INSECTICIDAL ACTIVITY OF BRUCEIN-C FROM BUAH MAKASAR (*Brucea javanica*) AGAINST *Helopeltis antonii* AND *Dacinus piperis*

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ABSTRACT

Cashew and pepper are kind of important commodity in Indonesia. In recent times, productivity of these plants decreased due to insect pest *Helopeltis antonii* and *Dacinus piperis*. Generally, farmers use synthetic insecticide to control these pests. The use of synthetic insecticide gives risk to human and environmental. Therefore, there is an urgent need to find natural insecticide to control cashew and pepper insect pest. Many researches showed that buah makasar (*Brucea javanica*) contains quassinoid compounds having insecticidal activities against several pests (Subeki *et al*., 2006; Latif *et al*, 2000; Daido *et al*., 1995; and Klocke *et al*., 1985). In this research was carried out extraction and isolation of brucein-C from *B. javanica* and assayed its activity against *H. antonii* and *D. piperis*. Result of this research showed that bruceine-C formulated with liposomes at concentration 200 ppm gave mortality of *H. antonii* and *D. piperis* with value of 98.3% and 91.7%, respectively. Residual contents of bruceine-C in the soil on the fifteenth and thirtieth day after application at concentration 200 ppm decreased to 80 ppm and 0 ppm, respectively. Application of bruceine-C has no lethal effect against non target insects. Hypothetical study on the technoeconomy showed that insecticide with active substance brucein-C have price 40,3% cheaper than synthetic insecticides. This research can be recommended to Indonesian government to produce natural insecticide from the original Indonesian plant buah makasar.

Keywords: *Brucea javanica*, Brucein-C, Buah makasar, *Dacinus piperis*, *Helopeltis antonii*,

INTRODUCTION

In Indonesia the losses caused by insect pests is about 10 to 60 % of the total cashew and pepper production evidencing the relevancy of the damages. One of the main pests associated to cashew and pepper are *Helopeltis antonii* and *Dacinus piperis* due to their high biotic potential and to their broad host range since they can also attack chocolate. Insects of *H. antonii* and *D. pipperis* have a large destructive potential in cashew and pepper plants because they are able to damage the young leaf, flower, and fruit within a week. The pest feeds outside cashew and pepper and as a result the fruit become black spot.

Cashew and pepper pest control has been realized in large scale by chemical products. Current researches and the increasing knowledge about the harm derived from the indiscriminate use of these products as environmental and human contamination, existence of residues in foods and the consumer concern with food quality have encouraged studies related to novel tactics of pest control like the use of natural insecticides. These natural insecticides, present several advantages in relation to synthetic compounds as their rapid biodegradation reducing the risks of environment and food contamination besides the easy way of obtaining and preparation (Vendramim and Castiglioni, 2000).

Several plants may have insecticidal activities against insects and among them, *Brucea javanica*) is a medicinal plant widely distributed in Indonesia. *B. javanica* locally known as buah makasar is used in the folk medicine to treat malaria, dysentery, and cancer. Several quassinoids with a broad range of bioactivity as antitumor, antiamoebic, and antimalarial, have been isolated previously from this plant (Lee, *et al*., 1979; Anderson, *et al*., 1991; Yang, *et al,* 1996; Wright, *et al.,* 1988; O'Neill, *et al*., 1987). These quassinoids also showed insecticidal activity against *Plutella xylostella, Tetranychus urticae, Myzus persicae, Meloidogyne incognita, Heliothis virescens,* and *Spodoptera frugiperda* (Okano *et al*., 1994; Latif *et al*, 2000; Daido *et al*., 1993, 1995; Park *et al*., 1987; Klocke *et al*., 1985). In spite of the many phytochemical and pharmacologic investigations, there are no reports on the insecticidal activity of this plant against *H. antonii* and *D. pipperis*. We therefore investigated insecticidal compounds of this plant and herein present data on brucein-C that possess insecticidal activity.

MATERIALS AND METHODS

General

Melting points were measured on a Yazawa micro melting point apparatus. IR spectra were recorded on a Perkin-Elmer 2000 Series FT-IR spectrometer. FAB-MS and HR-FAB-MS were obtained on a Jeol JMS-AX500 mass spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM-500 FT-NMR (500 MHz) and a Jeol JNM-EX 270 FT-NMR (270 MHz) spectrometer, respectively. Optical rotations were determined on a JASCO DIP-370 digital polarimeter. Column chromatography was performed on silica gel 60 (Spherical, 70-140 mesh ASTM, Kanto Chemical).

Silica gel 60 F_{254} pre-coated plates (Merck) were used for analytical TLC and pTLC.

Plant Material

Plant material was purchased from Bandar Jaya traditional market, Central Lampung, in February 2009. The material was identified by Herbalist Aris Winarso at the Herbal Medicine Research and Education Centre "Karya Tama", Lampung, Indonesia. Voucher specimen is deposited at the Laboratory of Bioactive Compound, Department of Agricultural Product Technology, Lampung University.

Extraction and Isolation

Air dried fruits of *B. javanica* (47 kg) were soaked in EtOH 70% for two weeks. The filtrate was filtered and concentrated to give 1 L. The concentrate was extracted with ethyl acetate to give ethyl acetate and aqueous fractions. The active fraction of ethyl acetate was evaporated to give residue and chromatographed on a silica gel column, eluted with $CHCl_3$ (3 L), MeOH/$CHCl_3$ (3:97, 3 L), MeOH/$CHCl_3$ (1:4, 3 L), and MeOH (3 L), successively. The active fraction of MeOH/$CHCl_3$ (1:4) was evaporated and subjected to column chromatography on silica gel, eluted with Hexane/EtOAc (3:7) to give six fractions. The sixth fraction was recrystallized from hexane to give brucein-C. Extraction and isolation of brucein-C was shown at Figure 1.

Preparation of Liposomes

Liposomes were prepared according to method of Chono (2006). Briefly phosphatidylcholine, cholesterol, and diacetylphosphate in a lipid molar ratio of 7:2:1 were dissolved along with [3H] cholesterylhexadecylether as a noexchangeable lipid phase maker in chloroform:methanol (9:1), followed by evaporation to obtain thin lipid film. The lipid film was completely hydrated by phosphate buffer saline (PBS, pH 7.4) to obtain liposomes. Liposomes were then extruded through polycarbonate filter with pore size of 200 nm.

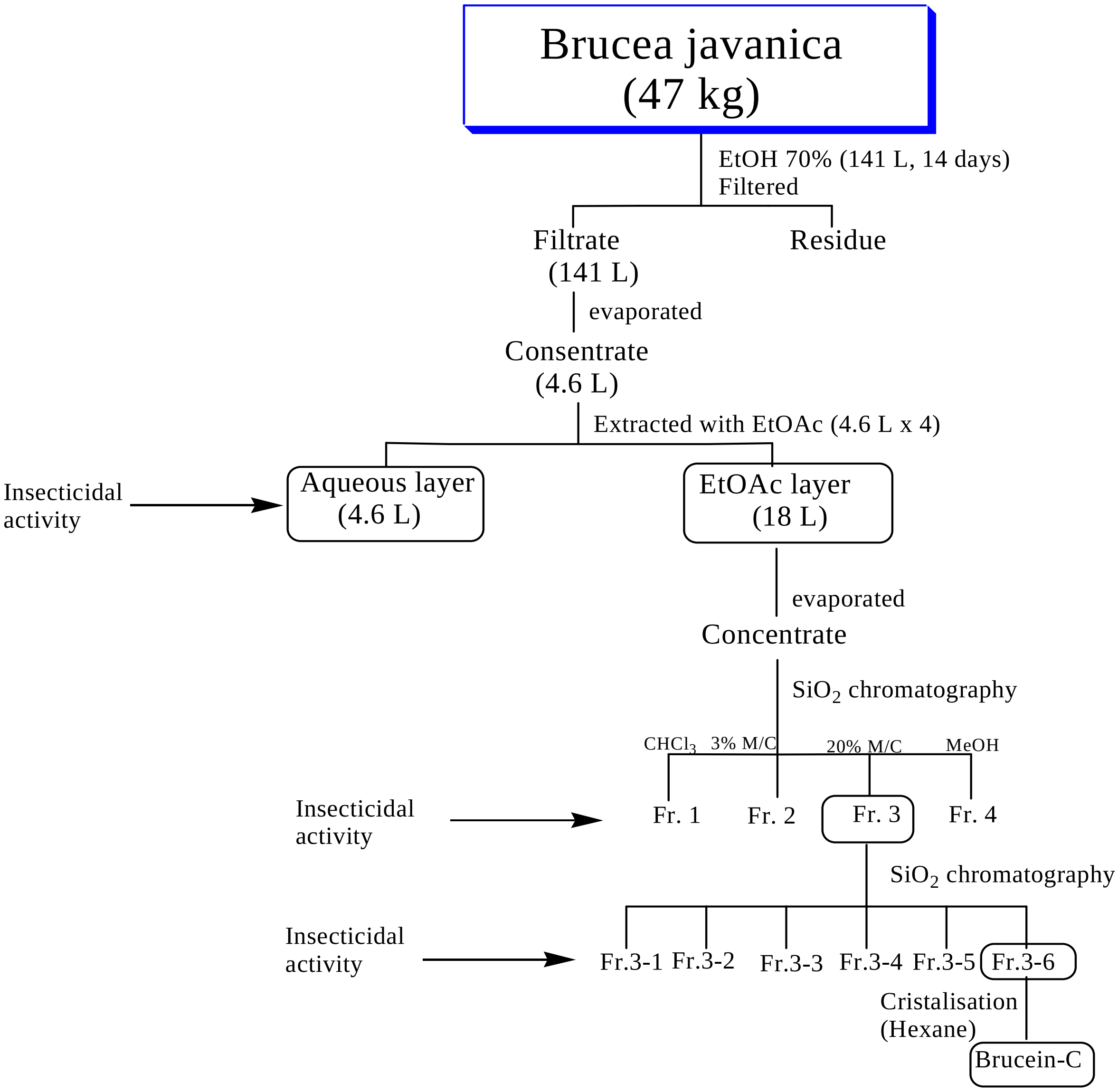

Figure 1. Extraction and isolation of brucein-C from *Brucea javanica*

Insecticidal activity

Insects of *H. antonii* and *D. pipperis* were obtained from field of cashew and pepper plantation. Each kind of insect was put on the cashew and pepper plants covered by net. Each plant contained 50 insects of *H. antonii* or *D. pippperis*. Adaptation of insects in the net were done for one day. Formulation of brucein-C was dissolved in water at concentration of 0, 100, 200, 300, 400, and 500 ppm and sprayed into net containg *H. antonii* and *D. pippperis*. Mortality of insect was evaluated each three hours for three days. Three replications were used per treatment.

Residue of Brucein-C

The experiments were done in the green house. Soil 1 kg were put in polybag and then sprayed with brucein-C at concentration 200 ppm. Content of brucein-C in the soil was evaluated at 0, 15, 30, 45, and 60 after application using HPLC (*High Performance Liquid Chromatography*). Three replications were used per treatment.

RESULTS AND DISCUSSION

Structure Elucidation

Brucein C was isolated from *B. javanica* fruits as a colorless amorphous solid, m.p. 255-256 ^{o}C, and $[\square]^{20}_{D}$ -53.0^{o} (*c* 0.8, pyridine). The IR spectrum displayed characteristic absorptions for hydroxyl (3425 cm^{-1}), □-lactone and ester (1740 cm^{-1}), and □,□-unsaturated carbonyl (1688 and 1644 cm^{-1}) groups. FAB-MS: *m/z* 563 [M-H]$^{+}$; HR-EI-MS *m/z* 563.2143 [M-H]$^{+}$ (calcd. for $C_{28}H_{35}O_{12}$, 563.2129). Data of ^{1}H-NMR and ^{13}C-NMR were shown at Table 1.

Table 1. ^{13}C and ^{1}H-NMR of brucein-C from *Brucea javanica*

Position	□C*	□H*
1	50.2	2.85 (d, 16.0)
2	194.5	
3	145.8	
4	130.5	
5	43.2	2.98 (br, d, 13)
6	30.1	2.31 (ddd, 43.8, 3.0, 3.0)
		1.89 (ddd, 14.8, 14.8, 3.0)
7	84.8	4.92 (br, s)
8	46.6	
9	42.2	2.28 (br, s)
10	42.2	
11	72.9	4.17 (d, 4.7)
12	76.5	4.19 (br, s)
13	82.9	
14	50.5	3.79 (br, s)
15	68.5	6.53 (br, s)
16	167.2	
18	13.4	1.84 (d, 1.8)

19	15.6	1.36 (s)
20	74.5	4.70 (d, 7.4)
		3.71 (d, 7.4)
21	172.1	
OMe	53.2	3.73 (s)
1’	168.5	
2’	113.1	6.07 (s)
3’	169.9	
4’	73.6	
5’	28.5	1.34 (s)
6’	28.5	1.32 (s)
7’	15.7	2.16 (d, 1.3)

*) Chemical shifts d (ppm) and coupling constants *J* in Hz in parentheses (500 MHz, CD_3OD).

Analysis [1]H-NMR showed spectrum resonance three metyl tertier (δ 1.34, 1.32, and 1.36), two olefinic metyl (δ 2.16 and 1.84), and a olefinic proton (δ 6.07). Analysis [13]C-NMR showed spectrum resonance at C-3 (δ 145.8), C-11 (δ 72.9), and C-12 (δ 76.5) indicated hidroxy corelated to carbon. Analysis [13]C NMR (δ 168.5, 113.1, 169.9, 73.6, 28.5, 28.5, and 15.7), COSY, and HMBC showed 4-hydroxy-3,4-dimethyl-2-pentenoyloxy corelated to C-15. Chemical structure and correlation of HMBC and [1]H-[1]H COSY were shown at figure 2 and 3.

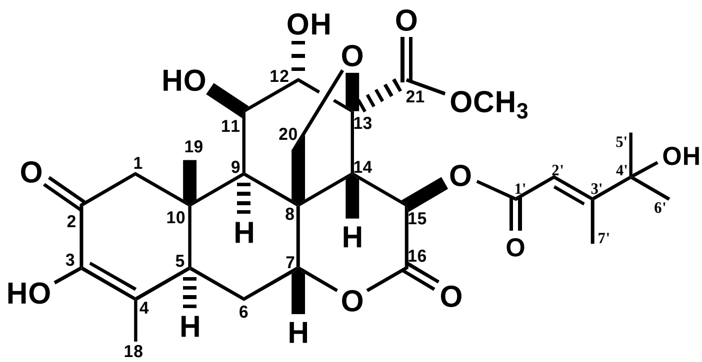

Figure 2. Chemical structure of brucein-C from *Brucea javanica*

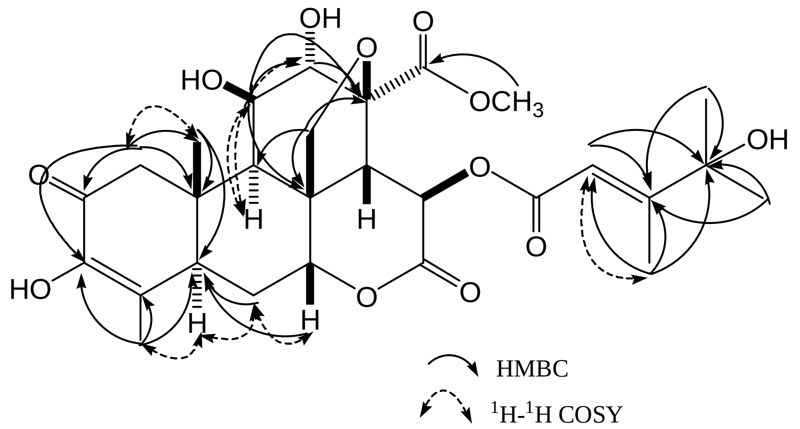

Figure 3. HMBC and [1]H-[1]H COSY correlation of brusein-C from *Brucea javanica*

Insecticidal activity

Insecticidal activities of brucein-C formulated in liposomes against *D. piperis* and *H. antonii* were shown at Figure 4. The results showed that brucein-C at concentration 200 ppm effectively reduced *D. piperis* and *H. antonii* with mortality value of 91.7% and 98.3%, respectively. Insecticidal activity of brucein-C at concentration higher than 200 ppm showed mortality value more than 90%. In contras, brucein-C at concentration 100 ppm showed mortality of *D. piperis* and *H. antonii* 25.0%.

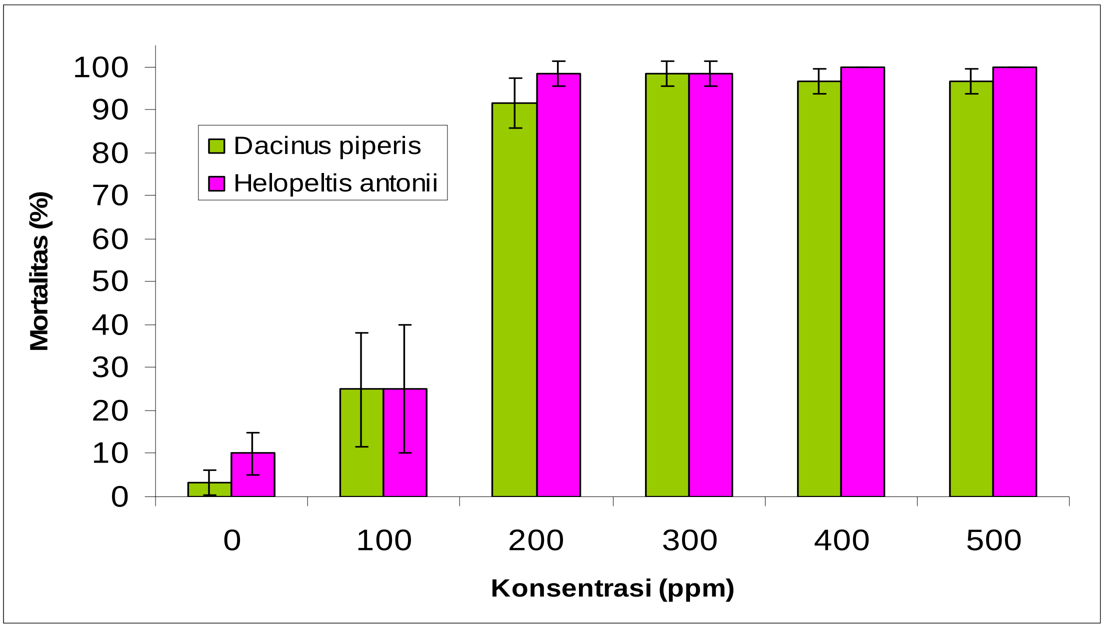

Figure 4. Insecticidal activity of brusein-C against *Dacinus piperis* and *Helopeltis antonii*

Insecticidal activities of brucein-C in order to kill pests of *D. piperis* and *H. antonii* were caused by its ability to inhibit protein synthesis of insect. Side chain of 4-hydroxy-3,4-dimethyl-2-pentenoyloxy in the brucein-C has ability to inhibit protein syntesis. Many researches previously showed that brucein-C have activity as insecticidal against *Plutella xylostella, Tetranychus urticae, Myzus persicae, Meloidogyne incognita, Heliothis virescens,* and *Spodoptera frugiperda*. (Okano *et al*., 1994; Latif *et al*, 2000; Daido *et al*., 1993, 1995; Park *et al*., 1987; Klocke *et al*., 1985).

Activity of Brucein-C against Non Target Insects

Experiments were done in order to know spectrum inhibition of brucein-C as insecticide. Experiments were done at field with put some insect in the net and sprayed with brucein-C at concentration 200 ppm. The results showed that brucein-C at concentration 200 ppm have no lethal effect against ant, grasshopper, butterfly, bee, and dragonfly. Therefore, brucein-C has narrow insecticidal spectrum. It is just kill pest of *D. piperis* and *H. antonii* and not kill other insect at concentration 200 ppm. Spectrum inhibitions of brucein-C against non target insects were shown at Table 2.

Table 2. Spectrum inhibitions of brucein-C against non target insects

	Amount of insect dead*				
Treatment	Ant	grasshopper	butterfly	bee	dragonfly
Control	0	0	1	0	0
Brucein-C (200 ppm)	0	0	0	0	0

*) Each insect is five

Residue Brucein-C in the soil

Residual analysis of brucein-C were done with sprayed brucein-C in the soil at concentration 200 ppm. Content of brucein-C in the soil were evaluated with HPLC at 0, 15, 30, 45, and 60 days after aplication. The result showed that content of brucein-C in the soil at fifteenth after aplication decreased to 80 ppm and at thirtieth to 0 ppm. Some microorganisme in the soil degradated brucein-C to other derivation. The fact indicated that brucein-C safe for environmental and biodegradable.

Hypothetical Technoeconomy

Hypothetical study of technoeconomy on the natural insecticide produced from *B. javanica* were done in laboratorium scale with 10 L containing active substance of brucein-C 5 g. Direct production costs consist of raw material, extraction/isolation, liposomes formulation, direct labor, and other. These costs not included laboratorium equipments. Direct costs of production brucein-C as insecticide were shown at table 3.

Table 3. Direct production costs of insecticide 10 L containing brucein-C 5 g

No	Production cost	Total (Rp)
1	Raw material (Production 5 g of brucein-C)	
	Brucea javanica (50 kg x Rp 500)	25.000
2	Extraction and isolation (Solvent 10 times used)	
	- EtOH 70% (4 L x Rp 25.000/10 times)	10.000
	- EtOAc (4 L x Rp 100.000/10 times)	40.000
	- MeOH (4 L x Rp 87.500/10 times)	35.000
	- $CHCl_3$ (4 L x Rp 112.500/10 times)	45.000
	- Hexane (4 L x Rp 120.000/10 times)	48.000
	- Silica Column chromatography (250g x Rp2.000/10 times)	50.000
3	Formulation	
	- Phosphatidylcholine (10 g x Rp 30.000)	300.000
	- Cholesterol (1,4 g x Rp 20.000)	28.000
	- Diacetylphosphat (1 g x Rp 20.000)	20.000
4	Other	
	-Water (10 L)	12.000
	- Electricity	38.000
5	Labor costs (2 people x Rp 40.000)	80.000
TOTAL PRODUCTION COSTS		**731.000**

Direct costs to produce insecticide 10 L containing brucein-C 5 g are Rp. 731.000. Sale price of the insecticide brucein-C are Rp. 73.100 per liter. Wereas syntetic insecticide such as lamda sihalotrin is Rp. 181.250 per liter. Therefore, the use of insecticide brucein-C cheaper 40.3% than commercial insecticide. Present several advantages in relation to natural insecticide as their rapid biodegradation reducing the risks of environment and food contamination besides the easy way of obtaining and preparation.

CONCLUSION

Brucein-C isolated from buah makasar (*B. javanica*) at concentration 200 ppm inhibited *H. antonii* and *D. piperis* with mortality value of 98.3% and 91.7%, respectively, biodegradable, and no lethal effect against non target insects.

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