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Batatasin III a derivative of dihydrostilbene compound from Yam Peel of Uwi Tuban and Its Antioxidant Activity

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Abstract A dihydrostilbene derivative compound, namely 3,3-dihydroxy-5-methoxybibenzyl or batatasin III (**1**) was isolated from the yam peel of *Dioscorea alata* L. The structure of compound 1 has been elucidated based on spectroscopy UV-Vis, 1D and 2D NMR Analysis. The IC_{50} of DPPH radical scavenging of this compound (206.82 µg/mL) lower than ethyl acetate extract (109.99 µg/mL), but higher than methanol extract (893.59 µg/mL**).**

1. Introduction

The genus *Dioscorea* (Dioscoreceae) comprises more than 600 species that are widely distributed in tropical and subtropical region such as Indonesia. Wild yam or *Dioscorea alata* occur in several part of Indonesia and the plants is commonly known as Uwi tuban. Many of these species are used local food crop rich in starch of Indonesia but the bioactivity of this species is still poorly understood. This genus has been shown to produce a number of secondary metabolite such as terpenoid [1], saponin [2], steroid [3] and phenolic compounds [4]. Previous research from other country reported that *Dioscore*a are used indigenously as traditional medicines to leprosy, tumor in Bangladesh [5], inflammatory diseases such as asthma, rhemathoid arthritis and bronchitis in Taiwan [6].

In continuation of the research of the phenolic compounds in this medicinal plant, our research group already reported from two species: *D. esculenta* L.successfully isolated two phenanthrene derivatives namely confusarin and nudol [7], methyl-3,4-dihydroxybenzoate and 9,10-dihydrophenanthrene from *D. alata* L.[8]. In this research, reported of 3,3-dihydroxy-5-methoxybibenzyl or batatasin III is a phenolic compound isolated from the methanol extract of the yam peel of *Dioscorea alata* L.(uwi tuban). Uwi Tuban is one of *D. alata* species which mostly cultivated in Tuban, East Java. The chemical structure of compound **1** was established by UV, IR, 1D and 2D NMR. The antioxidant activity against DPPH radical scavenging the isolated compound 1, methanol and ethyl acetate extract are also briefly described.

2. Materials and Methods

2.1 General experimental

NMR spectra were recorded on JEOL 600 ECA spectrometer using $CDCL_3$ at 600 (^1H) and 125 (^{13}C) MHz. UV and FTIR spectrum recorded in KBr powder with Shimadzu series 1800 spectrophotometer.

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Gravity Coloumn Chromatography (GCC) and radial chromatography were carried out using Si gel 60 GF254 and Si gel PF254 for TLC analysis and pre-coated silica gel plates (Merck, Darmstadt, Germany, Kieselgel 60 GF254 0,25 mm thickness) were used.

2.2 Plant Material

Sample of yam peels of uwi Tuban were collected in March 2015 from the district Tuban, East Java, Indonesia, and the specimen was deposited at the Department of Biology, Fac. Of Science and Technology, Universitas Airlangga. The yam peels were cleaned, dried under the shade , cut into small pieces and milled.

2.3 Extraction and Isolation

The dried yam peels of uwi Tuban (2.1 kg) were macerated in methanol at room temperature (3x24 hours). The methanol extract was evaporated under reduced pressure to give a dark brown residue (88 g). The crude methanol extract (88 g) was partitioned respectively with n-hexane and ethyl acetate. The ethyl acetate extract (16 g) was separated by vacum coloumn chromatography on silica gel using eluent the mixture of n-hexane and ethyl acetate with increasing polarity gradient and gravity coloumn chromatography with the same method of eluent. The subfraction that showed two spot on TLC test, then purified using radial chromatography using the mixture of chloroform-aceton (increasing polarity gradient) yielded pure compound that showed one spot on TLC test (15 mg)

3.4 DPPH Radical Scavenging

The antioxidant assay of compound **1** against DPPH (2,2-diphenyl-1-picrihidrazil) radical was measured by UV-Vis spectrophotometer λ 517 nm as described previously [10]. The inhibition precentage (%) of radical scavenging activity was calculated using the following equation

:

$$\text{Inhibition (\%)} = \left(\frac{Ao - As}{Ao}\right) x\ 100\%$$

Where Ao is the absorbance of the control (containing all reagents except the test compound), and As is the absorbance of the test compound.

3. Results and Discussions

Extraction of the dried milled of yam peel of uwi Tuban (2.1 kg) was carried out using methanol and then methanol extract was portitioned succesively with n-hexane and ethyl acetate. The ethyl acetate extract (16 g) was separated by gravity coloumn chromatography on silica gel and radial chromatography yielded 3,3-dihydroxy-5-methoxybibenzyl or batatasin III (15mg) (**Figure 1**)

The compound of 3,3-dihydroxy-5-methoxybibenzyl (**1**) was isolated as a brown liquid (15 mg). UV spectrum (MeOH) λ_{max} (log ε) 226.4 nm(3.67) and 274.2 nm (2.42). IR ν_{max} cm^{-1} 3334.93, 2937.59, 2858.51,1697.36, 1494.83, 1149.57, 1058.92, 692.44. 1H-NMR (600 MHz, $CDCL_3$) δ 2.84 (4H, m, H-6' a,b), 3.77 (1H, s, 5'-OMe), 6.28 (1H, s , H-2', H-4'), 6.35 (1H, s, H-6'), 6.66 (1H, s, H-2), 6.70 (1H, d, J = 12, H-4), 6.77 (2H, d, J = 6, H-6), 7.17 (3H, t, J= 6, 12, H-5). ^{13}C-NMR (150 MHz, $CDCL_3$) 160.79 (C 5'-OMe), 144.45 (C-1'), 143.57 (C-1), 129.56 (C-5), 120.92 (C-6), 115.43 (C-2), 112.94 (C-4), 108.09 (C-2'), 106.88 (C-6'), 99.17 (C-4'), 37.63 (C-6a) , 37.29 (C-6b). The ^{1}H-NMR (Table **1**) spectrum of **1** exhibited the presence seven aromatic proton at δ 6.28 (1H, s , H-2', H-4'), 6.35 (1H, s, H-6'), 6.66 (1H, s, H-2), 6.70 (1H, d, J = 12, H-4), 6.77 (2H, d, J = 6, H-6), 7.17 (3H, t, J= 6, 12, H-5), two methylene proton at δ 2.84 (4H, m, H-6' a,b). The ^{13}C-NMR spectrum showed the presence seven aromatic ring, two hydroxy aromatic ring (δ 156.51 and 155.45), one methoxy aromatic ring (δ 160.79), one methoxy (δ 55.31) and two methylene carbon (δ 37.29 and 37.62). Base on NMR data, it is predicted that compound **1** is a dihydrostilbene derivative (**Figure 1**). The methoxy position were confirmed to be C-5' by HMBC. And compound 1 contain two hydroxy (C-3' and C-3) by HSQC spectrum data. Based on futher comparison with published data (**Tabel 2**)[9], the structure of 1 was identified as 3,3-dihydroxy-5-methoxybibenzyl with the trivial name batatasin III. The complete of HMBC correlations consistent with the structure **1** are shown in **Table 1** and **Figure 2**.

Based on the literature study, this compound has never been reported yet from *D. alata* especially from Indonesia.

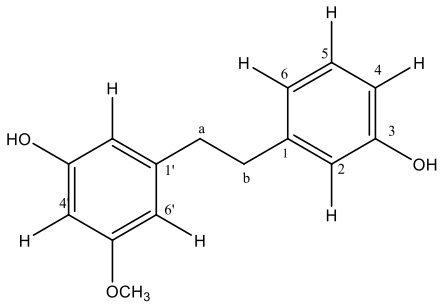

Figure 1. Structure of 3,3-dihydroxy-5-methoxybibenzyl

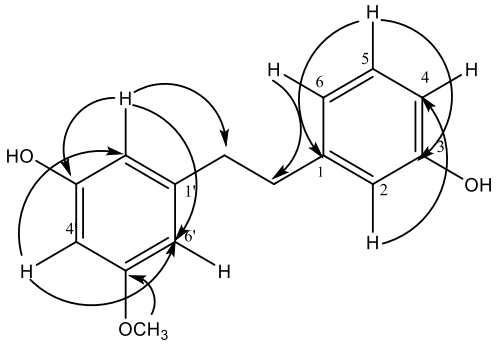

Figure 2. Selected HMBC correlation for compound **1**.

Table 1. NMR spectroscopic data of 3,3-dihydroxy-5-methoxybibenzyl in $CDCL_3$

No	^{1}H (m, *J* in Hz)	^{13}C	HMBC
1	-	143.45	
2	6.66 (IH, s)	115.43	
3	-	155.45	
4	6.70(IH, d, J=12)	112.94	
5	7.17 (1H, t, J=6;12)	129.56	C-1, C-3
6	6.77 (1H, d, J=6)	120.92	C-6'a,b , C-4, C-1
1'	-	144.45	
2'	6.28 (1H, s)	108.09	C-6' a,b , C-4', 3'OH
3'	-	154.45	
4'	6.28(1H, s)	99.71	C-6', 2'-OH, C-5' OMe
5'	-	160.79	
6'	6.35 (1H, s)	106.88	C-2', C-4', C-5' OMe
a	2.84 (2H, m)	37.29	C-6', C-1, C-1'
b	2.84 (2H, m)	37.62	C-6', C-1, C-1'
3-OH	5.22 (1H, sbr)	-	
3'-OH	5.22 (1H, sbr)	-	

5'-OMe	3.77 (3H, s)	55.31

Tabel 2. The comparison of chemical shift data of 3,3-dihydroxy-5-methoxybibenzyl from isolated compound *B. striata* [9]

Isolated compound from *D. alata*			3,3-dihydroxy-5-methoxybibenzyl rom *B. striata* [9]		
No	**^{1}H (m, *J* in Hz)**	**^{13}C**	**No**	**^{1}H (m, *J* in Hz)**	**^{13}C**
1	-	143.45	1	-	143.5
2	6.66 (IH, s)	115.43	2	6.65	121.2
3	-	155.45	3	-	129.7
4	6.70(IH, d, ***J***=12)	112.94	4	6.70	113.1
5	7.17 (1H, t, ***J***=6 ;12)	129.56	5	7.14 (1H, m)	155.7
6	6.77 (1H, d, ***J***=6)	120.92	6	6.75 (1H, d, ***J***=8)	115.5
1'	-	144.45	1'	-	144.6
2'	6.28 (1H, s)	108.09	2'	6.25	108.1
3'	-	154.45	3'	-	156.7
4'	6.28(1H, s)	99.71	4'	6.28	99.33
5'	-	160.79	5'	-	161.1
6'	6.35 (1H, s)	106.88	6'	6.32	107.0
a	2.84 (2H, m)	37.29	a	2.84 (2H, m)	37.8
b	2.84 (2H, m)	37.62	b	2.79 (2H, m)	37.5
3-OH	5.22 (1H, sbr)	-	3-OH		-
3'-OH	5.22 (1H, sbr)	-	3'-OH		-
5'-OMe	3.77 (3H, s)	55.31	5'-OMe	3.75(3H, s)	55.4

4. Conclusions

On antioxidant evaluation against DPPH radical scavenging of compound **1** exhibited IC_{50} values of 206.82 μg/mL suggested that compound **1** has moderate activity. This compound more active than IC_{50} of methanol extract (893.59 μg/mL) and less active than ethyl acetate extract (109.66 μg/mL).

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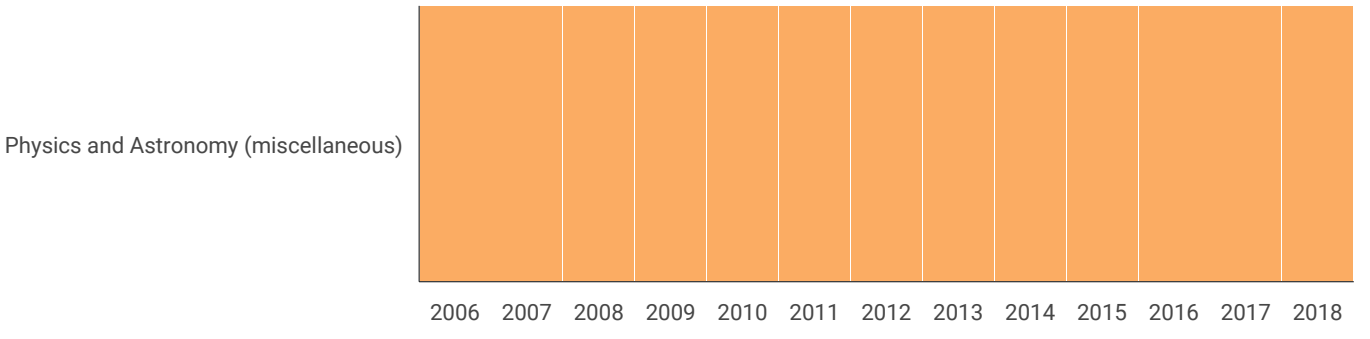

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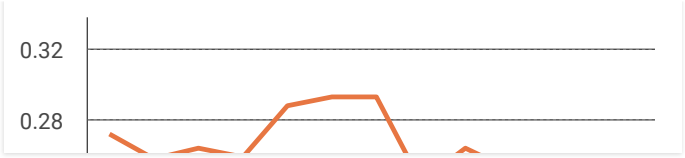

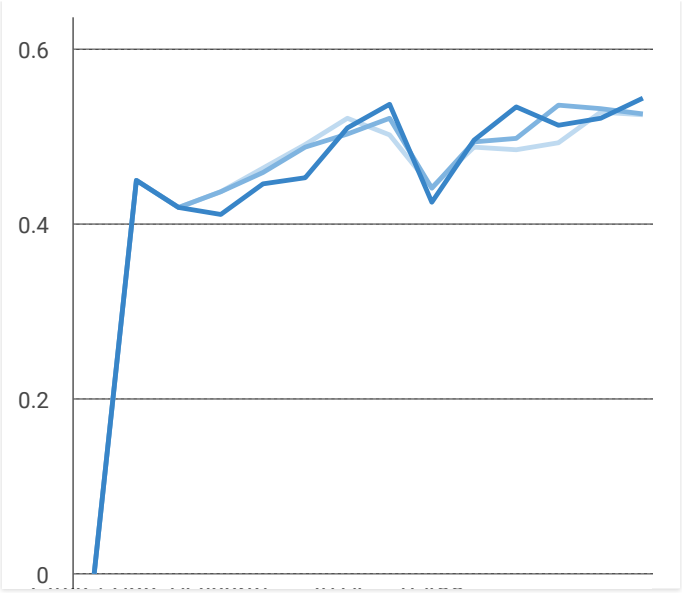

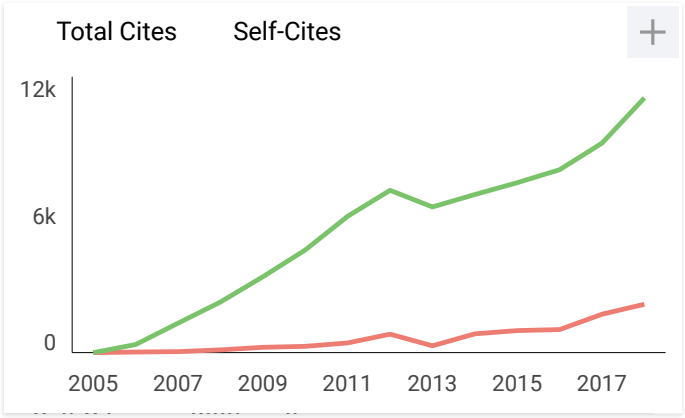

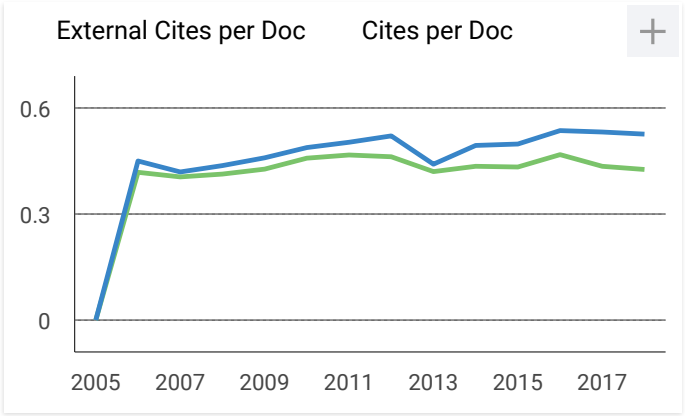

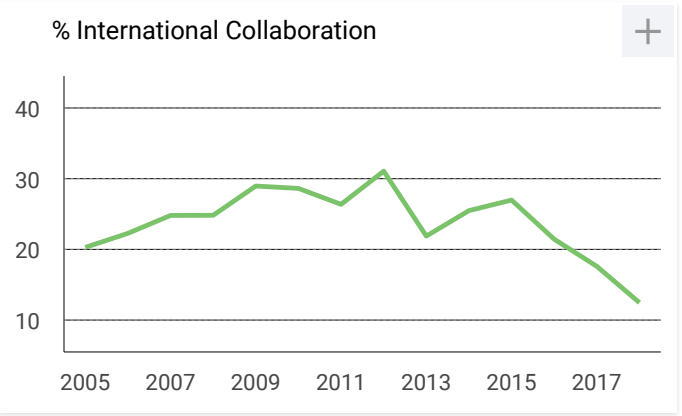

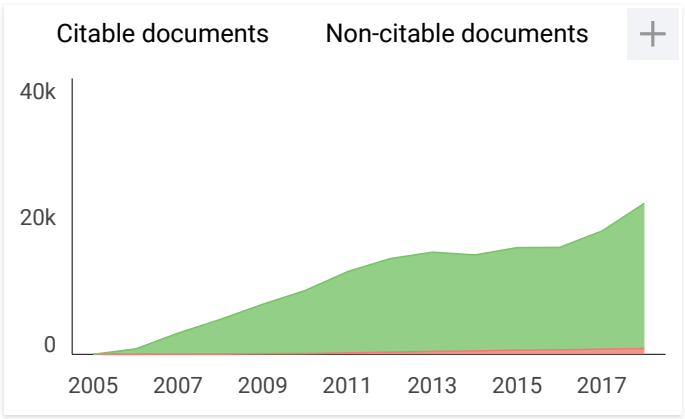

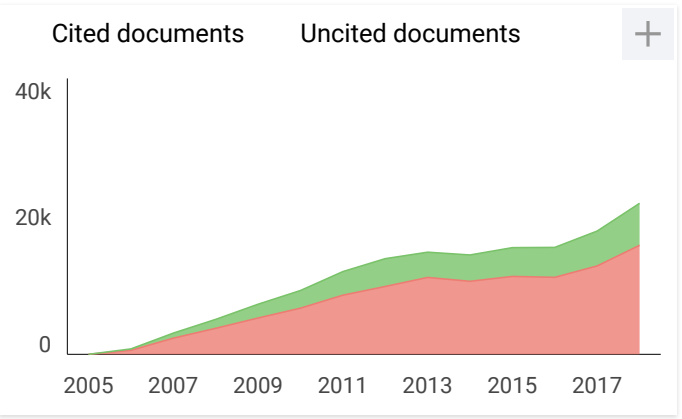

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