

ISOLATION OF SECONDARY METABOLITE COMPOUNDS FROM *Elaeocarpus mastersii* KING STEM BARK AND THEIR BIOLOGICAL ACTIVITY EVALUATION

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ABSTRACT

In this research, isolation of secondary metabolites of *Elaeocarpus mastersii* King stem bark was conducted by column chromatography and pure compounds were characterized by the IR, UV, and NMR spectra. The biological activity of the isolated compounds as antidiabetic was determined based on the α -glucosidase inhibition. The investigation led to isolate β -sitosterol (1), β -sitosterol glucoside (2), and cucurbitacin F(3). The evaluation of the α -glucosidase inhibitory assay showed that the isolated compounds had different inhibition performances caused by the interaction distinction with the active site of the enzyme. These results perform that *Elaeocarpus mastersii* King contained several types of secondary metabolite compounds and had the potential to be used as medicine.

Keywords: Cucurbitacin F, *Elaeocarpus mastersii* King, β -sitosterol, β -sitosterol Glucoside, α -glucosidase Inhibitory Activity

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INTRODUCTION

The development of research in plant science is increasing continuously, most plant species are used for medicinal uses. WHO (World Health Organization) has recorded more than 21,000 names of plants that have been reported as medical treatments worldwide.¹ The utilization of plants as traditional medicine is caused by the active compounds contained in the plants. These compounds are classes of secondary metabolite compounds. Most of these active compounds have been isolated from medicinal plants which have interesting activities such as 3-methyl-1H-benz[f]indole-4,9-dione², sentulic acid³, and clerodermic acid⁴. *Elaeocarpus mastersii* King (Elaeocarpaceae) is the endemic Riau (Sumatra) plant. Its bark is used for hypertensive and diabetic treatment. As far as our literature study of *E. mastersii* King, only six compounds have been successfully isolated from this plant, such as two antocyanins⁵, two cucurbitacins, and two ellagic acid derivatives.⁶ Several species of this genus show the biological activities as cytotoxic⁶, antioxidant⁷, and antibabesial activity.⁸ These activities are related to the various organic compounds of *Elaeocarpus* genus plants such as flavonoids⁷, ellagic acid derivatives⁸, alkaloids⁹⁻¹², terpenoids¹³⁻¹⁶, and the other aromatic compounds.¹⁷ As a part of our research of *E. mastersii* King^{18,19}, we proved the constituents of the stem bark from *E. mastersii* King and investigated their biological activity to inhibit the α -glucosidase enzyme.

EXPERIMENTAL

General Experimental Procedure

Si gel column chromatography and Thin-layer chromatography (TLC) were provided by Merck 60 (70-230 or 230-400 mesh) and Merck 60 GF₂₅₄ (0.25 mm). Fractionations were obtained from TLC analyses. NMR spectrums were afforded by the spectrometer of JEOL JNM-ECS 400 using tetramethylsilane (TMS) as an internal standard.

Plant Collection and Extraction

Plant of *E. mastersii* King was identified at Andalas Herbarium. Stem bark was collected in the Riau Province, Sumatra-Indonesia. The dried stem bark (13.5 Kg) was ground and extracted at room

temperature by maceration method using the step gradient polarity of hexane, ethyl acetate (EtOAc), and methanol (MeOH) solvent. The solvents were evaporated by the rotary evaporator to obtain the crude extracts of each fraction.

Isolation

The ethyl acetate extract (80 g) was separated by Si gel column chromatography with the gradient mixture of hexane-EtOAc-acetone-MeOH affording 11 fractions. Fraction 8 dissolved by dichloromethane (DCM) (1.42 g) was purified by Si gel column chromatography eluted with hexane-DCM-EtOAc-MeOH and obtained 16 fractions (8₁₋₁₆). Compound **1** (± 6 mg) was isolated from fraction 8₁₁ using Si gel column chromatography (with DCM-EtOAc as eluent) and was recrystallized by MeOH. Fraction 8₁₆ was subjected for further purification using Si gel column chromatography eluted with hexane-DCM-EtOAc-MeOH and continued by trituration method using EtOAc 100% to obtain compound **3** (± 6 mg). Compound **2** (± 30 mg) was gained from fraction 9 using the trituration method with EtOAc-MeOH solvent.

α -Glucosidase Inhibitory Activity

The α -glucosidase inhibitory activity was investigated by the method of Sancheti et al²⁰ with slight modifications. The sample was dissolved in DMSO solution. The sample solution of 10 μ L was mixed with 50 μ L of phosphate buffer (100 mM, pH 7.0), 25 μ L of 0.5 mM 4-nitrophenyl α -D-glucopyranoside (dissolved in 100 mM phosphate buffer, pH 7.0), and 25 μ L of 0.04 Units mL⁻¹ α -glucosidase solution obtained by the dilution of a stock solution (1000 μ g/mL) with phosphate buffer (10 mM, pH 7.0). The mixture was incubated at 37°C for 30 minutes. The addition of 100 μ L sodium carbonate solution (200 mM) was used after the incubation process to terminate the enzymatic reaction. The absorbance was measured at 410 nm as the amount of p-nitrophenol released in the reaction.

RESULTS AND DISCUSSION

β -sitosterol (1)

This compound had molecular formula of C₂₉H₅₀O, white powder with a melting point of 133-134°C. UV (MeOH) $\lambda_{\max}$ nm: 210. IR $\nu_{\max}$ (KBR) cm⁻¹: 3341 ($\nu_{\max}$, OH), 2938 ($\nu_{\max}$, C-H), 1658 ($\nu_{\max}$, C=C), 1040 ($\nu_{\max}$, C-O). ¹H NMR (500 MHz, CDCl₃) and ¹³C NMR (125 Mhz, CDCl₃) spectroscopic data, see Table-1.

β -sitosterol glucoside (2)

This compound had molecular formula of C₃₅H₆₀O₆, white powder with a melting point of 286-288°C. UV (MeOH) $\lambda_{\max}$ nm: 210. IR $\nu_{\max}$ (KBR) cm⁻¹: 3386 ($\nu_{\max}$, OH), 2936 ($\nu_{\max}$, C-H), 1672 ($\nu_{\max}$, C=C), 1027 ($\nu_{\max}$, C-O). ¹H NMR (500 MHz, DMSO-D₆) and ¹³C NMR (125 MHz, DMSO-D₆) spectroscopic data, see Table-1.

Cucurbitacin F (3)

This compound had the molecular formula of C₃₀H₄₆O₇, white powder with a melting point of 190-192°C. UV (MeOH) $\lambda_{\max}$ nm: 210, 231. IR $\nu_{\max}$ (KBR) cm⁻¹: 3343 ($\nu_{\max}$, OH), 2928 ($\nu_{\max}$, C-H), 1683 ($\nu_{\max}$, C=O), 1052 ($\nu_{\max}$, C-O). ¹H NMR (500 MHz, Acetone-D₆) and ¹³C NMR (125 Mhz, Acetone-D₆) spectroscopic data, see Table-1.

Table-1: NMR Chemical Shifts of Compound 1-3.

Carbon	1 ^{21,22}		2 ²³		3 ^{24,25}	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
C-1	37.43	1.86; 1.05	36.83	0.81; 1.8	33.43	1.03; 1.81
C-2	31.85	1.95; 1.86	29.27	1.8; 1.15	70.40	3.54
C-3	71.99	3.53	76.91	3.05	80.57	2.85
C-4	42.48	2.28	39.28	2.12; 1.98	42.01	-
C-5	140.94	-	140.44	-	141.35	-
C-6	121.92	5.35	121.22	5.33	118.63	5.75
C-7	32.08	1.54; 1.48	31.42	1.5; 1.95	23.41	1.98; 2.39
C-8	32.08	1.43	31.37	1.38	42.99	1.92

C-9	50.30	0.93	49.60	0.90	48.30	-
C-10	36.68	-	36.21	-	33.63	2.49
C-11	21.26	1.43; 1.48	20.60	1.51	214.77	-
C-12	39.95	1.95; 1.13	38.30	0.98; 2.34	48.42	2.56; 3.31
C-13	42.50	-	41.86	-	48.10	-
C-14	56.94	0.99	56.17	0.98	50.55	-
C-15	26.23	1.13	23.87	1.53; 0.8	45.29	1.39; 1.84
C-16	28.44	1.86; 1.23	27.80	1.27; 1.8	70.18	4.48
C-17	56.23	1.05	55.42	1.09	58.09	2.60
C-18	12.04	0.67 s	11.68	0.65	19.20	0.89
C-19	18.96	1.01 s	19.11	0.95	19.29	1.07
C-20	36.33	1.33	35.48	1.35	78.58	-
C-21	19.21	0.91	18.62	0.91	24.00	1.38
C-22	34.12	1.05; 1.33	33.34	1.15; 1.31	203.62	-
C-23	24.49	1.54	25.43	1.15; 0.8	119.93	6.80 d (J = 19.5 Hz)
C-24	46.01	0.94	45.14	0.92	153.98	6.98 d (J = 19.4 Hz)
C-25	29.32	1.65	28.70	1.64	70.27	-
C-26	20.01	0.83 d (J = 7.1 Hz)	19.72	0.8	27.87	1.31
C-27	19.59	0.81 d (J = 7.1 Hz)	18.94	0.91	27.87	1.32
C-28	23.24	1.23	22.61	1.26	18.41	0.96
C-29	12.17	0.86	11.79	0.8	20.92	1.17
C-30	-	-	-	-	24.03	1.30
C-1'	-	-	100.77	4.22	-	-
C-2'	-	-	73.47	2.89	-	-
C-3'	-	-	76.91	3.47	-	-
C-4'	-	-	70.11	3.01	-	-
C-5'	-	-	76.75	3.12	-	-
C-6'	-	-	61.10	3.38 dd (J = 4.55, 11.0 Hz) 3.65 dd (J = 4.55, 11.05 Hz)	-	-

The data had been confirmed by the reported data.²¹⁻²⁵

The HMBC (Heteronuclear Multiple Bond Connectivity) spectrums are used to confirm the structure correctly. It exhibits information about the correlation or relationship between protons and adjacent carbons through two, three, and sometimes four bonds.²⁶ The HMBC spectrums of β -sitosterol(1) and cucurbitacin F(3) were shown in Fig.-1.

The HMBC spectrums of the isolated compound (Fig.-1) confirmed that compounds **1** and **3** were β -sitosterol and Cucurbitacin F, respectively. The β -sitosterol compound was indicated by the correlation between the specific protons and carbons, such as C-3 (C-OH) with H-1 and H-4; C-5 and C-6 (olefin carbon) with H-1, H-4, H-19; and proton at methyl carbon of H-18 with C-12 and C-13; H-19 with C-1, C-5, C-9, and C-10; H-21 with C-22, C-20, and C-17; H-26 with C-27 and C-25; H-27 with C-24, C-25, and C-26; and H-29 with C-24 and C-28 (Fig.-2A). Whereas the cucurbitacin F was identified by the connection between protons and the specific carbons, such as carbon with OH substitutions at C-2 with H-3 and H-29, C-3 with H-28 and H-29, C-20 with H-16, olefins at C₅=C₆ with H-28 and H-29, and C₂₃=C₂₄ with H-23, H-26, and H-27, and carbonyl at C-11 with H-10, H-12, and H-19, and C-22 with H-17, H-21, H-23, and H-24 (Fig.-2B).

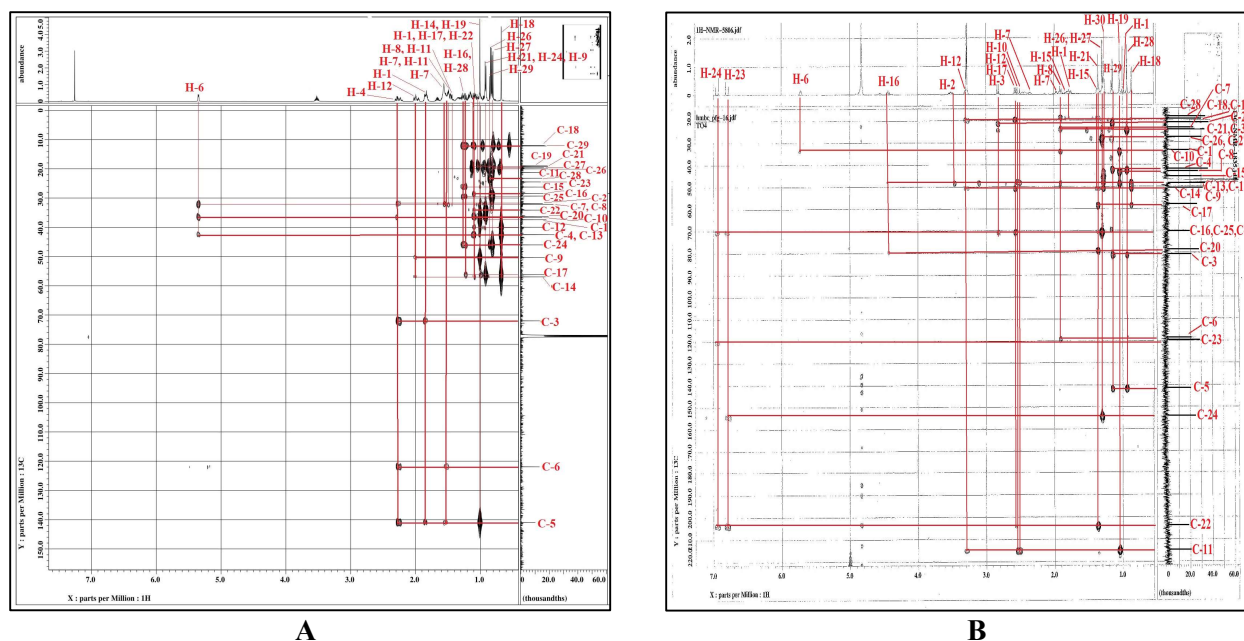


Fig.-1: HMBC Spectra of Isolated Compounds of *E. mastersii* King Stem Bark; β -sitosterol (A) and Cucurbitacin F (B)

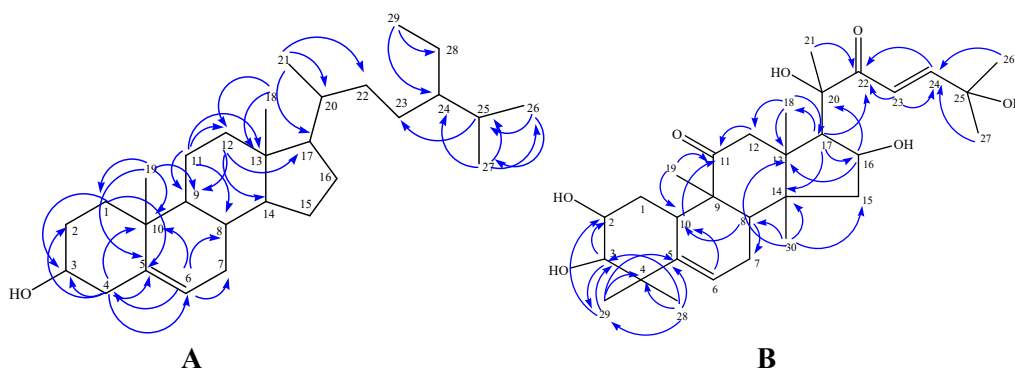


Fig.-2: Key HMBC (H $\rightarrow$ C) of Isolated Compounds; β -sitosterol (A) and Cucurbitacin F (B)

All of the spectroscopy data confirmed that the isolated compounds **1**, **2**, and **3** were assigned as β -sitosterol, β -sitosterol glucoside, and cucurbitacin F, respectively (Fig.-3). Sterol compounds of β -sitosterol and β -sitosterol glucoside were firstly reported from the plant *E. mastersii* King.

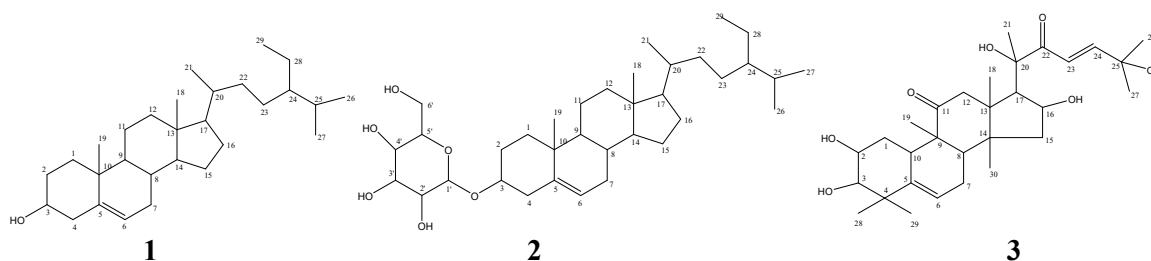


Fig.-3: Isolated Compounds of *E. mastersii* King Stem Bark; β -sitosterol (**1**), β -sitosterol glucoside (**2**), and Cucurbitacin F (**3**)

α -Glucosidase Inhibitory Activity of Isolated Compounds

The inhibition of the α -glucosidase enzyme was used to determine the antidiabetic activity of the isolated compounds (Fig.-4). The principle of this evaluation is based on the decrease in the amount of p-nitrophenol produced due to the performance of the inhibitor agent.²⁰

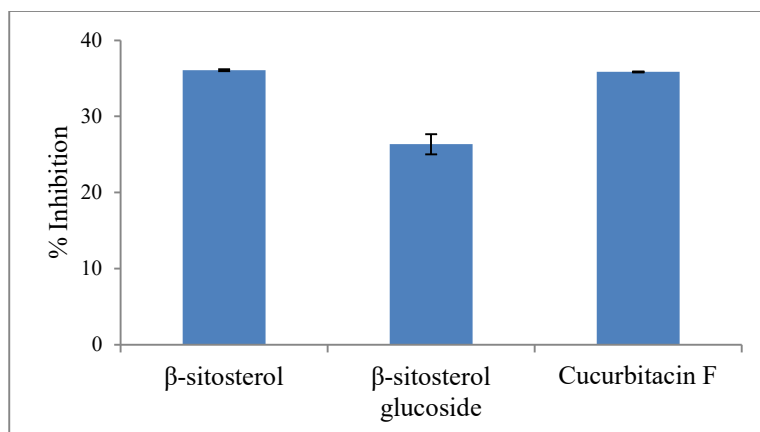


Fig.-4: The α -Glucosidase Inhibition Percentage of the Isolated Compounds from *E. mastersii* King

As shown in Fig.-4, each isolated compound had a different inhibition performance, such as the β -sitosterol inhibition percentage of 36.08 ± 0.12 % at the concentration of 2.4×10^{-3} mol/L, a β -sitosterol glucoside of 26.35 ± 1.33 % at the concentration of 1.7×10^{-3} mol/L, and cucurbitacin F of 35.85 ± 0.06 % at the concentration of 1.9×10^{-3} mol/L.

Sterol compounds such as β -sitosterol and β -sitosterol glucoside had been reported to have potential α -glucosidase inhibitor activity.^{27, 28, 29} Some previous studies suggested the inhibition mechanism of α -glucosidase by β -sitosterol and β -sitosterol glucoside was through the hydrophobic interactions that blocked the entry of the substrate resulting in the change of the conformation of the active site of the α -glucosidase enzyme^{30,31}, in which β -sitosterol had the interactions with the enzyme site of MET 385, ILE 143, HIS 203, PHE 282, and TYR 388³⁰ and β -sitosterol glucoside had the interactions with the enzyme site of LYS 156, TYR 158, and PHE 303.³¹ Meanwhile, cucurbitacin F had not been known clearly about its inhibition mechanism. It was due to the limitations of the study of cucurbitacin F in inhibiting the α -glucosidase enzyme.

CONCLUSION

This study exhibited that the *E. mastersii* King stem bark had various types of secondary metabolite compounds such as terpenoid and steroids. Based on the spectroscopic data, those compounds were β -sitosterol, β -sitosterol glucoside, and cucurbitacin F. All of those compounds revealed different inhibition performances as an α -glucosidase inhibitors.

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