Identification of Cadalene-β-Carboxylic Acid From Barks of Bawang Hutan (*Scorodocarpus borneensis* Becc.)

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Abstract

One sesquiterpene compound has been isolated and identified from ethylacetate extract of bawang hutan (*Scorodocarpus borneensis Becc.*) barks. The barks were macerated with methanol, and then partitioned with mixture of ethylacetate-water (1:1). Fractionation of the ethylacetate phase by column chromatography gave pure compound. Based on data interpretation from of ultra-violet (UV) spectra, Fourier Transform Infra Red (FT-IR), NMR 1D (1H and 13C-NMR); NMR 2D (HMQC, COSY, HMBC) and comparison with literature, the pure isolated compound was determined as a sesquiterpene compound, cadalene-β-carboxylic acid which exhibit LC_{50} of 42.32 ppm.

Keywords: bawang hutan, *Scorodocarpus borneensis*, Olaceaeae, brine shrimp lethality test

Introduction

Indonesia is well-known for its abundant plant natural product resources. Since thousand years, Indonesian people have used medicinal plants in order to overcome many health problems. The knowledge about medicinal plants is inherited from generation to generation.

Cancer, one of the major threats in health issues, is a disease where cells of body tissue showed abnormal, uncontrolled and fast growing. Several cancer common therapies such as surgery, radiotherapy and chemotherapy in general have not yet to give satisfactory results as they exhibit great side effects and require expensive costs. Therefore, it is necessary to find other alternative treatment for curing cancer, such as using medicinal plants (Kintzios, 2004).

Many researches keep looking for anticancer compounds from biodiversity available in Indonesia, both by reviewing traditional usage or chemo-taxonomical approach. The discovery of anticancer medicine from plant requires reliable tests starting from pre-screening to clinical test (Soeksmanto *et al.*, 2010).

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One of potential plants as medicine source is bawang hutan (*Scorodocarpus borneensis* Becc.) from Kalimantan, Indonesia. Some researches on the isolation of bioactive compounds in bawang hutan were pionereed by Kubota *et al.* (1994a, 1994b) and Wiart *et al.* (2001). The result showed that the fruit of bawang hutan contained sulphuric compounds identified as methyl methylthiomethyl disulphide and bis (methylthiomethyl) disulphide which gave characteristic aroma similar to garlic, making the native people frequently use it as spice. It also contains other compounds such as methyl thiomethyl (methylsulphonil) methyldisulphide that has antimicrobial activity against some bacteria 2,4,5-trithiahexane; 2,4,5,7-tetrathiaoctane; 2,4,5,7-tetrathiaoctane-2,2-dioxide; 2,4,5,7-tetrathiaoctane-4,4-dioxide which can prevent platelet aggregation in rabbit (Wiart *et al.*, 2001; Lim *et al.*,1999). However, many researches on this plant give no focus on this plant.

Based on those studies, it is very important to isolate and characterize one of the chemical compounds isolated from ethylacetate phase of bawang hutan barks using Brine Shrimp Lethality Test (BSLT) method as leading test. This method was used in preliminary screening of active anticancer compound from natural product based on positive correlation between toxicity and potency for anti-cancer (Meyer *et al.*, 1982). The aim of this research

was to isolate and characterize one of bioactive compounds from ethylacetate phase of Bawang hutan (*Scorodocarpus borneensis* Becc.) barks.

Materials and Methods

General. Melting point was measured by using Melting Point Electromantle 9100. UV spectrum was measured using UV-Vis Shimadzu UV-1700 Spectrometer. IR spectrum was obtained by using FTIR 8400S Shimadzu Spectrometer, 13C-NMR and $_1$H-NMR spectrum were determined using JEOL ECA 500 Spectrometer operated at 500 MHz ($_1$H) and 125 MHz (^{13}C). Column Chromatography was performed by using silica gel Merck 60 GF_{254} (230–400 mesh), while thin layer chromatography analysis was conducted with aluminium plate coated by silica gel Merck Kieselgel 60 GF_{254} 0.25 mm. All eluent used in this experiments were distilled technical grade eluents.

Plant Material. Plant material used was bark of bawang hutan (*Scorodocarpus borneensis* L, *Olecaceae*) collected from Samarinda (East Kalimantan, Indonesia). Plant identification was performed at Herbarium Bogoriensis, Indonesian Institute of Sciences (LIPI) Cibinong, Indonesia. Plant specimen was then kept in the institute.

Extraction and Isolation. Dry sliced barks of *S. borneensis* (2 kg) were extracted using reflux method (3´3) with methanol as eluent. The methanol extract resulted from extraction (74.05 g) were partitioned with mixture of water: ethylacetate (1 : 1) three times. Twenty two grams of ethylacetate extract were fractionated by column chromatography using SiO_2 and mixture of *n*-hexane: ethylacetate (50: 1 ~ 1 : 1) with 10 fractions (SBEA-1 ~ SBEA-10) as the results. Fraction SBEA-4 was then further fractionated using similiar method (SiO_2, n-hexane : ethylacetate (10 : 1 ~ 1 : 1) resulted 7 fractions (SBEA-4-1 ~ SBEA-4-7). Toxicity test for those 7 fractions showed that fraction SBEA-4-2 (10 mg) with LC_{50} 42.32 ppm is the most active fraction amongst all fractions tested. Thin layer chromatography also showed that the fraction had migh has single spot and it was considered pure for further spectrometric analysis.

Biological Assay. Eggs of Brine shrimp (*Artemia salina*) were hatched in 100 mL saline water (NaCl at 3.8%) using Beaker glass at 37°C. After 48, 10 larvaes were put into test vial filled with 10 mL of saline water. Ten µL sample solution was placed into test vials with 3 replicates for each concentration. One hundred µL blank added with saline water to 1 mL were used as control. Observation was performed after 24 h by counting dead larvae and the survival. LC50 value was determined by simple computing program for probit analysis with trust level at 95%. An extract or a fraction was considered active when it has LC50 < 1000 µg/mL and has LC50 < 30 µg/mL for pure compound (Meyer *et al.*, 1982).

Results and Discussion

Compound SBEA-4-2 was obtained as white needle crystal with uncorrected melting point. Molecular formula was determined as $C_{15}H_{16}O_2$based on GC-MS data with *m/z* 220 [M]+. UV spectrum in methanol showed maximum absorbance at λ max 291.0, 328.5 and 342.0 nm. Based on NMR data (Table 1) SBEA-4-2 had 8 degree of unsaturation. The IR spectrum showed absorbance band from hydroxyl group (3502 cm^{-1}), carbonyl (1694 cm^{-1}), olifenic (1619 cm^{-1}), and C-O from esther (1102 cm^{-1}).

The ^{13}C-NMR spectrum and DEPT data gave carbon signals consisted of 3 methyl carbon, 5 double bonded methyne carbon, 5 quartenary carbon, 1 single bonded carbon, and 1 carbonyl carbon. ^{1}H-NMR spectrum showed a septet signal at δH 3.85, typical for proton which has resonance with 2 methyl groups: 4 aromatic proton signals at δH 8.10 (d, J = 2 Hz; H-8), δH 8.15 (d, J = 2 Hz; H-7), δH 7.39 (d, J = 2 Hz; H-3), δH 7.42 (d, J = 2 Hz; H-2).

Based on spectrum data above, compound SBEA-4-2 had aromatic ring adjacent one to another. The results of NMR 2D data analysis (1H-1H COSY, HMBC) were summarized on Figure 1. The ^{1}H-^{1}H COSY spectrum showed that aromatic proton at δH 8.10 ppm (H-8) was correlated with δH 8.15 ppm (H-7), δH 7.42 ppm (H-2) correlated with δH 7.39 ppm (H-3), and δH 3.85 ppm (H-11) correlated with two methyl groups at δH 1.43 ppm (12-CH_3 and 13-CH_3). The NMR 2D HMBC spectrum showed correlation between proton and carbon to 2–3 bonds, i.e at δH 8.10 ppm (H-8) with δC 125.59 (C-8), 125.91 (C-6), 130.79 (C-10), and δH 8.15 ppm (H-7) with δC 128.16 (C-5), 172.47 (C-15), 135.84 (C-9), and

δH 2.69 ppm (H-14) with δC 132.32 (C-1), 135,84 (C-9), 129,46 (C-2) and δH 1.43 ppm (H-12/H-13) with δC 28.63 (C-11), and δH 3.85 ppm (H-11) with δC 129.46 (C-2);144.91(C-4);122.60 ppm (C-3).

Based on data above and comparison NMR data on Table 1, we concluded that compound SBEA-4-2 is cadalene ?- carboxylic (Figure 2) (Wiart *et al.*, 2001).

Results of toxicity test for ethylacetate extracts and compound SBEA-4-2 to shrimp larvae were summarized at Table 2. The results showed that ethylacetate extracts and SBEA-4-2 pure compound had toxicity with LC_{50} value of 31.17 ?g/mL and 42.32 ?g/mL respectively.

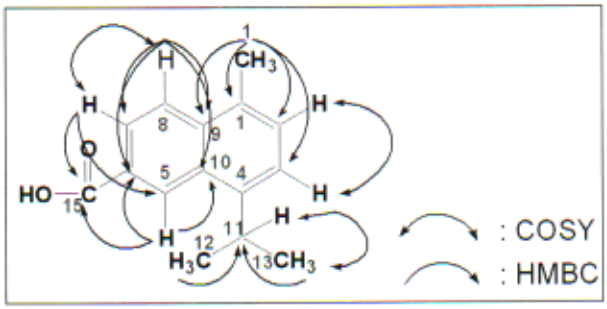

Figure 1. COSY and HMBC experiment for SBEA-4-2 compound.

Table 1. Chemical shift of (1H dan 13C NMR) for compound SBEA-4-2.

No	δc (ppm,125 MHz)		δH (ppm,500 MHz)	
	SBEA 4.2	Reference*	SBEA 4.2	Reference*
1	132.32	132.1	-	-
2	129.46	129.3	7.42 (d)	7.46 (d)
3	122.60	122.4	7.39 (d)	7.44 (d)
4	144.91	144.7	-	-
5	128.16	128.0	9.05 (s)	9.08 (d)
6	125.91	125.8	-	-
7	124.84	124.6	8.15 (d,d)	8.18 (d,d)
8	125.59	125.4	8.10 (d)	8.12 (d)
9	135.84	135.7	-	-
10	130.79	130.7	-	-
11	28.63	28.5	3 . 8 5 (sept)	3.88 (sept)
12	23.98	23.8	1.43 (d)	1.46 (d)
13	23.98	23.8	1.43 (d)	1.46 (d)
14	19.66	19.5	2.69 (s)	2.72 (s)
15	172.47	172.7	-	-

Table 2. Toxicity Test Results for ethylacetate extract of *S. borneensis* (SBEA-1 - SBEA-10 and SBEA-4-1 - SBEA-4-7)

No	Sample	LC_{50} (ppm)
1	Ethylacetate extract	31.17
2	SBEA-1	108.78
3	SBEA-2	103.97
4	SBEA-3	60.46
5	SBEA-4	28.25
6	SBEA-5	35.73
7	SBEA-6	45.70
8	SBEA-7	42.12
9	SBEA-8	57.14
10	SBEA-9	63.72
11	SBEA-10	81.44
12	SBEA-4-1	381.55
13	SBEA-4-2	42.32
14	SBEA-4-3	53.99
15	SBEA-4-4	186.36
16	SBEA-4-5	265.08
17	SBEA-4-6	209.08
18	SBEA-4-7	124.80

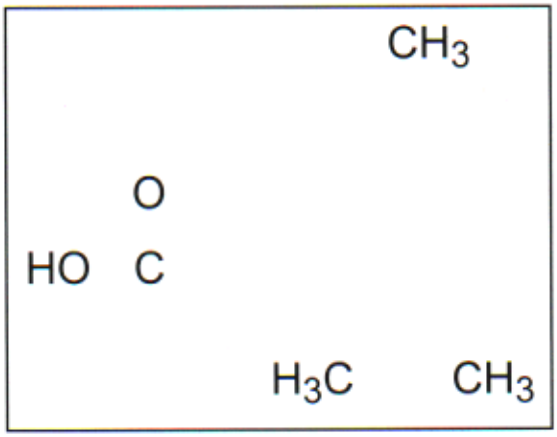

Figure 2. Chemical structure of cadalene – β – carboxylic acid

Conclusions

One compound known as cadalene – β – carboxylic acid had been identified from ethyl acetat extract of Bawang Hutan (*Scorodocarpus borneensis* Becc.)

Acknowledgment

We would like to thank Herbarium Bogoriensis, Research Center for Biology, Indonesian Institute of Sciences (LIPI), Indonesia for identification of the plant sample. We also thank Yulvivil Tridaris for her assistance during this experiment.

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