (94.40 ppm), C1 (108.10 ppm), C2/6 (161.36 ppm) and C4 (165.78 ppm). Like that also H2'/6' (7.6 ppm) correlated with the C2'/6' (133.09 ppm), C4' (163.13 ppm) and C3'/5' (115.62 ppm). H_{OCH3} (3.77 ppm) correlated with C4 (165.78 ppm) (Fig 2).

From the spectroscopy analysis it could be ascertained that the isolated compound was 2,6,4'-trihydroxy-4-methoxybenzophenone with molecular formula $C_{14}H_{12}O_5$ (Fig. 3). 2,6,4'-trihydroxy-4-methoxy benzophenone was aglucon of benzophenone glucoside (6,4'-dihydroxy-4-methoxybenzophenone-2-O-β-D-glucopiranoside) **(2)** from fruit and bark of mahkota dewa. Invention of this isolated compound was first on mahkota dewa. Compound of 2,6,4'-trihydroxy-4-methoxybenzophenone reference (Table 1) was not isolated product but hydrolysis product from benzophenone glucoside, isolated compound from fruit of mahkota dewa [6]. This benzophenone aglucon was non polar so that out first on the chromatography column in fraction 1.

Table 3. Antioxidant activities of ethyl acetate extract, 2,6,4'-trihydroxy-4-methoxybenzophenone and quercetin using the free radical-scavenging assay (DPPH)

Compounds	Concentrations (µg/mL)	Antioxidant activities(%)	Liner Regression equation	IC_{50} (µg/mL)
Ethyl acetate extract*	50	24.74	-	101.6
2,6,4'-trihydroxy-4-methoxybenzophenone	10	48.09	Y = 2.2386 X + 26.34	10.57 ± 0.72
	12	53.48		
	14	58.32		
	16	62.56		
	18	65.94		
Quercetin	1.2	1.95	Y = 19.657 X + 6.9034	2.93 ± 2.12
	1.8	17.29		
	2.4	36.79		
	3.0	54.21		
	3.6	66.52		

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

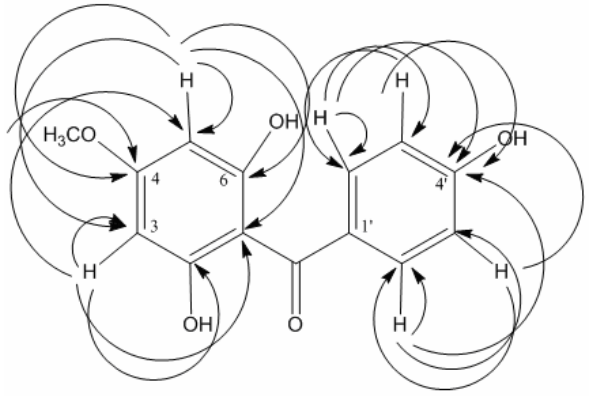

Fig 2. Correlation HMBC of 2,6,4'-trihydroxy-4-methoxy benzophenone

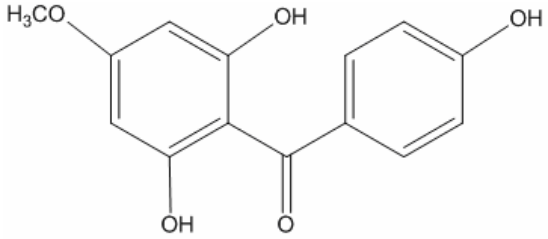

Fig 3. Structure of 2,6,4'-trihydroxy-4-methoxybenzo phenone

2,6,4'-trihydroxy-4-methoxybenzophenone from ethyl acetate extract of mahkota dewa was different with phalerin (4,5,dihydroxy-4'methoxybenzophenone-3-O-β-D-glucopyranoside), isolated compound from leaves of mahkota dewa, previously. Phalerin from methanol extract of leaves of mahkota dewa has different layout groups [9]. In ^{1}H-NMR spectrum of phalerin was appeared singlet signals of H2 and H6, glucose binding at C3, but at H-NMR of this isolated compound was appeared singlet signals of H3 and H5, no binding with glucose (aglucon benzophenone).

Ethyl acetate extract (semi-polar) of leaves of mahkota dewa was produced benzophenone aglucon (non polar), while the methanol extract (polar) of leaves of mahkota dewa was produced benzophenone glucoside, phalerin (polar), this was in accordance with "like dissolves like" principle.

2,6,4'-trihydroxy-4-methoxybenzophenone obtained from the leaves of the mahkota dewa was in 0.0015%. This compound derived from the leaves was more advantageous because the leaves can be obtained throughout the year and the harvesting does not damage the plant.

In this research, 2,6,4'-trihydroxy-4-methoxy benzophenone as well as the ethyl acetate extract showed antioxidant activity on DPPH with IC_{50} were 10.57 and 101.06 µg/mL, respectively. IC_{50} value of 2,6,4'-trihydroxy-4-methoxybenzophenone was lower 10 times than the ethyl acetate extract, or it had higher antioxidant activity 10 times than the ethyl acetate extract. This means that ethyl acetate extract contains antagonist compound that decrease antioxidant activity. 2,6,4'-trihydroxy-4-methoxybenzophenone (IC_{50} 10.57 ± 0.72 µg/mL) could change the colour of DPPH from violet to yellow very fast, almost to the standard antioxidant activity of quercetin (IC_{50} 2.93 ± 2.12 µg/mL) (Table 3).

Antioxidant activity was caused by the presence of phenolic groups that can capture free radical of DPPH. If one of the OH group in this compound was bound to the sugar (example glucose), the OH groups that can capture free radical DPPH was reduced. The antioxidant activity of this compound were far less than it aglucon. Thus, molecule of sugar inhibited antioxidant activity. In the form of aglucon, benzophenone compound have a very strong activity, almost to the standard antioxidant activity of quercetin. When compared with its aglucon, benzophenone glucoside of 6,4'-dihydroxy-4-methoxybenzophenone-2-O-D-glucopyranoside from fruit of mahkota dewa

exhibited the lowest antioxidant activity with IC_{50} 232.4 µg/mL [5].

CONCLUSION

Based on ^{1}H-NMR and ^{13}C-NMR (1D and 2D) spectra and comparison with spectra from previous research, compound obtained from ethyl acetate extract of leaves of mahkota dewa was identified as benzophenone group (2,6,4'-trihydroxy-4-methoxybenzo phenone) in the form of red-brown spherical crystal in 8 mg. This compound showed strong antioxidant activity on DPPH with IC_{50} of 10.57 ± 0.72 µg/mL

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REFERENCES

1. Harmanto, N., 2005, *Mahkota dewa, Gods Heritage Drugs*, 1st ed., Agro Media Pustaka, Jakarta.
2. Sumastuti, R., and Sonlimar M., 2006, Cytotoxic Effect of Fruit and Leaf Extracts *Mahkota dewa [Phaleria macrocarpa (Scheff.) Boerl.] of Cells Hela*, Pharmacology, Faculty of Medicine Universitas Gadjah Mada, Yogyakarta, http://www.tempo.co.id/medika/arsip/122002/art-3.htm, accessed on May 08th 2006.
3. Lisdawati, V., 2007, *Mahkota Dewa Fruit-toxicity, Antioxidant Effects, and Anticancer Effect by Pharmacological Screening Tests*, http://www.mahkotadewa.com/Indo/info/makalah/Vivi201002.htm, accessed on October 14th 2008.
4. Oshimi, S., Zaima, K., Matsuno, Y., Hirasawa, Y., Iizuka, T., Studiawan, H., Indrayanto, G., Zaini, N.C., and Morita, H., 2008, *J. Nat. Med*., 62, 2, 207–210
5. Hakim, R.W., Nawawi, A., Adnyana, I.K., Achmad, S.A., Makmur, L., Hakim, E.H., Sjah, Y.M., and Kitajima, M., 2004, *Bull.* Soc. *Nat. Prod. Chem.*, 4, 67–70.
6. Tambunan R.M., and Simanjuntak, P., 2006, *Indonesian Pharmaceutical Industry Magazine*, 17, 4, 184–189.
7. Lisdawati, V., Wiryowidagdo, S., Kardono, L. and Broto S., 2007, *Buletin Penelitian Kesehatan*, 35, 3, 115–124.
8. Winarno, H., and Katrin W.E, 2009, *Indo. J. Chem.*, 9, 1, 142–145.
9. Hartati, M.S., Mubarika, S, Gandjar, I.G., Hamann, M.T., Rao, K.V., and Wahyuono, S., 2005, *Indo. J. Pharm.*, 16, 1, 51–57.
10. Zhang, Y.B., Yu, X.J., and Liu, H.M., 2006, *J. Asian Nat. Prod. Res.*, 8, 119–123.
11. Ferrari, J., Terreaux, C., Sahpaz, S., Msonthi, J.D., Wolfender, L., and Hostettmann, K., 2000, *J. Phytochem.*, 54, 883–889.
12. Molyneux, P., 2004, *J. Sci. Technol*., 2004, 26, 2, 211–219.
13. Creswell, CJ, Runquist, OA, Campbelll, MM, 2005, *Spectral Analysis of Organic Compounds*, ed 10, ITB, Bandung,
14. Silverstein, R.M., Bassler, G.C., and Morrill, T.C., 2005, *Spectrometric Identification Organic Compounds*, 7th ed., New York, John Wiley and Sons Inc.