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Methyl-3,4-dihydroxybenzoate and 9-10-dihydrophenanthrene-2,4,7-triol two phenolic compounds from Dioscorea alata L. and their antioxidant activity

N. S. Aminah, A. Yulvia, and M. Tanjung

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Methyl-3,4-Dihydroxybenzoate and 9-10-Dihydrophenanthrene-2,4,7-Triol Two Phenolic Compounds from *Dioscorea alata* L. and Their Antioxidant Activity

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Abstract. Two phenolic compounds namely: methyl-3,4-dihydroxybenzoate (**1**) and 9,10-dihydrophenanthrene-2,4,7-triol (**2**) had been isolated for the first time from the tuber of Dioscorea alata L. The extraction of two compounds were done by maceration method using methanol as solvent, followed by partition with n-hexane and ethyl acetate. The ethyl acetate extract was separated and purified using various chromatographic techniques yielded pure compounds. The structure of isolated compounds were determined based on spectroscopic data, including UV-Vis, 1D and 2D NMR spectra. Compounds (**1**), (**2**) and ascorbic acid as a comparator were evaluated for their antioxidant properties against DPPH, showing their IC_{50} were 9,41 ± 0,08; 23,52 ± 0,05; and 10,95 ± 0,08 ppm, respectively.

INTRODUCTION

Dioscorea alata L. is a plant used as an alternative food for produce carbohydrates which grow throughout the Indonesian archipelago [1]. *Dioscorea* is one genus of the family Dioscoreaceae which has 600 species which spread in China, Taiwan and in the tropical countries [2]. *Dioscorea* plants produce secondary metabolites include saponins, steroids, terpenoids, arilpropanoid, alkaloids and stilbenoid [3] [4]. Secondary metabolites of *Dioscorea* show activity as antimicrobial, anti-inflammatory, anti-cancer, allergy, antineoplastic and antioxidant [5] [6] [7] [8] [9] [10] [11].

Based on literature, research of phytochemical compounds that contained in *Dioscorea alata* L. until now has not been reported as well as an antioxidant activity, and on this occasion will be reported the discovery of two phenolic compounds are methyl-3,4-dihydroxybenzoate (**1**) and 9,10-dihydrophenanthrene-2,4,7-triol (**2**). The two compounds has not been reported yet from this species. It will also be reported to the antioxidant activities of the two compounds toward the reagent DPPH (2,2-diphenyl-1-picrylhydrazyl).

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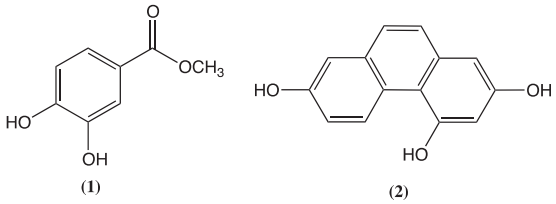

FIGURE 1. Chemical structures of two isolated compounds

`MATERIAL METHODS

Gravitation column chromatography (GCC) was carried out using Merck Si gel 60 (700-200 mesh), radial chromatography were carried out using Merck Si gel 60 PF_{254} , and for TLC analysis, pre-coated Si gel plates (Merck Kieselgel 60 GF_{254}, 0.25 mm) were used. UV and IR spectra were measured with a Shimadzu UV-1800 and FT IR Spectrum One Perkin-Elmer instruments, respectively. ^{1}H and ^{13}C-NMR spectra were recorded with either JEOL ECA 400, operating at 400 (^{1}H) and 100 (^{13}C) MHz, using residual and deuterated solvent peaks as reference standards. The determination of antioxidant activity uses the inhibition of free radical against DPPH using spectroscopy method.

Samples of plants which used in this research is the tuber of *Dioscorea alata* L. which obtained from Porong market, Sidoarjo, East Java, Indonesia. The specimen of plant identified in Herbarium Bogoriense Cibinong Science Center, Bogor, Indonesia. and a voucher specimen has been deposited at the herbarium.

The powdered tuber of *Dioscorea alata* L. (10,0 kg) was maserated with methanol for 2 x 24 hours and then concentrated under reduced pressure to give a gummy brownish extract. The methanol extract was partitioned with n-hexane and ethyl acetate. The ethyl acetate extract (10,1 grams) was fractionated by gravitation column chromatography with the eluent mixture n-hexane-ethyl acetate (9:1 to 7:3) yielded four main fractions A-D. Separation of fraction B (256 mg) with radial chromatography (eluent: n-hexane-chloroform 8:2 and chloroform-ethyl acetate 9:1) was derived compound **1** (2 mg). Separation and purification of fraction D (317 mg) using radial chromatography (eluent: n-hexane-ethyl acetate 8,5:1,5 to 7,5:2,5) was derived compound **2** (36 mg).

DPPH scavenging activity assay was used to determine compound's inhibiton capacity. Its reaction priciple was based on mechanism of free radicals inhibition by hydrogen transfer, the antioxidant activity of sample expressed in IC_{50} (Inhibiton Concentration 50%). A total of 500 µL of test Solutions in various concentration (10-500 ppm), 500 µL of 0.2 M acetate buffer pH 5.5, and 1000 µL of methanol are mixed in a test tube. Added to this mixture is 500 µL of $5x10^{-4}$M DPPH. The mixture was homogenized using a vortex in a dark room (resistant to UV light) and has incubated for 30 minutes. The mixture was measured by a spectrophotometer UV absorbance at λ_{max} 517 nm. Ascorbic acid is used as positive control [12]. To prevent the sample from light disturbance, the test tube wrapped with aluminum foil. Inhibiton Capacity was measured by this equation:

$$\% \text{ inhibisi} = \frac{A.blanko - A.sampel}{A.blanko} \text{ x } 100\%$$

RESULTS AND DISCUSSION

Methyl-3,4-dihydroxybenzoic (**1**), yellow powder. UV spectrum (MeOH) □□□□□nm (log ε) 262,5 nm (3,67) and 284,5 nm (3,58). **(**MeOH + NaOH**)** 306, 0 nm (3,97). ^{1}H-NMR ($CDCl_3$, 400 MHz) and ^{13}C-NMR ($CDCl_3$, 100 MHz) see Table-1.

9, 10-dihydrophenanthrene-2, 4, 7-triol (**2**), yellow gel form. UV spectrum (MeOH) λ_{maks} nm (log ε): 277, 5 (4, 43) and 294, 5 (4, 25) nm. **(**MeOH + NaOH**)** 291, 0 nm (4,20). ^{1}H-NMR (DMSO-*d6*, 400 MHz) and ^{13}C-NMR (DMSO-*d6*, 100 MHz) see Table-2.

Compound **1** was obtained as a white solid. ^{1}H-NMR spectrum of phenolic compound **1** shows three aromatic proton signals of ABX system that on δ_H (ppm) chemical shift. It is doublet meta signals at δ_H 6,91 (J = 2,4 Hz); doublet doublet ortho and metha signal at pada δ_H 7,56 (*dd*, J = 7,6; 2,4 Hz) and doublet para signals at δ_H 7,57 ppm (J = 7,6 Hz). The third signal of aromatic protons in ABX system show the characteristic of aromatic

compound structure which having substituents attached to the aromatic nucleus. Residual proton signals in the NMR spectrum at δ_H 3,88 ppm with a singlet proton multiplicity for a methoxy substituent. ^{13}C-NMR spectrum of compound **1** showed eight carbon signals which separate perfectly. Three metin carbon signals at δ_C 123,8; 116,5 and 114,8 ppm confirms that compound **1** has three substituents attached to the aromatic nucleus, the methyl carbon at δ_C 52,1 ppm is the carbon of the methoxy, and four quaternary carbon signals consist by one of the ester carbonyl on δ_C 167,5 ppm, and two oxiaryl groups of hydroxyl -OH on δ_C 148,6 and 142,9 ppm. Based on the ^{13}C-NMR spectrum data suggested phenolic compound **1** is a derivative of the methylbenzoic which having two hydroxyl groups attached to the aromatic nucleus of methylbenzoic structure. Additionally, justification of compound **1** is a derivative of methylbenzoic which have two hydroxy groups were determined by 2D NMR spectrum. The HMBC spectrum measurements can be seen in Figure-2 and Table-1.

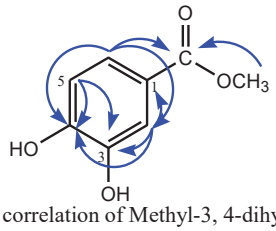

FIGURE 2. The HMBC correlation of Methyl-3, 4-dihydroxybenzoic compound

TABLE 1. *NMR data ($CDCl_3$) of compound 1*

C	Compound (1)		
	δ_H (multiplicity, *J* in Hz)	δ_C	HMBC (H⇔C)
1	-	122,6	-
2	6,91(*d*, 2,4)	116,5	C-1; C-3; C-4
3	-	142,9	-
4	-	148,6	-
5	7,57(*d*, 7,6)	114,8	C-1; C-3, C-4
6	7,56(*dd*, 7,6; 2,4)	123,8	C-2;C-4, C=O
C=O	-	167,5	-
-OCH3	3,88 (*s*)	52,1	C=O

Compound **2** was obtained as a yellow gel. ^{13}C-NMR spectrum data showed 14 carbon signals separated completely consisting of seven quaternary carbon signals at δ_C 113,3; 125,9; 138,7; 140,2; 155,1; 155,6 and 156,3 ppm, five metin carbon signals (CH) at δ_C 102,2; 107,0; 113,0; 114,6 and 128,9 ppm, and a methylene carbon signals (CH_2) at δ_C 30,1 and 30,7 ppm. Quaternary carbon signals at δ_C155,1; 155,6 and 156,3 ppm are characteristic of oxiaryl carbon signal. Based on the analysis of ^{13}C-NMR spectra indicated that compound **2** is a dihydrostilbene derivative that has three -OH hydroxy substituents. ^{1}H-NMR spectrum data showed five aromatic proton signals in the area, characteristic for compounds which have two aromatic nuclei. Two proton aromatic signals in the form of a pair of doublet meta (J = 2.4 Hz) at δ_H 6,21 and 6,08 ppm. Three proton aromatic signals of ABX system at δ_H 7,99 (*d, J* = 9,3 Hz), 6,53 (*dd, J* = 9,3; 2,4 Hz) and 6,52 (*d, J* = 2,4 Hz). Signals of proton at δ_H 2,45 ppm with a multiplet multiplicity is two signals of methylene protons.

The signal at δ_H 2,45 ppm consists of two methylene proton signals which supported by ^{13}C-NMR dan HMQC spectrum. Based on the results of ^{1}H-NMR spectrum on the compound **2** is two aromatic proton signals doublet meta and three aromatic proton signals with ABX system. It is recommended that the compound **2** is 9,10-dihydrophenanthrene-2,4,7-triol. Position of the hydroxyl groups at C-2, C-4 and C-7 is determined based on the analysis of 2D NMR spectrum (HMQC and HMBC). The multiplet of methylene proton signals at δ_H 2,45 ppm showed a correlation with the two carbon signals at 30,1 and 30,7 ppm. This proves the proton signals at δ_H 2,45 ppm has two methylene at the C-9 and C-10. HMBC spectrum analysis shows the correlation between doublet meta proton signals (J = 2,4 Hz) at δ_H 6,08 ppm and two quaternary carbon signals at δ_C 156,3 (C-2) and 113,3 ppm (C-

4a), one metin signal δ_C 102,3 ppm (C-3) and methylene carbon signal at δ_C 30,7 ppm (C-10). Based on the results of these measurements are known proton signals at δ_H 6,08 ppm (H-1). The correlation between the doublet meta proton signals (J = 2,4 Hz) at δ_H 6,21 ppm with three quaternary carbon signals δ_C 156,3 (C-2); 113,3 (C-4) and 113,1 ppm (C-8) and one metin signal δ_C 107,0 ppm (C-1). Based on the results of these measurements are known proton signals at δ_H 6,21 ppm is H-3. Based on the correlation data between the aromatic proton signals on the H-1 and H-3 with a carbon signal it can be ascertained that the other aromatic proton signal of ABX system at δ_H 7,99 ppm is at H-5, 6,53 ppm is at H- 6 and 6,52 ppm is at H-8. The chemical shift at δ_H 7,99 ppm in H-5 more desheilding than any other aromatic proton signals due to the biphenyl unit. The correlation between the proton signals and carbon signals of compounds **2** in the HMBC spectrum can be seen in Figure-3 and Table-2.

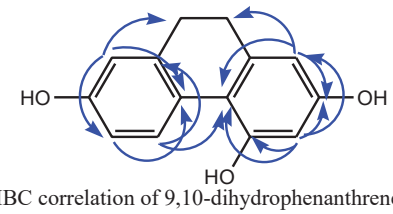

FIGURE 3. The HMBC correlation of 9,10-dihydrophenanthrene-2,4,7-triol compound

TABLE 2. *NMR data compound 2 (DMSO-d6)*

C	Compound (2)		
	δ_H (multiplicity, *J* in Hz)	**δ_C**	**HMBC (H⇔C)**
1	6,08 (*d, J* = 2,4 Hz)	107,0	C-2; C-3; C-4a; C-10
2	-	156,3	-
3	6,21 (*d, J* = 2,4 Hz)	102,3	C-1; C-2; C-4, C-4a
4	-	155,6	-
5	7,99 (*d, J* = 9,3 Hz)	128,9	C-4a; C-7; C-8a
6	6,53 (*dd, J* = 9,3; 2,4 Hz)	114,6	C-4b; C-8
7	-	155,1	-
8	6,52 (*d, J* = 2,4 Hz)	113,1	C-4b; C-6, C-9
9	2,45 (*m*)	30,1	C-4b; C-8; C-10a
10	2,45 (*m*)	30,7	C-1; C-4a; C-8a
4a	-	113,3	-
4b	-	125,1	-
8a	-	138,7	-
10a	-	140,2	-

The antioxidant activity tests of the compounds **1** and **2** toward the radical DPPH reagent exhibit IC_{50} values respectively 9,41 ± 0,08 and 23,52 ± 0,05 ppm. Compound **1 is** very potensial as antioxidant compound. The activity was showed more active compare with ascobic acid (10, 95 ± 0,08 ppm) as positive control and also compound **2**.

CONCLUSION

Two phenolic compounds, methyl-3,4-dihydroxybenzoic (**1**) and 9,10-dihydrophenanthrene-2,4,7-triol (**1**) had been isolated for the first time from the tubers of *Dioscorea alata* L. The antioxidant activities test of the compound (**1**) and (**2**) against DPPH reagent showed potential activity.

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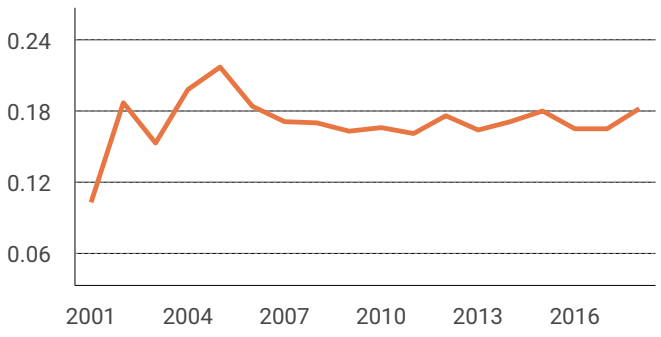

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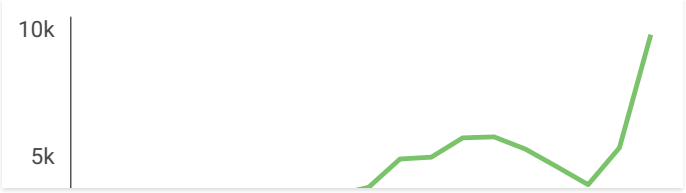

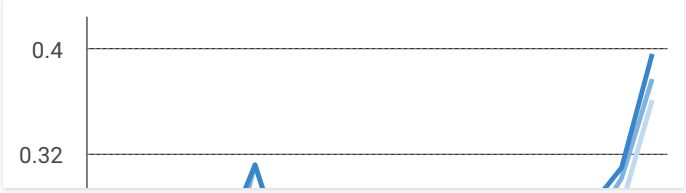

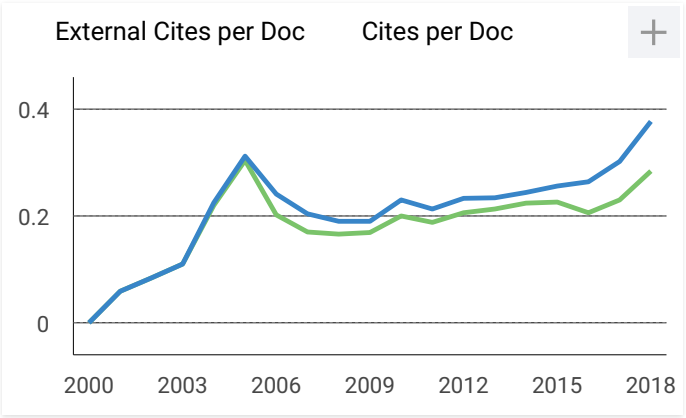

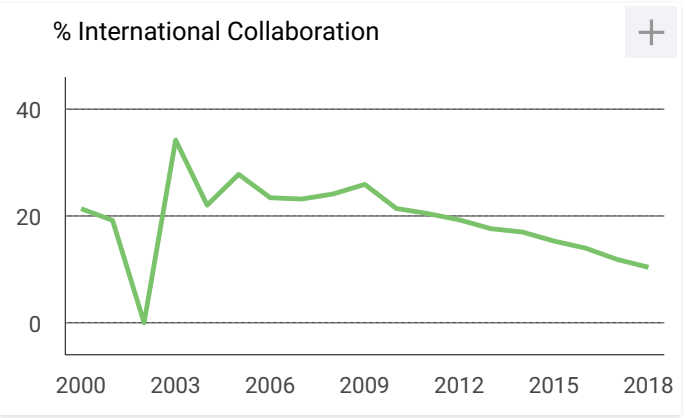

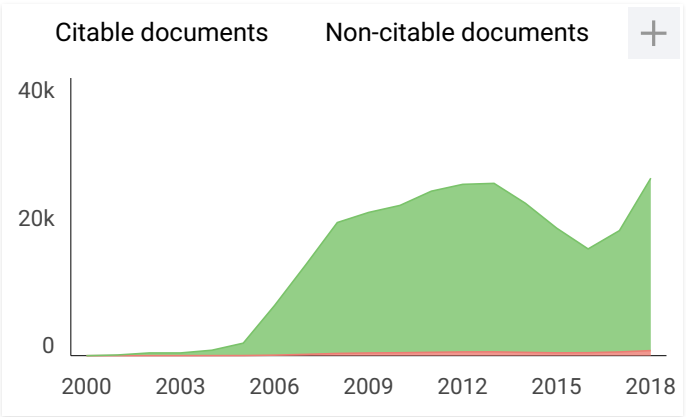

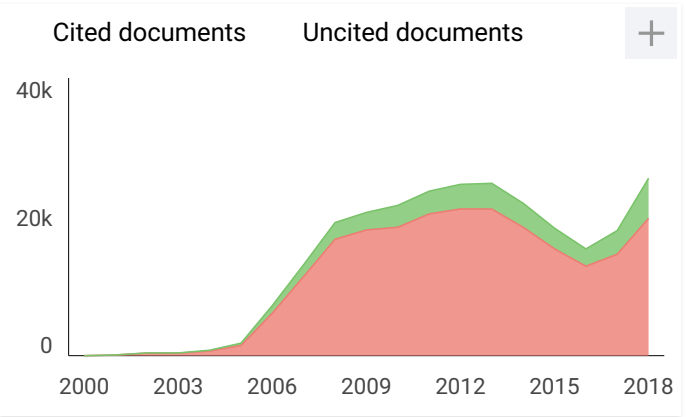

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