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IDENTIFICATION AND BIOLOGICAL ACTIVITY TEST SOME ISOLATED COMPOUNDS FROM STEM BARK OF MELINJO (*GNETUM GNEMON*)

(Identifikasi dan Uji Aktivitas Biologi Beberapa Senyawa Hasil Isolasi dari Kulit Batang Melinjo (Gnetum Gnemon))

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Abstract

Isolation and structure elucidation of two compounds, namely resveratrol (**1**), and 3-methoxyresveratrol (**2**) from stem bark of Melinjo *(Gnetum gnemon)* had been done. The isolation of those compounds was carried out by chromatographyc method and structure elucidation was performed by interpretation of spectroscopic data, including UV, IR, ^{1}H and ^{13}C NMR 1D and 2D, and FABMS. The result of this study showed that activity each compounds as radical hydroxyl scavenger of resveratrol (**1**), and 3-methoxyresveratrol (**2**), with an IC_{50} 45,17 and 60,12; μg/ml respectively. Each compound showed significant activity as UV-B protection. Activity test as UV-B protection showed that resveratrol and methoxyresveratrol have maximum protections (SPF 8,03 and 12,34 respectively), each compounds on 50 μg/ml.

Key word : melinjo; Gnetum gnemon; natural antioxidant; UV-B protection

INTRODUCTION

Gnetum gnemon is a species of Gnetaceae which can be found at several places in Indonesia and the local name is " melinjo". The plant ussually can be used as food source. Several oligostilbenoid compounds had been isolated from some species of Gnetaceae. Oligostilbenoid compound isolated from gnetaceaous plant showed interesting structure and have different characteristic molecular structure than the other oligostilbenoids from Dipterocarpaceae. Therefore, from this research can be found bioactive compounds and can be used as lead compound in pharmaceutical industry [1-8]. In our continuing phytochemical study of the tropical plants occuring in Indonesia, we have examined phenolic constituents of *Gnetum gnemon*. This paper will report our first investigation of two compounds from stem bark of *Gnetum gnemon*, namely

resveratrol (**1**), and 3-methoxyresveratrol (**2**). The structure of this compound based on the analysis spectrum of UV, IR, MS and NMR included ID and 2D NMR.

EXPERIMENTAL

General Experimental Procedure

UV and IR spectra were measured with varian cary 100 Conc and Shimadzu 8300 FTIR respectively. ^{1}H and ^{13}C NMR spectra were recorded with Jeol JNM A-5000 spectrophotometers, operating at 600.0 MHZ (^{1}H) and 150.0 MHZ (^{13}C) using residual and deuterated solvent peaks as internal standards. MS spectra were obtained with a JMS-AM 20 spectrometer, using the mode FAB. Vacuum liquid chromatography was carried out using Merck Si gel Merk 60 GF_{254} (230-400 mesh), column chromatography using Si-gel Merk 60 (200-400 mesh) and TLC analysis on precoated Si gel plates Si-gel Merk Kieselgel 60 F_{254} 0.25 mm, 20x20 cm.

Plant Material

Samples of the stem bark of *Gnetum gnemon* were collected in Desember 2005 from the Sleman, Yogyakarta vilage. Determination of these plants have been done by staff at Laboratory of Biology UGM and a voucher specimen had been deposited at the Herbarium.

Extraction and Isolation

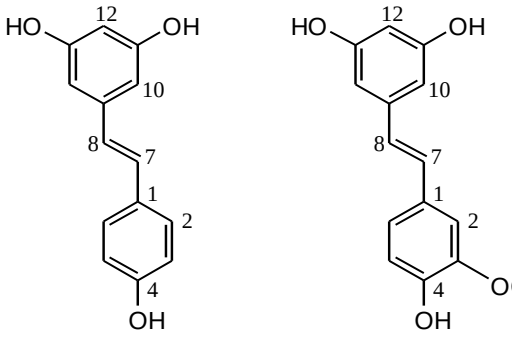
The isolation was carried out by extraction 9.5 kg dried powdered plant materials with methanol at room temperature for 24 hours (3x). The extract was concentrated at a low pressure, then partision with hexane, chloroform, and ethyl acetate respectively. Fractionated and purification of chloroform fraction (65 g) by repeated chromatography to give isolat **1** (200 mg). A portion of ethyl acetate fraction (40 g) was then subjected to fractionated by VLC (silica gel GF 60 Merk 250 g; ϕ: 10 cm, t = 10 cm), using n-hexane, n-hexane-EtOAc, EtOAc, Me_2CO, and MeOH of increasing polarity as eluents to give twenty fractions. These fractions were combined to give two major fractions A (4,96 g) and B (10,4 g). Fraction B (10,4 g) was repeatedly separated and purified by column chromatography. From this method we obtained isolated **2** (350 mg). Identification these compounds by spectroscophy UV, IR, NMR (1D and 2 D) and FAB MS.The biological activity as antioxidant was conducted by invitro using Fenton method [9], and activity test as sun protection by invitro with Walters methode[10].

RESULT AND DISCUSSION

Resveratrol (**1**) was obtained as a white yellow powder, UV (MeOH) $\lambda_{max.}$ 217 and 307 nm, IR (KBr) $u_{max.}$: 3276; 1587-1444;

989; 833 cm^{-1}, ^{1}H and ^{13}C NMR (Me_2CO-d_6, 600.0 and 150 MHz) see in Table 1.

3-Methoxyresveratrol (**2**) was obtained as a white yellow powder, UV (MeOH) $\lambda_{max.}$ 229 and 325 nm, IR (KBr) $\upsilon_{max.}$: 3415; 1598-1514; 989cm^{-1}, ^{1}H and ^{13}C NMR (Me_2CO-d_6, 600.0 and 150 MHz) see in Table 1. FABMS isolat **3** showed molecular ion at *m/z* 258 $[M]^+$ $[C_{15}H_{14}O_4]$.



1 **2**

Table 1. ^{1}H and ^{13}C NMR data of two compounds in acetone-d_6

No. carbon	Isolated 1		Isolated 2		
	δ_H (*m*, *J* Hz) ppm	δ_C ppm	δ_H (*m*, *J* Hz) ppm	δ_C ppm	HMBC (H→C)
1	-	129,95	-	130,5	
2	δ 7,42 (*d,* 8,5)	128,75	7,20 (*d*, 2,0)	110,2	C-5; C-6
3 OCH_3)	6,83 (*d* 8,5) -	116,43 -	- 3,89 (*s*)	148,6 56,26	-
4	-	158,24	-	140,8	-
5	6,83 (*d,* 8,5)	116,43	6,99 (*d*, 6,2)	121,2	C-6; C-4
6	δ 7,42 (*d,* 8,5)	128,75	7,01 (*dd*, 6,2; 2,0)	129,4	C-2; C-5
7	δ 7,03 (*d,* 16,0)	126,86	6,82 (*d*, 8,3)	115,9	C-1; C-9
8	6,86 (*d,* 16,0)	129,09	6,93 (*d*, 8,3)	127,4	C-10;14; C-1
9	-	140,88	-	147,5	-
10	6,54 (*d,* 2,5)	105,64	6,54 (*d,* 2,0)	105,6	C-12; C-8; C-13
11	-	159,65	-	159,5	-
12	6,26 (*t,* 2,5; 2,5)	102,68	6,27 (*d*, 2,0)	102,6	C-10; 14; C-13
13	-	159,65	-	159,5	-
14	6,53 (*d,* 2,5)	105,64	6,54 (*d*, 2,0)	105,6	

From the chloroform and ethyl acetate extract of stem bark *G. gnemon*, after separated and repeatedly purification by extensive chromatography resulted two compounds. Compound **1** was obtained as a white yellow powder, maxima of absorption were observed at 217 and 307 nm in the UV spectrum attributable to the conjugated phenol cromophor. The IR spectrum exhibited hydroxyl group (3276 cm^{-1}), C=C

aromatic (1587-1444 cm^{-1}), 989 (trans olefenic), and monosubtituen benzene (833 cm^{-1}). The ^1H NMR spectra showed two sets of AA'BB' system of aromatic protons assignable to two independent 4-hydroxyphenyl groups at 6. δ 7,42 (2H, *d, J* = 8,5 Hz) ppm and 6,83 (2H, *d, J* = 8,5 Hz), two sets of *meta*-coupled aromatic protons at δ 6,54 (1H, *d, J* = 2,5 Hz); 6,53 (1H*, d, J* = 2,5 Hz); and 6,26 (1H, *t, J* = 2,5; 2,5 Hz) assignable to units 3,5-dihydroxibenzene. They also displayed two signal protons trans coupling at δ 7,03 (1 H, *d, J* = 16,0 Hz) and 6,86 (1H, *d, J* = 16,0 Hz) exhibited of olefenic unit. The ^{13}C NMR spectrum showed 10 signal carbon which exhibited of 14 carbon atom. Furthermore, 14 signal carbon showed 3 carbon oksiaril at δ 159,65 (2 C); 158,24 (1C) ppm, 9 carbon metin at δ 129,09 (1C); 128,75 (2C); 126,86 (1C); 116,43 (2C); 105,64 (2C); 102,68 (1C) ppm; and two carbon quarterner at δ 140,88 (1C) at 129,95 (1C) ppm. Spectrum NMR (^1H and ^{13}C) of 2 has similar with resveratrol in literature data [2].Therefore, it may be concluded that the **1** is a resveratrol

Compound **2** was obtained as a white yellow powder, maxima of absorption were observed at 229 and 325 nm in the UV spectrum attributable to the conjugated phenol cromophor. The IR spectrum exhibited hydroxyl group (3415 cm^{-1}), C=C aromatic (1598-1514 cm^{-1}), and 989 (trans olefenic). The positive ion FABMS exhibited an $[M]^+$ ion at *m/z* 258 consistent with a molecular formula $C_{15}H_{14}O_4$ for a resveratrol derivative and supported by the NMR data. ^{13}C NMR spectra showed three signals for oxyaryl carbon at 140,8 (C-4), and 159,5 (C-11; 13). Additionally, the ^{13}C NMR also exhibited one oxyalkyl carbon at 56,26 indicating that C-3 attached with methoxyl fungtional group. The ^1H NMR spectrum of **2** in acetone-d_6 exhibited signals for of 1,3,4-trisubstitutebenzene at 7,20 (*d*, 2,0); 6,99 (*d*, 6,2); and 7,01 (*dd*, 6,2; 2,0). The ^1H NMR spectrum also showed two sets of meta-coupled aromatic protons signals at δ 6,54 (2H, *d, J* = 2,0 Hz) and 6,26 (1H, *t, J* = 2,0; 2,0 Hz). Additionally, the ^1H NMR spectrum exhibited signals for a set of aliphatic proton at δ δ 6,93 (1 H, *d, J* = 8,3 Hz) and 6,82 (1H, *d, J* = 8,3 Hz), characteristic for *trans*-olefenic proton, and signals assignable aliphatic protons at δ 3,89 (s) characteristic for methoxyl proton. These spectral data indicated that compound **2** is 3-methoxy resveratrol (rampotigenetin) (3). The HMQC spectrum supported complete assignment of all proton-bearing carbon signals of compound **2** (Table 1).

Activity test as antioxidant conducted by radical scavenger activity from chloroform and ethyl acetate with Halliwel methode [9], showed at table 2.

Table 2. Data activity test as radical scavenger

No	Sample	IC_{50} μg/ml	Note
1	Chloroform fraction	214,56	Active
2	Ethyl acetate fraction	1606,41	Less active
3	Resveratrol	45,17	More active
4	3-Methoxy resveratrol	60,12	More active
5	Vitamin C	83,87	More active
6	BHT	1328,10	Less active

Note : IC_{50} < 100 μg/ml : more active; 100 -1000 μg/ml : active; dan 1000-5000 μg/ml : less active; > 5000 μg/ml : not active

The data showed that the activity as radical hidroxyl scavenger from chloroform and ethyl acetate more lower than vitamin C but more high than BHT. The activity test of isolate 1 and 2 as antioxidant showed high activity. Each compound showed significant activity as UV-B protection. Activity test as UV-B protection showed that resveratrol and methoxyresveratrol have maximum protections (SPF 8,03 and 12,34 respectively), each compounds on 50 μg/ml. Therefore, These results suggest that the compounds from stem bark of *melinjo* may be useful as potential sources of natural antioxidants and UV-B protection.

CONCLUSION

From the non polar fraction extract acetone stem bark of *Gnetum gnemon* can be isolated three phenolic compounds, namely resveratrol (1) and 3-methoxyresveratrol (2). Each compound showed significant activity as antioxidant and UV-B protection.

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