TWO ISOPHALERIN COMPOUNDS FROM ETHYL ACETATE OF LEAVE AND FRUIT OF MAHKOTA DEWA (*Phaleria macrocarpa* (Scheff.) Boerl.) AND ITS ANTIBACTERIAL ACTIVITY

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ABSTRACT

*Mahkota dewa plant (Phaleria macrocarpa (Scheff.) Boerl.) which is belong to family of Thymelaeaceae is one of Indonesia's traditional medicines. The aim of this research is to isolate secondary metabolites from ethyl acetate extract of leave and fruit of mahkota dewa and to determine the molecular structure of isolated compounds using spectroscopic method and to know the antibacterial activity of the isolated compound. Sample was extracted with methanol, concentrated then extracted by n-hexane, chloroform and ethyl acetate. The compounds were separated and purified with column chromatography. The compound **1** was isolated from ethyl acetate extract of leave as white needle amorphous solid as 45 mg. The compound was identified by spectroscopic as 4,6-dihydroxy-4'-methoxybenzophenon-2-O-β-D-glucopyranoside and named isophalerin B. From the test results of antibacterial activity showed that the compound **1** (10 mg/mL) in ethanol has a weak activity against the bacteria S. aureus and E. coli. The compound **2** was isolated from ethyl acetate extract of fruit as peach needle crystal as 10 mg. The compound was identified by spectroscopic as 4,6-dihydroxy-4'-methoxybenzophenon-2-O-α-D-glucopyranoside and named isophalerin A.*

Keywords: *mahkota dewa; Phaleria macrocarpa; isophalerin; antibacterial; benzophenon*

ABSTRAK

*Tumbuhan Mahkota dewa (Phaleria macrocarpa (Scheff.) Boerl.) yang termasuk dalam famili Thymelaeaceae adalah salah satu tumbuhan obat tradisional Indonesia. Tujuan penelitian ini adalah untuk mengisolasi metabolit sekunder dari ekstrak etil asetat daun dan buah mahkota dewa dan untuk menentukan struktur molekul senyawa hasil isolasi menggunakan metode spektroskopi serta untuk mengetahui aktivitas antibakteri dari senyawa hasil isolasi. Sampel diekstrak dengan metanol, dipekatkan kemudian diekstrak dengan n-heksana, kloroform dan etil asetat. Senyawa dipisahkan dan dimurnikan dengan kromatografi kolom. Senyawa **1** diisolasi dari ekstrak etil asetat daun sebagai padatan amorf jarum putih sebanyak 45 mg. Senyawa diidentifikasi dengan spektroskopi sebagai 4,6-dihidroksi-4'-metoksibenzofenon-2-O-β-D-glukopiranosida dan dinamai isofalerin B. Hasil test aktivitas antibakteri menunjukkan bahwa senyawa hasil **1** (10 mg/mL) dalam etanol mempunyai aktivitas lemah melawan bakteri Staphylococcus aureus dan Eschericia coli. Senyawa **2** diisolasi dari ekstrak etil asetat buah sebagai kristal jarum krem sebanyak 10 mg. Senyawa diidentifikasi dengan spektroskopi sebagai 4,6-dihidroksi-4'-metoksibenzofenon-2-O-α-D-glukopiranosida dan dinamakan isofalerin A.*

Kata Kunci: *mahkota dewa; Phaleria macrocarpa; isofalerin; antibakteri; benzofenon*

INTRODUCTION

Mahkota dewa plant (*Phaleria macrocarpa* (Scheff.) Boerl.) which is belong to family of 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. 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 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 not yet owned enough information reference in order to be applied optimally. The research on mahkota dewa has been much conducted, mostly about the bioactivity tests (antimicrobial, brine shrimp, cytotoxic, pharmacological

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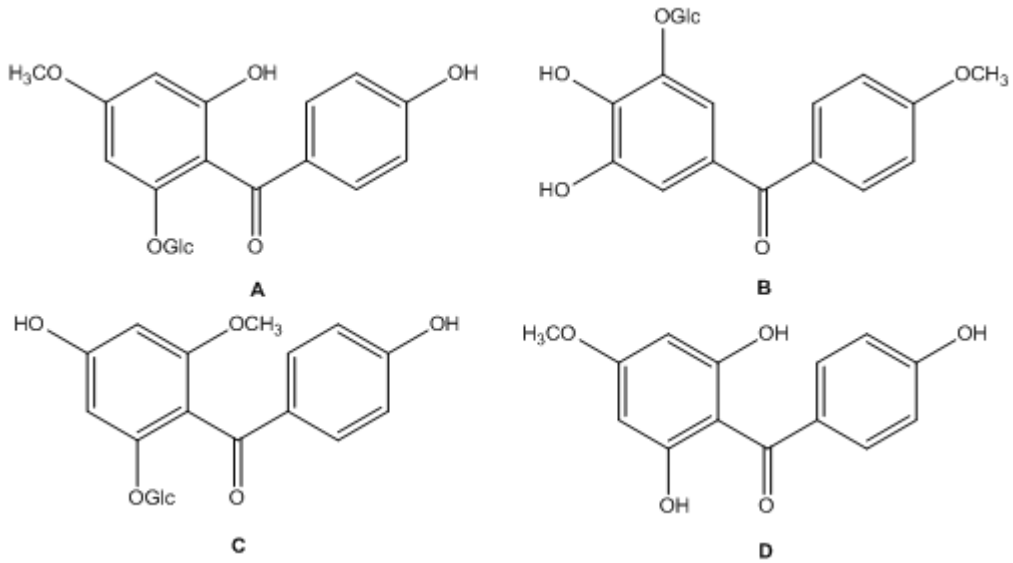

Fig 1. Structure of benzophenone glucopyranosides and benzophenone aglucon were isolated from mahkota dewa

and antioxidant activity) of the extract or fraction of seed and fruit [3].

Mahkota dewa used in treating diseases, among others: pain heart kidney, hypertension, heart disease, diabetes, rheumatism, arthritis and bacterial infectious disease such acne, eczema secondary infections, dysentery, cough and fever. Leaves of mahkota dewa effective as analgesic, antibacterial and antihistamines. Extract of mahkota dewa leaves by tube dilution method has antibacterial activity against *Staphylococcus aureus* [4]. In this research, It has been conducted antibacterial activity assay of isolated compound from ethyl acetate extract of mahkota dewa leaves by agar diffusion method against *Staphyllococcus aureus* and *Escheria coli.*

Chemical contents of mahkota dewa fruit were benzophenone glucoside (6,4'-dihydroxy-4-methoxy benzophenone-2-O-β-D-glucopiranoside) **(A)** from chloroform extract of ripe fruit [5], from ethyl acetate extract of red fruit, from *n*-butanol extract of the fruit of mahkota dewa [6-7]. Compound **(A)** was also isolated from ethyl acetate extract of stem bark of mahkota dewa [8].

Phalerin (4,5-dihydroxy-4'-methoxybenzophenone-3-O-β-D-glucopiranoside) **(B)** from the methanol extract of leave of mahkota dewa was obtained. This compound 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) **(C)** has been isolated [10]. Benzophenone aglycon of compound **(A)** (2,6,4'-trihydroxy-4-methoxy benzophenone **(D)** has been isolated from ethyl acetate extract of leave of mahkota dewa [11]. Structure of benzophenone glucopyranosides and benzophenone aglycon from mahkota dewa is given on Fig. 1. Benzophenone glucoside has been isolated from *Gnidia invoclurata* which also included into the family Thymelaeaceae **(A)** [12].

This paper reported the isolation of phenolic compounds from the ethyl acetate extract of leave and fruit of mahkota dewa and its antibacterial activity. Structural elucidation of isolated compounds was performed by means of spectroscopy analyses. Test of antibacterial activity employed in this research was diffusion method.

EXPERIMENTAL SECTION

Materials

Leave and fruit of mahkota dewa was 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 were consisted of methanol (technical, distilled) and pa (Merck), *n*-hexane (technical, distilled), ethyl acetate (technical, distilled), chloroform pa (Merck), acetone pa (Merck), ethanol pa (Merck), $FeCl_3$ (Merck), Mg powder (Merck), HCl (Merck), KOH (Merck), NH_4OH (Merck), dragendorff, acetic anhydride (Merck), sulfuric acid (Merck), ethanol 70%, Nutrient Agar (NA) media 20 g/L, physiologist NaCl, bacteria of gram positive *S. aureus* and bacteria of gram negative *E. coli*.

Instrumentation

Melting point apparatus (Electrothermal 9100), UV-vis spectrophotometer UV-vis (Spectronic 3000), Fourier Transformation-Infra Red (FT-IR) spectrophoto

meter (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. 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), Autoclave Omron Auto type STMN-Y222, Petri dish, test tubes, beaker glass, discs of paper, tweezers, needle ose, aseptic cabinet, magnetic stirrer, spectronic 21.

Procedure

Extraction, isolation and identification

The dried leave (0.5 kg) of mahkota dewa was extracted using macerator (drip pan) with methanol (6.5 L) by heating (60 °C) for 7 h then stand up overnight at room temperature. The residue was macerated again 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 (6 x 320 mL) to give *n*-hexane extract. Residue of extract methanol was partitioned with chloroform (10 x 640 mL) to give chloroform extract. Residue was partitioned again with ethyl acetate (13 x 200 mL) to give ethyl acetate extract (25 g). Ethyl acetate extract (18 g) was then separated by column chromatography on silica gel using gradient elution (*n*-hexane, ethyl acetate and methanol) to give 5 fractions. The fourth fraction was recrystallized to give white needle amorphous solid (Compound **1**).

The dried fruit (0.5 kg) of mahkota dewa was extracted using macerator (drip pan) with methanol (4.6 L) by heating (60 °C) for 7 h then stand up overnight at room temperature. The residue was macerated again for 3 times and all the filtrates were combined (12.5 L) and concentrated using vacuum distillation and rotary evaporator. The methanol extract was partitioned with *n*-hexane (10 x 600 mL) to give *n*-hexane extract. Residue of extract methanol was partitioned with chloroform (4 x 450 mL) to give chloroform extract. The residue was fractionated with ethyl acetate (7 x 300 mL) to give ethyl acetate extract (6 g). Ethyl acetate extract (2.5 g) was then separated by column chromatography on silica gel using gradient elution (*n*-hexane, ethyl acetate and methanol) to give 11 fractions. The first fraction was refractionated by column chromatography to give 5 fraction. The fifth fraction was recrystallized to give peach needle crystal solid (Compound **2**).

Antibacterial activity

The antibacterial activity was carried out by employing 24h cultures of *Staphylococcus aureus* and *Escherichia coli*. Activity of isolated compound and extract was tested separately using agar diffusion method [13]. All the tools, the media were sterilized in an autoclave at a temperature of 121 °C, pressure 1 atm, for 15 min. Test bacteria were rejuvenated from stock pure cultures by growing on italics NA agar and then incubated for 18-24 h at 37 °C. Preparation of test bacterial suspension on adolescent culture stock by taking it with the needle of ose and suspended in physiological NaCl solution and then vortexed until homogeneous. Then the turbidity is measured to obtain 25% T at 580 nm for bacteria with spectronic-21. Concentration of the isolated compound was 10 mg/mL ethanol, while ciprofloxacin was 1 mg/mL. Each test bacteria (*S. aureus*, *E. coli*) was taken 0.1 mL by pipette of cooked, placed in the middle petri dish. Then the agar media that was still liquid (50-55 °C) poured into petri dish as 15 mL, shaken until a homogeneous mix with the test bacterial suspension and then allowed to solidification. Then 10 μL solution of the isolated compound (10 mg/mL) was placed on paper discs using a micro pipette and grown in media seeding. Incubation for 24 h at 37 °C in an incubator in the inverted position. The reading of a positive result if around the paper disc there are areas transparent without growth of bacteria. The inhibition area measured with a shove. As a control used sterile paper disc that has been poured solvent [14].

RESULT AND DISCUSSION

The ethyl acetate extract of the leave of mahkota dewa was fractionated by column chromatography. The fraction containing the major component was purified by recrystallization to give compound **1** with white needle amorphous solid as 45 mg (burning point 187-195 °C). Its spot gave dark fluorosence at TLC plate (UV_{366}) with Rf of 0.65 at TLC chromatogram with eluent of ethyl acetate; 0.80 with ethyl acetate : methanol (7:3); 0.85 with ethyl acetate : methanol (5:5). The compound was dissolved in methanol.

The ethyl acetate extract of the fruit of mahkota dewa was fractionated by column chromatography. The fraction 1.5 was purified by recrystallization to give compound **2** with peach needle crystal solid as 10 mg. Its spot gave dark fluorosence at TLC plate (UV_{366}) with Rf of 0.4 at TLC chromatogram with eluent of *n*-hexane : ethyl acetate; 0.90 with ethyl acetate. The compound was dissolved in methanol.

Compound **1** and **2** were tested with some chemical reagents ($FeCl_3$ for phenolic, Shinode (Mg and HCl) and NH_4OH for flavonoid, Lieberman Burchard (acetic anhydric, sulfuric acid) for triterpenoid and steroid, KOH in ethanol for coumarin). The results were positive for $FeCl_3$ (blue-black) and negative for

Table 1. Data ^{13}C-NMR (CD_3OD, 125 MHz) and ^{1}H-NMR (CD_3OD, 500 MHz) of compound **1** and benzophenone glucoside [5]

No. atom C	*Compound 1 (CD_3OD-d_4)*		*Benzophenone glucoside) [5]*	
	^{13}C-NMR δ (ppm)	^{1}H-NMR δ (ppm), *J* (Hz)	^{13}C-NMR δ (ppm)	^{1}H-NMR δ (ppm), *J* (Hz)
C1	111.8	-	112.4	-
C2	158.6	-	158.9	-
C3	96.8	**6.17** (d, 1H, 2.0)	97.0	**6.38** (d, 1H, 2.0)
C4	164.3	-	163.9	-
C5	94.9	**6.40** (d, 1H, 2.0)	94.8	**6.16** (d, 1H, 2.0)
C6	159.1	-	158.3	-
C7	197.2	-	196.7	-
C1'	132.0	-	129.3	-
C2'/6'	133.7	7.69 (dd, 2H, 8.2 2.0)	134.0	7.65 (d, 2H, 8.7)
C3'/5'	116.0	6.79 (dd, 2H, 8.2 2.0)	117.5	6.68 (d, 2H, 8.7)
C4'	163.9	-	169.0	-
C1"	102.6	4.87 (d, 1H, 12.4)	102.7	4.91 (d, 1H, 7.8)
C2"	74.9	3.31 (t, 1H)	74.8	3.85 (dd, 2H)
C3"	77.9	3.37 (t, 2H)	77.8	3.38 (m, 2H)
C4"	71.3	3.25 (t, 1H)	71.3	3.25 (dd, 1H)
C5"	78.4	3.37 (t, 2H)	78.4	3.38 (m, 2H)
C6"	62.7	3.84 (dd, 1H)	62.6	3.85 (dd, 2H)
		3.64 (k, 1H)		3.64 (dd, 1H)
OCH_3	56.0	3.81 (s, 3H)	55.9	3.80 (s, 3H)
OH	-	-	-	-
CD_3OD	49.1(t)	3.3 (k)	-	-
H_2O	-	4.87	-	-

other reagents. This indicated compound **1** and **2** were classified to the phenolic compound.

$UV_{(MeOH)}$ spectrum of compound **1** and **2** showed maximum absorbances at λ 210, 290 and 210, 293 nm, respectively. Absorbance at 210 nm was peak of the methanol solvent, absorbance at 290/293 nm described the system was substituted aromatic ketone. Compound **1** could be considered as major constituent in the leave of mahkota dewa, because the methanol extract of the leave has also absorbance in almost the same region. There was wavelength 207 and 286 nm.

IR spectrum $_{(KBr)}$ of compound **1** showed absorption bands at 3363 cm^{-1} and 3209 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 1504 and 1442 cm^{-1} indicated the present of C=C in the aromatic system. Strong absorption bands at 1604 and 1278 cm^{-1} represented group of hydroxyl aryl ketone or aromatic ketone. Characteristic absorption band at 1651 cm^{-1} strongly correlated with C=O group. Strong absorption band at 1087 cm^{-1} came from the vibration of ether [15].

Analyses to elucidate for the structure of compound **1** were done by ^{1}H-NMR and ^{13}C-NMR spectrometers (Table 1, Fig. 2 and Fig. 3). The ^{1}H-NMR (CD_3OD 500 MHz) data indicated the presence of 16 H atoms where there were signals at δ 6.17 and 6.40 ppm (doublet, 1H, *J* = 2.0 Hz) which respectively derived from H3 and H5 of the first aromatic ring. H3 and H5 were in meta position each other. The second ring had proton aromatics symmetrically at δ 7.69 and 6.79 (doublet-doublet, 2H, *J* = 8.2 and 2.0 Hz) which respectively derived from H2'/H6' and H3'/H5' which had the same chemical environment.

Protons of glucose were indicated by signal at δ 4.87 ppm (doublet, 1H, *J* = 12.4 Hz which came from H1", whose signal was closed by H_2O proton signal. Chemical shift at δ 3.31 ppm (triplet, 1H) was derived from the H2" and the signal at δ 3.25 ppm (triplet, 1H) was derived from H4". Signal at δ 3.37 ppm (triplet, 2H) derived from the H3" and H5". Doublet-doublet signal at δ 3.84 ppm (1H) was derived from H6"a. Multiplet signal at δ 3.64 ppm (1H) was derived from H6"b. Furthermore the value *J* H (12.4 Hz) between H1" and H2" indicated that the two protons coupling was axial-axial (β) as sugar molecules tends to form a stable conformation in which the OH groups were nearly equatorial. The structure was projected upwards anomeric OH (cis to the CH_2OH end) called β-anomer, thus bonding the sugar in this compound **1** was β-glucoside [16].

The signal at δ 3.81 ppm (singlet, 3H) was derived from OCH_3 protons which has the same chemical environment. Proton signal of 6OH does not appear in the ^{1}H-NMR spectrum. Signal at δ 3.3 ppm (multiplet, 1H) came from the proton it CD_3OD-d_4 solvent. The signal at δ 4.87 ppm (doublet, 2H) came from dissolved water in deuterated solvent CD_3OD-d_4.

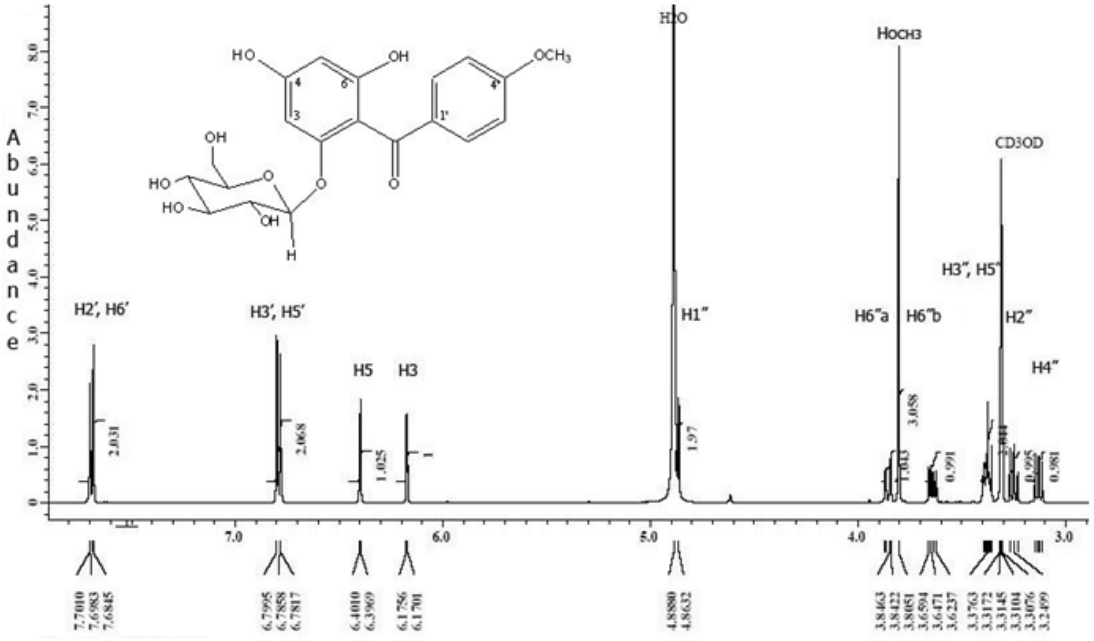

Fig 2. ^{1}H-NMR spectrum of compound **1** (CD_3OD-d_4, 500 MHz)

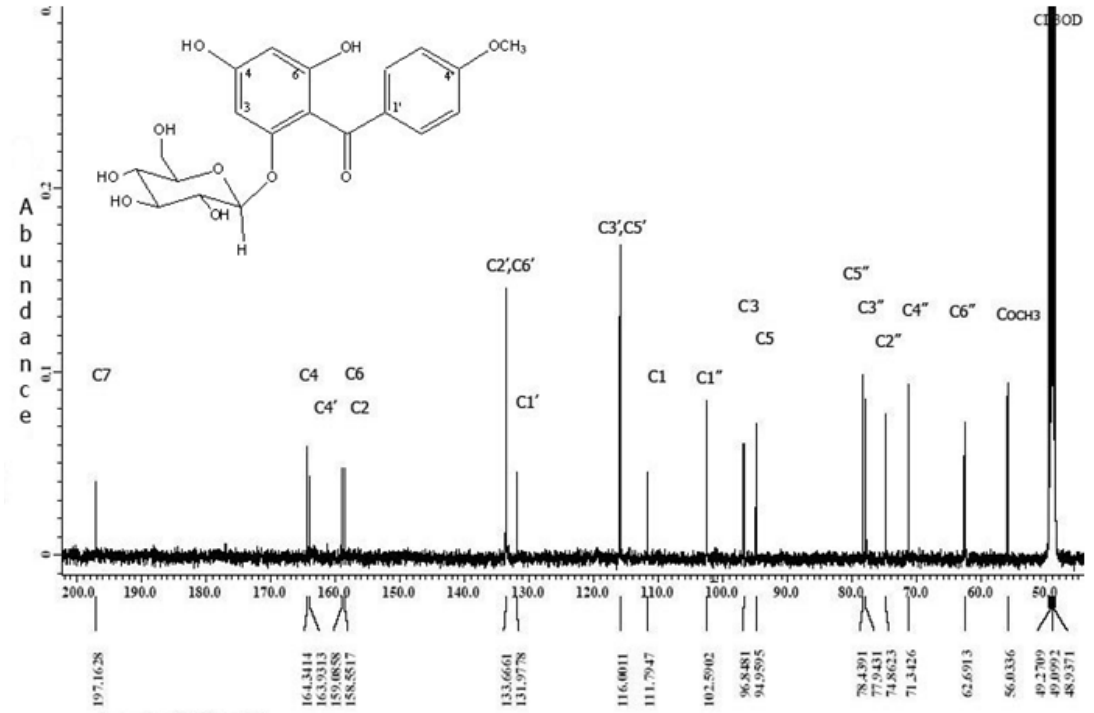

Fig 3. ^{13}C-NMR spectrum of compound **1** (CD_3OD-d_4, 125 MHz)

Based on ^{13}C-NMR (CD_3OD 125 MHz) and Distortionless Enhancement Polarization Transfer (DEPT) analysis, isolated compound contained 20 carbon atoms which consisted of one C=O (C_7), six quaternary C, eleven CH (methine carbon or tertiary C), one methylene carbon (secondary C) and one CH_3 (methyl carbon/ primary C). The exist of C=O (C_7) at δ 197.2 ppm was supported by IR peak at 1651, 1604 cm^{-1} and UV absorbance at λ 290 nm indicating the C=O from substituted aromatic ketone.

Chemical shifts at 164.3, 163.9, 159.1, 158.6 132.0 and 111.8 ppm were specific for 6 quaternary carbon atoms. Chemical Shifts at 133.7 (2), δ 116.0 (2), δ 96.8 and δ 95.0 ppm were specific for 6 methine

Table 2. Data ^{13}C-NMR (CD_3OD, 125 MHz) and ^{1}H-NMR (CD_3OD, 500 MHz) of compound **1** and compound **2**

No. atom C	*Compound 1 (CD_3OD-d_4)*		*Compound 2 (CD_3OD-d_4)*	
	^{13}C-NMR δ (ppm)	^{1}H-NMR δ (ppm), *J* (Hz)	^{13}C-NMR δ (ppm)	^{1}H-NMR δ (ppm), *J* (Hz)
C1	111.8	-	111.8	-
C2	158.6	-	158.6	-
C3	96.8	6.17 (d, 1H, 2.0)	96.9	6.17 (d, 1H, 2.0)
C4	164.3	-	164.3	-
C5	94.9	6.40 (d, 1H, 2.0)	95.0	6.40 (d, 1H, 2.0)
C6	159.1	-	159.1	-
C7	197.2	-	197.2	-
C1’	132.0	-	132.0	-
C2’/6’	133.7	7.69 (dd, 2H, 8.2 2.0)	133.6	7.69 (dd, 2H, 8.5, 2.5)
C3’/5’	116.0	6.79 (dd, 2H, 8.2 2.0)	116.0	6.79 (dd, 2H, 8.5 2.5)
C4’	163.9	-	163.9	-
C1”	102.6	4.87 (d, **1H, 12.4**)	102.6	4.87 (d, **1H, 2.8**)
C2”	74.9	3.31 (t, 1H)	74.9	3.13 (t, 1H)
C3”	77.9	3.37 (t, 2H)	77.9	3.37 (t, 2H)
C4”	71.3	3.25 (t, 1H)	71.3	3.24 (t, 1H)
C5”	78.4	3.37 (t, 2H)	78.4	3.37 (t, 2H)
C6”	62.7	3.84 (dd, 1H)	62.7	3.85 (dd, 1H)
		3.64 (k, 1H)		3.64 (k, 1H)
OCH_3	56.0	3.81 (s, 3H)	56.0	3.80 (s, 3H)
OH	-	-	-	-
CD_3OD	49.1(t)	3.3 (k)	49.1	3.3 (k)
H_2O	-	4.87	-	4.91

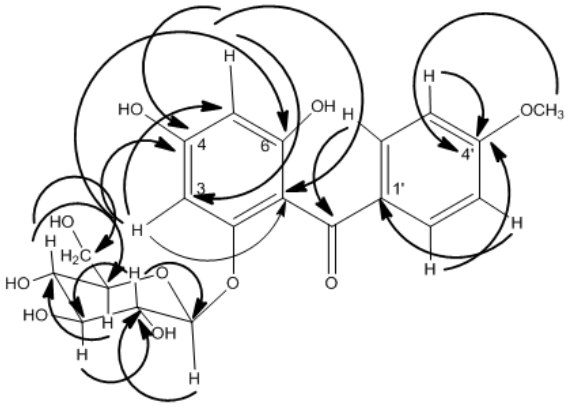

Fig 4. Correlation HMBC of isophalerin B

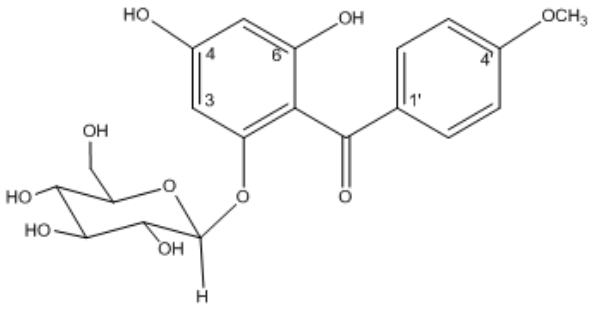

Fig 5. Structure of isophalerin B

carbon atom (tertiary C). Chemical shifts at 102.6, δ 78.4, δ 77.9, δ 74.9, δ 71.3 and δ 62.7 ppm represented 6 methine and methylene carbon atoms specific for glucoside moiety. Chemical shift at 56.0 ppm was specific for 1 methyl carbon atom (C primary). Solvent of CD_3OD-d4 appeared as triplet at δ 49.6, 49.4 and 49.2 ppm.

Data ^{1}H-^{13}C HMQC spectrum could provide correlation between proton with the carbon which it was attached. Example: H_{OCH3} (3.81ppm) correlated with C_{OCH3} (56.0 ppm), H2” (3.13 ppm) correlated with C2” (74.9 ppm) and so on (Table 3).

The spectrum of ^{1}H-^{1}H COSY represents autocorrelation spectra by connecting the dots signals from protons contained in the spectra. As H3 (6.17 ppm) coupled each other with H5 (6.40 ppm) and H2’/6’ (7.69 ppm) coupled each other also with H3’/5’ (6.79 ppm).

HMBC spectrum provided information about the correlation between each proton with the neighboring carbon atom to 2 bond (^{1}H-C-^{13}C) or 3 bond (^{1}H-CC-^{13}C). Example: H5 (6.40 ppm) correlated with C3 (96.8 ppm), C1 (111.8 ppm), C2 (158.6 ppm) and C4 (164.3 ppm). Like that also H2’/6’ (7.69 ppm) correlated with the C2’/6’ (133.7 ppm), C4’ (163.9 ppm) and C7 (197.2 ppm). H3” (3.37 ppm) correlated with C4” (71.3) and C2’ (74.9 ppm).

From the spectroscopy analysis it could be ascertained that the compound **1** was isophalerin B or 4,6-dihydroxy-4’-methoxybenzophenone-2-O-β-D-gluco pyranoside with molecular formula $C_{20}H_{22}O_{10}$ (Fig. 5). Benzophenone glucoside was more polar from its aglucon, this is caused by a number of OH groups

Table 3. Data ^{13}C-NMR (CD_3OD 125 MHz) and ^{1}H-NMR (CD_3OD 500 MHz) and correlation HMQC, COSY and HMBC compound **1**

No. Atom C	^{13}C-NMR δ (ppm)	^{1}H-NMR δ (ppm), *J* (Hz)	HM QC	COSY	HMBC
C1	111.8	-	-	-	-
C2	158.6	-	-	-	-
C3	96.8	6.17 (d, 1H, 2.0)	C3	H5	C5, C1,C6, C4
C4	164.3	-	-	-	-
C5	94.9	6.40 (d, 1H, 2.0)	C5	H3	C3, C1, C2, C4
C6	159.1	-	-	-	-
C7	197.2	-	-	-	-
C1'	132.0	-	-	-	-
C2'/6'	133.7	7.69 (dd, 2H, 8.2 2.0)	C2'/C6'	H3'/H5'	C2'/6', C4', C7
C3'/5'	116.0	6.79 (dd, 2H, 8.2 2.0)	C3'/C5'	H2'/6'	C3'/5', C1', C4'
C4'	163.9	-	-	-	-
C1"	102.6	4.87 (d, 2H, 12.4)	C1"	H2"	C2
C2"	74.9	3.31 (t, 1H)	C2"	H4",H3"	C3", C1"
C3"	77.9	3.37 (t, 2H)	C3"	H2", H6"	C4", C2"
C4"	71.3	3.25 (t, 1H)	C4"	H2"	C6", C5"
C5"	78.4	3.37 (t, 2H)	C5"	H6a", H6"a	C4", C2"
C6"	62.7	3.84 (dd, 1H)	C6"	H6"b	-
		3.64 (k, 1H)		H3",H6"a	C_{OCH3}
OCH_3	56.0	3,81 (s, 3H)	C_{OCH3}	-	**C4'**
OH	-	-	-		
CD_3OD	49.1(t)	3.3 (k)	C_{CD3OD}	-	-
H_2O	-	4.87	-		-

Table 4. Measured result of growth inhibition diameter of test bacteria by extract and Compound **1** in ethanol

No.	Sample (in ethanol)	d growth inhibition of bacteria (mm)	
		Stapyllococcus aureus	*Escheria coli*
1.	Blank	6.0	6.0
2.	Crude methanol extract	9.0	10.0
3.	Ethyl acetate extract	8.0	7.0
4.	Compound 1	7.3	7.2
5.	Ciprofloxacin	18.0	18.0

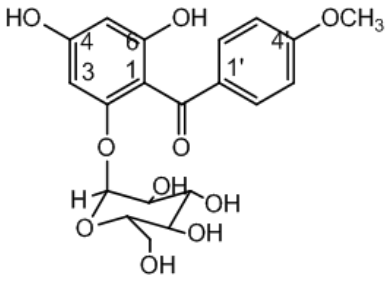

Fig 6. Structure of isophalerin A

contained in glucose.

Value of chemical shift of compound **2** at ^{1}H and ^{13}C-NMR very similar with isophalerin B from leave of mahkota dewa, except value of *J* proton anomeric of its sugar is different by significantly (Table 2). Proton anomeric of glucose shown by signal at δ 4.87 ppm (d, 1H, *J* = 2.8 Hz),. Value of *J* H (0-5 Hz) between H1" and H2" showing 2 proton which coupling equatorial-equatorial (α) as sugar molecule having conformation form which OH group at axial position. Structure projecting OH anomeric group downwards (trans to CH_2OH end) referred α-anomer, so that sugar bending at this compound is α-glucoside (Silverstein, et al., 2005). Based on spectroscopy data analysis contended that compound **2** is isophalerin A with sugar bending at this compound is α-glucoside. This compound is isomer of isophalerin B. Structure of isophalerin A presented at Fig. 6.

From the results of testing the antibacterial activity of compound **1** by agar diffusion method showed that this compound had activity against bacteria used. Diameter of inhibition of compound **1** against bacteria (average of 3 times measurements) in ethanol showed at Table 4.

Tests for antibacterial activity used 2 types of bacteria that represent the class of bacteria of gram positive and negative. From the test results of antibacterial activity from leave of mahkota dewa (Table 4) shows that crude methanol extract, ethyl acetate extract and the compound 1 (10 mg/mL) in ethanol has a weak activity against the bacteria *S. aureus* and *E. coli*. This happened because compound **1** is compound of polar (dissolve in methanol/ethanol) so it does not easy to penetrate to cell membrane of bacterium which having character of non polar. So

activity of compound **1** against both the bacteria is weak. Compound of bactericide come from compound of phenol will have big activity if the compound more dissolves in fat or less dissolves in water.

Antibacterial activity category to be 'weak' if it has a 7-11 mm inhibition zone and 'medium' if it has a 12-16 mm inhibition zone with diameter of discs 6 mm [17-18]. The presence of ciprofloxacin antibiotic aims to demonstrate the compound model has a patented that has very strong antibacterial activity. Ciprofloxacin is a class of antibiotics with broad spectrum, so it active against bacteria of gram positive and negative

Activity test of antibacterial to Compound **2** cannot be done because this compound obtained only little. But this compound estimated will have weak activity like at isophalerin B.

CONCLUSION

The compound **1** obtained from ethyl acetate extract of leave of mahkota dewa was identified and named as isophalerin B (4,6,-dihydroxy-4'-methoxybenzophenone-2-O-β-D-glucopiranoside) in the form of white needle amorphous as 45 mg. This compound showed weak of antibacterial activity against *S. aureus* and *E. Coli* on Diffusion agar of method. The compound **2** obtained from ethyl acetate extract of fruit of mahkota dewa was identified and named as isophalerin A (4,6,-dihydroxy-4'-methoxybenzophenone-2-O-α-D-glucopiranoside) in the form of peach needle crystal as 10 mg.

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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, 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. Suryani, L., and Stepriyani, S., 2007, *Mutiara Medika*, 7(1), 23–28.
5. 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.
6. 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.
7. Tambunan R.M., and Simanjuntak, P., 2006, *Indones. J. Pharm.*, 17(4), 184–189.
8. Winarno, H., and Katrin, W.E, 2009, *Indones. 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, *Indones. J. Pharm.*, 16(1), 51–57.
10. Zhang, Y.B., Yu, X.J., and Liu H.M., 2006, *J. Asian Nat. Prod. Res.*, 8(1-2), 119–123.
11. Susilawati, Matsjeh, S., Pranowo, H. D., and Anwar, C., 2011, *Indones. J. Chem.*, 11(2), 180–185.
12. Ferrari, J., Terreaux, C., Sahpaz, S., Msonthi, J.D., Wolfender, L., and Hostettmann, K., 2000, *Phytochemistry*, 54(8), 883–889.
13. Ramakrishnan, G., Kothai R., Jaykar B., and Rathnakumar, T.V., 2011, *Int. J. PharmTech. Res.*, 3(2), 1000–1004.
14. Dey, P.M., and Harborne, J.B., 1991, *Methods in Plant Biochemistry: Assay for Bioactivity*, Vol. 6, Academic Press Limited, London.
15. Creswell, C.J., Runquist, O.A., and Campbelll, M.M., 2005, *Spectral Analysis of Organic Compounds*, 10th ed., ITB, Bandung.
16. Silverstein, R.M., Bassler, G.C., and Morrill, T.C., 2005. *Spectrometric Identification Organic Compounds*, 7th ed., John Wiley and Sons Inc., New York.
17. Ontengco, D.C., and Dayap, L.A., 1996, Invitro Antibacterial Activity of Rind Essential Oil of *Citrus Grandis* (L) Osbeck vs Common Clinical Pathogen, *Proceeding ASOMPS VIII*, 10-13 June 1996, Melaka, 231–235.
18. Mattana, C.M., Satorres, S.E., Sosa, A., Fusco, M., and Alcará L.E., 2010, *Braz. J. Microbiol.*, 41(3), 581–587.