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# SOME PHENOLIC COMPOUNDS FROM STEM BARK OF MELINJO (*GNETUM GNEMON*) AND THEIR ACTIVITY TEST AS ANTIOXIDANT AND UV-B PROTECTION

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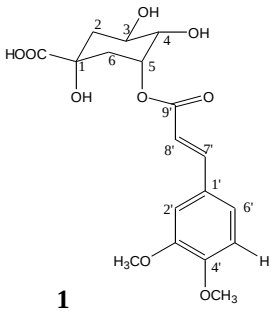
**Abstract—** Isolation and structure elucidation of three phenolic compounds, namely 3,4-dimethoxychlorogenic acid (1), resveratrol (2), and 3-methoxyresveratrol (3) from stem bark of Melinjo *(Gnetum gnemon)* had been done. The isolation of those compounds was carried out by chromatographyc method 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 3,4-dimethoxychlorogenic acid (3), resveratrol (2), and 3-methoxyresveratrol (3), with an $IC_{50}$ 523,7; 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), and 3,4-dimethoxychlorogenic acid has minimum protection (SPF 2,55), each compounds on 50 μg/ml.

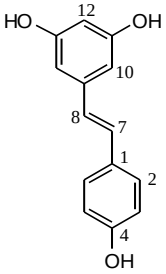
*Key word : melinjo; Gnetum gnemon; natural antioxidant; UV-B protection*

## 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 three phenolic derivatives from stem bark of *Gnetum gnemon*, namely 3,4-dimethoxychlorogenic acid (1), resveratrol (2), and 3-methoxyresveratrol (3). The structure of this compound based on the analysis spectrum of UV, IR, MS and NMR included ID and 2D NMR.




**1**

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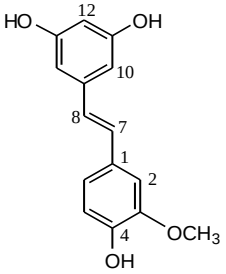


**3**

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## RESULTS AND DISCUSSION

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 two compounds i.e. isolat 1 (40 mg), and isolat 2 (200 mg). Identification these compounds by spectroscophy UV, IR, NMR (1D and 2 D) and FAB MS, concluded that isolat 1 as 3,4-dimethoxychlorogenic acid (1) and isolate 2 as resveratrol (2). 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 one compound, namely 3-methoxyresveratrol (3) (350 mg). 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].

3,4-Dimethoxychlorogenic acid (**1**) was obtained as a white powder, UV (MeOH) $\lambda_{max.}$ 242, 286 and 317 nm, IR (KBr) $\upsilon_{max.}$ : 3388 ; 2918-2850 ; 1724 ;1598-1514 and 989 $cm^{-1}$, $^1H$ and $^{13}C$ NMR ($Me_2CO$-$d_6$, 600.0 and 150 MHz) see in discussion.

Resveratrol (**2**) was obtained as a white yellow powder, UV (MeOH) $\lambda_{max.}$ 217 and 307 nm, IR (KBr) $\upsilon_{max.}$ : 3276; 1587-1444; 989; 833 $cm^{-1}$, $^1H$ and $^{13}C$ NMR ($Me_2CO$-$d_6$, 600.0 and 150 MHz) see in discussion.

3-Methoxyresveratrol (**3**) was obtained as a white yellow powder, UV (MeOH) $\lambda_{max.}$ 229 and 325 nm, IR (KBr) $\upsilon_{max.}$ : 3415; 1598-1514; 989$cm^{-1}$, $^1H$ 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 $[M]^+$ $[C_{15}H_{14}O_4]$.

Table 1. $^1H$ and $^{13}C$ NMR data of compound **3*** in acetone-$d_6$

| No. carbon | $\delta_H$ ( *m*, *J* Hz) ppm | $\delta_C$ ppm | HMBC (H→C) |
|---|---|---|---|
| 1 | - | 130,5 | |
| 2 | 7,20 (d, 2,0) | 110,2 | C-5; C-6 |
| 3<br>($OCH_3$) | -<br>3,89 (s) | 148,6<br>56,26 | - |
| 4 | - | 140,8 | - |
| 5 | 6,99 (d, 6,2) | 121,2 | C-6; C-4 |
| 6 | 7,01 (dd, 6,2; 2,0) | 129,4 | C-2; C-5 |
| 7 | 6,82 (d, 8,3) | 115,9 | C-1; C-9 |
| 8 | 6,93 (d, 8,3) | 127,4 | C-10;14; C-1 |
| 9 | - | 147,5 | - |
| 10,14 | 6,54 (d, 2,0) | 105,6 | C-12; C-8; C-13 |
| 11 | - | 159,5 | - |
| 12 | 6,27 (d, 2,0) | 102,6 | C-10; 14; C-13 |
| 13 | - | 159,5 | - |

[a]measured in methanol-$d_4$ at 600 MHz ($^1H$) and 150 MHz ($^{13}C$) .

From the chloroform extract of stem bark *G. gnemon*, after separated and repeatedly purification by extensive chromatography resulted two compounds. 3,4-dimethoxychlorogenic acid (**1**) was obtained as a white powder. Its UV spectrum showed absorption maxima at 242, 286 and 317 nm suggesting the presence of unconjugated phenolic chromophore. The IR spectrum exhibited hydroxyl group (3388 $cm^{-1}$), 2918-2850 (C-H aliphatic); 1724 (C=O acid); C=C aromatic (1598-1514 $cm^{-1}$), 989 (C=C trans olefenic) and monosubtituen benzene (833 cm-1), these spectra characteristic absorptions for supporting **1** to be a phenol derivative. The $^1H$ NMR spectrum of **1** in acetone-$d_6$ exhibited signals in aliphatic and aromatic range. In aromatic range, the $^1H$ NMR spectrum exhibited signals for a set of ABX system at δ 7,33 (1H, *d*, *J* = 1,85 Hz); 7,12 (1H, *dd*, *J* = 1,85; 8,0 Hz); and 6,85 (1H, *d*, *J* = 8,0 Hz) ppm, characteristic for 1,3,4-trisubstituted benzene. The $^1H$ NMR spectrum also showed two sets olefenic proton at δ 7,60 (1H, *d*, *J* = 15,9 Hz) and 6,41 (1H, *d*, *J* = 15,9). Additionally, the $^1H$ NMR spectrum exhibited signals in aliphatic range δ 3,92 (3 H, *s*) and 3,87 (3H, *s*) characteristic for methoxyl group; and a set signal at δ 5,61 (1H, *s*); 4,14 (1H, *m*); 3,50 (1H, m); 2,10 (1 H , m); 2,18 (1H, m); 2,17 (1H, m); dan 1,95 (1 H, m) exhibited of siclohexane ring. These spectral data indicated that compound **1** has a phenolic derivative with siclohexane skeleton as part of its structure. $^{13}C$ NMR spectra showed signals for acid carbon at 178.2 ($COO^-$); carbon aromatic ring at 138.0; 115.0; 148.0; 150.0; 112.5; and 123.8; olifenic carbon at 142.0 and 113.0; and carbonil carbon at 169.2 ppm. Additionally, the $^1C$ NMR spectrum exhibited signals in aliphatic range at 77.0; 39.0; 71.5; 68.0; 41.0;

exhibited carbon of siclohexan ring, and 58.0 and 55.0 ppm exhibited of methoxyl carbon group. Spectrum NMR ($^1$H and $^{13}$C) of 1 has similar with chlorogenic acid [11], but isolate **1** has methoxyl group at position 3 and 5 in aromatic ring. Therefore, it may be conclude that **1** is 3,4-dimethoxychlorogenic acid .

Compound **2** 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 $^1$H 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 ($^1$H and $^{13}$C) of 2 has similar with resveratrol in literature data [2].Therefore, it may be concluded that the **2** is a resveratrol

Compound **3** 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 $^1$H NMR spectrum of **1** 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 $^1$H 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 $^1$H 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 **3** is 3-methoxy resveratrol (rampotigenetin) (3). The HMQC spectrum supported complete assignment of all proton-bearing carbon signals of compound **3** (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 | 3,4-Dimetoksichlorogenat acid | 523,7 | Active |
| 4 | Resveratrol | 45,17 | More active |
| 5 | 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 2 and 3 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), and 3,4-dimethoxychlorogenic acid has minimum protection (SPF 2,55), 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.

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