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Anticancer Activity of Bioactive Compounds from Fruits of Bawang Hutan (*Scorodocarpus borneensis* Becc)

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In this research, dehydroxyscorodocarpin B was isolated from fruits of Bawang hutan and tested its anticancer activity against L_{1210} line with resulted anticancer activity IC_{50} value.

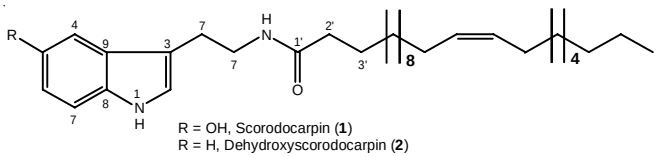
Keywords: Bawang hutan, Dehydroxyscorodocarpin B, Anticancer.

INTRODUCTION

One of the Indonesian native medicinal plants is Bawang hutan (*Scorodocarpus borneensis* Becc) that widely grow in tropical forest of Sumatra island and Kalimantan island. Parts of this plant are used as spice for its aroma and properties that similar with garlic (*Allium sativa* L). Fruits of Bawang hutan can be used as antibacterial and antifungal agents as they contain flavonoid, saponin, steroid and methylthiomethyl compounds[1,2]. Leaves of Bawang hutan are used as diarrhea medicine. Abe and Yamauchi[3] reported that the leaves of Bawang hutan contain several compounds *viz.*, methylthiomethyl and flavonoids. Methylthiomethyl sulphide compound has polysulphide pathway similar with garlic species (*Allium sativa L*) has anticancer activity[2,3].

Many developing epidemiolocical researchers reported that garlic and other plants containing organosulphuric compounds can prevent cancer in human, including colon cancer. Some researches showed that people consumed garlic have lower risk to get stomach and colon cancer[4].

Fruits of Bawang hutan contain scorodocarpin B and dehydroxyscorodocarpin B compounds[5] (Fig. 1).



R = OH, Scorodocarpin (**1**)
R = H, Dehydroxyscorodocarpin (**2**)

Fig. 1. Scorodocarpin B and dehydroxyscorodocarpin B

This research was conducted to evaluate the anticancer activity of scorodocarpin B and dehydroxyscorodocarpin B compounds, which are found in the fruits of Bawang hutan (*Scorodocarpus borneensis* Becc).

EXPERIMENTAL

Plants of Bawang hutan were collected from Samarinda Botanical Garden and determined by Herbarium Bogoriense, Research Centre for Biology, Indonesian Institute of Sciences (LIPI) Bogor, Indonesia. The speciments were determined as *Scorodocarpus borneensis* Becc. from *Olacaceae* family.

Extraction of bawang hutan by maceration: 500 g sliced fruits of Bawang hutan were macerated with 1 L of ethanol for 24 h, filtered and macerated again for 7 times to get optimum maceration with no colour appeared in the extracts. All extracts were collected and evaporated to get dry crude extracts (ethanol extracts).

Treatment of ethanolic extract with *n*-hexane:water: Ethanolic extract was partitioned with *n*-hexane:water (1:1) using separation funnel. *n*-Hexane fraction and water fraction obtained were evaporated to get dry extracts.

Active compounds purification using column chromatography method: Column chromatography for *n*-hexane fractions were done using mixture of *n*-hexane:ethyl acetate (20:1~1:1) for gradient column chromatography; and *n*-hexane:ethyl acetate (2:1) for isocratic column chromatography. Silica gel F_{254} were used as stationary phase.

Antioxidant activity test using free radical scavenging method. Antioxidant activity test were done using 1,1-

diphenyl-2-picrylhyidrazil (DPPH) as reagent and vitamin C as positive control according to free radical scavenging method. Measurements were performed using spectrophotometer at wavelength 515 nm.

Preparation of medium for anticancer test using Leukimia cell line L_{1210}: 4.7 g Eagle's MEM medium were dissolved in 475 mL water to make solution A. Meanwhile, 1.3 g $NaHCO_3$ were dissolved in 50 mL water and added with 0.3 g glutamine to make solution B. 25 mL to make 500 mL medium for screening process. This medium were filtered with sterile millipore 0.2 μm and stored in refrigrator. To make medium for propagation of cell line L_{1210}, 85 mL of this medium were added with 15 mL foetal bovine serum. All preparation were done aseptically in sterile room.

Preparation of cell stock: One tube of frozen cells was soaked in warm water (37 °C) for few minutes. The cells were transfered to new tube containing 4 mL medium. The tube was stirred for 30 s and centrifuged at velocity 1000 rpm for 1 min. Medium were removed from the tube and replaced with 4 mL new medium. This cleaning process were repeated for 3 times.

The cell line L_{1210} were transferred to incubator vessel by rinsing tube with 2 mL medium for 3 times. Medium were added to get final volume 10 mL before incubated at 37 °C in CO_2 incubator for 48 h. The CO_2 level in the incubator was 5 %. The cell propagation will be observed under microscope for measuring viable cell/mL medium and observing contamination. Viable cells were appeared as clear sphere with blue spot of nucleus in the center, while the dead ones appeared as irregular dark blue spot after addition of tryphan blue (1 mL for 1 mL cell, diluted to concentration 2×10^5 cells/mL).

Anticancer activity test for extracts against cell line L_{1210}: The test for extracts were done *in vitro* according to procedure used by Fujimoto *et al.*[6] with 2 replication. 10 μL of sample solution were added to multiwell plate tissue culture containing 2×10^5 cells/mL each. 10 μL of aquadest were used as control. The plate was incubated in CO_2 incubator at 37 °C for 48 h. Measurement were done under microscope using haemocytometer.

RESULTS AND DISCUSSION

Antioxidant activity test for extracts using free radical scavenging method: The results (Table-1) showed that *n*-hexane fraction had highest antioxidant activity with IC_{50} 60.075 ppm. Anticancer activity test followed for *n*-hexane fraction and ethyl acetate fraction gave IC_{50} 15,33 ppm and 34,3479 ppm respectively. According to National Cancer Institute (2003), a sample is considered as potential for anticancer if the sample has IC_{50} value less than 20 ppm. Based on the results, further step for purification using column chromatography method was done to *n*-hexane fraction.

TABLE-1
RESULTS OF ANTIOXIDANT ACTIVITY TEST FOR EXTRACTS

Fraction	Concentration (ppm)			
	25	50	100	IC_{50}
n-Hexane	24.24	43.40	78.59	**60.075**
Ethyl acetate	19.89	34.98	75.91	66.795
Water	18.28	26.65	53.67	93.905

Purification of *n*-hexane fraction by column chromatography method. Purification of *n*-hexane fraction by column chromatography method were done using *n*-hexane and ethyl acetate as eluent. The process gave total 9 fractions. These fractions were tested for their antioxidant activity. Two fractions showed highest activity, *i.e.* fractions 8 and 9 with IC_{50} 49.313 ppm and 65.279 ppm, respectively.

Fractions 8 and 9 were purified further by column chromatography method using *n*-hexane and ethylacetate (2:1) as eluent and gave 5 fractions with fraction B8.4 showed pure compound at R_f 0.26. Those fractions were tested further for their antioxidant activity. Fraction 8.4 showed highest activity with IC_{50} value 42.296 ppm (Table-2) while fraction B9.4 showed highest activity with IC_{50} value 51.157 ppm (Table-3).

TABLE-2
RESULTS OF ANTIOXIDANT ACTIVITY TEST FOR FRACTION 8

Fraction	Concentration (ppm)			
	25	50	100	IC_{50}
B-8.1	16.69	33.73	55.91	86.787
B-8.2	12.72	25.32	41.93	119.556
B-8.3	18.02	31.89	52.85	92.719
B-8.4	36.08	60.78	79.78	**42.296**
B-8.5	23.28	40.93	66.66	69.535

TABLE-3
RESULTS OF ANTIOXIDANT ACTIVITY TEST FOR FRACTION 9

Fraction	Concentration (ppm)			
	25	50	100	IC_{50}
B-9.1	17.31	30.69	56.43	87.510
B-9.2	27.60	44.55	77.34	58.590
B-9.3	11.53	22.79	50.56	99.648
B-9.4	32.54	49.21	82.63	**51.157**
B-9.5	19.69	33.61	64.56	76.152

Further step for pure active fractions is structure elucidation using spectral data of UV spectroscopy, FT-IR, RMI (1D, 2D) and LC-MS. Fraction B8.4 (isolate 8.4.1) was identified as dehydroxyscorodocarpin B and fraction B9.4 (isolate 9.4.1) was identified as scorodocarpin B[5].

The pure compounds obtained were tested further for their anticancer activity using cell line L_{1210} (limphoid leukemia). Limphoid leukemia cell line L_{1210} is tumor cells isolated from rat spleen. This cells can propagated and widely spreaded to other organs and causing death in 8-11 hari with growth level 100 %[7].

Doxorubisin was used as positive control with IC_{50} value 0.1763 ppm. Doxorubisin usually used as antiproliferation standard in cancer cells test. In this test, dehydroxyscorordocarpin B gave highest anticancer activity with IC_{50} value 1.1061 μg/mL. Meanwhile, scorodocarpin B gave highest anticancer activity with IC_{50} value 1.7053 μg/mL.

According to National Cancer Institute (2003), a sample is considered as potential for anticancer if the sample has IC_{50} value less than 20 ppm. Based on the results, dehydroxyscorodocarpin B and scorodocarpin B isolated from fruits of Bawang hutan (*Scorodocarpus borneensis* Becc) will be a potential anticancer agent in the future.

Conclusion

Active fractions isolated from fruits of Bawang hutan (*Scorodocarpus borneensis* Becc) were determined as scorodocarpin B and dehydroxyscorodocarpin B compounds based on spectral data. Dehydroxyscorodocarpin B and scorodocarpin B showed activity as potential anticancer agent with IC_{50} value 1.1061 µg/mL and 1.7053 µg/mL respectively against cell line L_{1210}.

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