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Potential antioxidant and anticancer effect of *Apis dorsata* Binghami Crude Venom from Minahasa, North Sulawesi

Mokosuli Yermia Samuel, Rudi Alexander Repi and Rantje Lilly Worang

Abstract

This current research aims to find the characteristics and composition of the crude venom *Apis dorsata* Binghami, its activity of free radical DPPH scavenging, and cytotoxic activity. The analysis of honey bee venom peptide composition was performed using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Phytochemical contents were analyzed using Harborne method, antioxidant activity was measured using DPPH free radical scavenging method and the anticancer activity *in vitro* was analyzed for cytotoxic effects on cancerous cells of murine leukemia p388 cell line. The results showed that bee venom AD1 has 5 bands of molecules with a molecular weight of 33,53 kDa; 32,21 kDa; 21,51 kDa; 6.1 kDa and 2.67 kDa; AD2 has molecules of 33,52 kDa; 21,51 kDa; 14,43 kDa; 6,14 kDa and 2.43 kDa. *Apis dorsata* crude venom of Minahasa in North Sulawesi contains hyaluronidase/phospholipase A; phospholipase; lysophospholipase or protease inhibitor 5 antigens, and melitin. An unknown tupe of peptide was also identified on fourth band. The analysis of phytochemicals revealed that the crude venom also contains flavonoids and polyphenols. AD1 antioxidant activity was present (IC₅₀:103,28 ppm), as well as the AD2 (IC₅₀:139,13 ppm) compared to control BHT (IC₅₀:142,38 ppm). Cytotoxic activity was observed in murine leukemia P388 cells by AD1 (IC₅₀:36,12 µg/ml), AD2 (IC₅₀:48,59 µg/ml) and positive control Canamycin (IC₅₀:42,06 µ g/ml). Thus, *Apis dorsata* Binghami crude venom from Minahasa, North Sulawesi is potential to be developed as a source of bioactive antioxidant and anticancer.

Keywords: Crude bee venom, *Apis dorsata* Binghami, radical scavengging, cytotoxic, Minahasa

1. Introduction

Apis dorsata Binghami is Sulawesi endemic honey bees, known for its giant honey bees (Hadisoesilo, 1997; Raffiudin, 2002) [12]. Until currently, *A. dorsata* Binghami has received little scientific attention compared to its closest relative, *Apis mellifera*. In addition to producing honey, propolis and bee pollen, honey bees also produce bee venom (BV). The biochemical composition of BV is very unique and has been an interesting research topic to date. Overall bee venom of bees consists of around 120 active chemical components, with the components of the 40s is already detected, including 11 peptides, 5 enzymes, 3 amine, carbohydrates, fat and amino acids. Many of the current research reports found that the most peptides that play a role in bee venom is melittin, apamin, mast cell degranulating peptide (MCDP) and adolapin (NCBI, 2010) [31]. Honeybee venom is a complex mixture between the low molecular weight polypeptide enzymes. A number of enzymes that was reported to be contained in honey bee venom include phospholipase A2, hyaluronidase, phosphomonoesterase acid esterase, α -D-glucosidase, lysophospholipase, α -galactosidase and α -asetilaminodeosiglucosidase, and arylamidase (Hassanein and Hegab, 2010) [14]. Until now, there has been little research about the bioactivity of *A. dorsata* Binghami venom in the field of pharmacology. It was stated that bee venom is a form of evolution that takes place among bees which caused this species occupies almost every area on planet earth. This has led to the study of the bioactivity of bee venom into the an interesting and broad research field of study (Zalat *et al.*, 2002) [39]. In our preliminary study, we found that the composition of the honey bee venom in Minahasa influenced by season, habitat and food source within radius of 700-1000 meters from the natural nesting site (Mokosuli, 2013) [28, 44]. Therefore the analysis of the bioactive contents of crude venom is very important in an effort as can be used as raw material for bio-pharmacy.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) technique has been mostly used to examine the composition of the honey bee venom peptide. SDS-PAGE is currently used by many researchers to classify insects based on the composition of their toxicity. With this technique the molecular composition in a sample of venom can be analyzed (Zalat *et al.*, 2002; Hassanein and Hegab, 2010) [39, 14]. The biochemical composition of bee venom is highly influenced by food source, namely the types of plants as a source of nectar and pollen that is available on the habitat where bees live (Puradidjaja and Muntasib, 1989) [41]. However, climate, habitat and the type plants as a source of food, greatly influence the composition of the honey bee venom (Chmielewska and Szczesna, 2004; Mokusuli, 2013) [5, 28, 44].

North Sulawesi, more specifically the Minahasa region, has many endemic plants species as a source of pollen and nectar for establishment and development of the bees. In other research, we have found these types of plants are the main food source *A. dorsata* Binghami on different areas in Minahasa, North Sulawesi. Each plant has its own characteristics of secondary metabolites including process and composition of nectar and pollen that was formed and subsequently became the ingredients of honey bees feed (Heldt and Heldt, 2005) [15]. Therefore, the composition of secondary metabolites, produced honey bee namely honey, propolis, wax, venom are also heavily influenced by the kinds of available plants in its natural habitat. Bees that live in one area will have a different component of secondary metabolites. Hadisoesilo (1997) [12] states that a worker bees, *Apis cerana* and *Apis nigrocincta* living in Sulawesi have distinct foraging activity and different types of plants. Previous studies conducted by the researchers found the antibacterial activity, antioxidant and anticancer of bee venom *Apis nigrocincta* Smith (Mokusuli *et al.* 2013) [28, 44]. *Apis nigrocincta* Smith body size is smaller than *A. dorsata* Binghami so that the volume of the generated venom are also smaller. On the other hand, *Apis nigrocincta* Smith tends to make nesting area at secondary forest or plantation area while the *A. dorsata* Binghami prefer old-growth forests for nesting. The range of the fly in search of food (water, pollen and nectar, propolis) *A. dorsata* Binghami is further (about 1000 up to 1200 m of nests) than *Apis nigrocincta* Smith (around 500 up to 700 m from the nest) (Hadisoesilo, 1997; Mokusuli, 2013) [12, 28, 44].

Cancer is a disease that results from disruption of cellular metabolism mainly due to damage of genetic materials that

exist within the cell (McKelvey and Evans, 2003) [25]. The prevalence of cancer is reported to be high and increasing. In Indonesia, the prevalence is estimated to be 100 per 100,000 people per year or about 200,000 per year (Puspitasari *et al.* 2003; Mokusuli, 2008) [42, 27]. The most dominant cellular mechanisms triggering cancer and other degenerative diseases such as diabetes, atherosclerosis and gout are oxidative stress triggered by free radicals or oxidants. Therefore the free radical scavenging activity can prevent degenerative damage on cells primarily on DNA. Honey bees BV has been used since a long time on the diseases related to the immune response, but still received very little research attention especially on its antioxidant and anticancer activity (References?).

Bee venom can serve as a modulator of rheumatoid arthritis that arise from radical oxygen species (ROS) activity (Murakami, *et al.*, 1997; Oren and Shai, 1997; Hassanein and Hegab, 2010) [26, 32, 14]. BV has been known to induce apoptosis in many types of cancer cell culture (Reference?). BV induces morphological changes and a decrease in the percentage of viable cells in a culture of cervical cancer cells. Flow cytometric analysis showed that BV induces the production of ROS, improving the content of cytoplasmic Ca^{2+} , reduces mitochondrial membrane potential by releasing cytochrome oxidase, and promoting the activation of caspase-3 that induce apoptosis. BV also induces an increase in the tumor suppressor gene p53, Fas, p21 and Bax, but lowers oncogenes and Bcl-2 (Wan *et al.*, 2008) [38]. BV significantly inhibits the growth of cancer cells in the lungs (Reference?). BV honey bees also inhibits vascular endothelial growth factor (VEGF) that induces cell proliferation (Huha, *et al.*, 2010) [16]. This current research aims to analyze the venom composition *A. dorsata* Binghami from Minahasa, North Sulawesi, its free radical scavenging activity (antioxidant) and cytotoxic activity on cancer cells *in vitro*.

2. Materials and Methods

2.1 Samples Collection: *A. dorsata* obtained from from Kombi Forest (Minahasa) and Kaweruan Forest (North Minahasa) (Fig 1). As many as 200 individual *A. dorsata* Binghami were collected from a natural nesting by using net. Living *A. dorsata* Binghami from each location were transported to the Molecular Biology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, State University of Manado. Isolation of bee venom was carried out by accommodating the liquid venom in eppendorf vial and stored at temperature 0 °C before use.

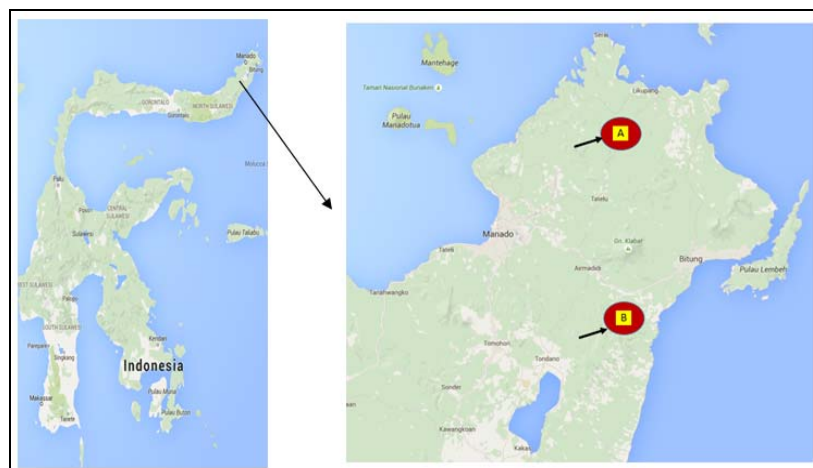


Fig 1: Location *A. dorsata* Binghami sampling in natural nesting, (A) the forest of Kaweruan and (B) forest of Kombi

2.2 Materials: The materials used include: acrylamid (Merck), ddH₂O, bisacrylamid, ammonium peroxodisulphate, a protein marker 4.6-100 kDal (Merck), TEMED, ethanol p.a. (Merck), aquadest,, 1-diphenyl-2-picirilhydrazil (DPPH) (Merck), butyl hydroxy toluene (BHT), methanol (Merck) pa. Murine p388 leukemia cancer cells were obtained from the Laboratory of Chemistry of Natural Materials, Institute of Tecnology Bandung (ITB) Indonesia, media Rosewell Park Memorial Institute (RPMI) 1680, fetal bovine serum, canamycin, dye reagent [3-(4,5-dimethyl thiazol-2-yl)-2,5 diphenyl tetrazolium bromide], a solution of 10% SDS-0.01 N HCL. Tools used 1 set of electrophoresis vertical model TV100YK-MODSYS, a set micropipette Eppendorf, centrifuge Eppendorf, UV-Vis Spectrophotometer Perkin Elmer Lamda 35, ultrasentrifuse Eppendorf 5430R, Waterbath mammert, incubator mammert, digital scales, hot plate, nanospectrophotometer, microplate reader, CO₂ incubator, glasses and other devices.

2.3 SDS PAGE: SDS-PAGE using a modification of the method of Laemmli (1970) [43] with poliacylamid concentrations of 17.5% was performed. The composition of the gel separator prof 17.5% while collecting gel composition Prof. 5%. After the gel was created, the sample and the protein marker were injected to existing wells. Running was performed for 4 hours on a 60 volt, 20 mA. Protein gel would sink with the help of an electric current that moves from the negative to the positive pole. After running, the gel was passed on to the silver staining. The gel was then soaked in a solution of fixation for approx. 2 hours, while being agitated slowly. The gel was then washed with a solution of washing solution for 20 minutes (repeated 3 times) without agitation. Gel was rinsed with free-ion aquadest (ddH₂O) for 10 seconds. The gel is then soaked in a solution of sensitize for 1 minute. Gel washed again in ddH₂O for 20 seconds (repeated 3times). Gel was then incubated into the fridge with AgNO₃ 0.1% (silver nitrate) for 20 minutes and washed or soaked with ddH₂O for 20 seconds and repeated 3 times. Gel was then soaked with a developing solution, while agitated up to a staining dye. Gel was added with a stop solution for 5 minutes then rinsed with ddH₂O for 5 minutes. Scaning was done and subsequent data analyses were performed.

2.4 Antioxidant test: Crude veom bee was made in the distribution of concentrations of 10, 50, 100, 200 and 800 ppm. Each was put into test tubes. Each of the test tubes was added with 500 µ l DPPH solution 1 mm in methanol. Volume was accomplished to 5.0 ml, then incubated at 37°C for 30 minutes, then absorption is measured at a wavelength of 515 nm. As positive controls used with BHT concentration adjusted. IC₅₀ values were calculated each regression equation using the formula (Kikuzaki and Nakatani, 1993) [19].

$$\% \text{ inhibition} = \frac{[\text{Control absorption} - \text{Semple absorption}]}{[\text{Control absorption}]} \times 100 \%$$

Cytotoxic test: Analysis of anticancer activity was performed *in vitro* in murine leukemia P388 cells, using methods developed by the Tokyo University of Pharmacy & Life Science Hachioji Japan and ITB. P388 cells were grown in RPMI Medium 1640 (attachment 10) with 5% FBS (Fetal Bovine Serum) and canamycin (100 µ g/ml). Cells (3 x 10⁵ cells per well) at microplate culture contains 100 µ L per well of growth media and incubated at a temperature of 37°C for 24 hours in humidity 95% water and 5% CO₂ atmosphere. The cell culture used to test anticancer activity have viability ± 95%. Solution test of a total of 10 µ L with different concentration was then added to the cell culture day after transplant. On the third day, 20 µ L solution was added of the dye 3-(4,5-dimethyl thiazol-2-yl) -2,5-diphenyl tetrazolium bromide) as much as 5 mg/ml per well. After 4 h incubation 100 µ L of a solution of 10% SDS-0,01N HCL was added into each of the wells. Crystal formazan was then added in each well, dissolved with stirring using micropipette. Optical density measurement was done using a microplate reader on two areas of wavelengths (550 and 700 nm). All stages were conducted in triplo.

2.5 Data Analysis Techniques: Isolation and characterization of the results of bee venom were analyzed qualitatively. The antioxidant activity IC₅₀ value is concentration required to scavenge DPPH free radical by 50%. The data were analyzed by linear regression equations using SPSS 20 (IBM). The anticancer activity IC₅₀ value is the concentration of the extract that is required for the inhibition of growth of murine P388 leukemia cancer cells by 50%. The data were analyzed by linear regression equations using SPSS 20 (IBM).

3. Results

3.1 Characteristic and Composition of *A. dorsata* Binghami Venom

The characteristics of the honey bee venom that were freshly taken from the worker bees are white, clear, odorless, tasteless as if burning on tongue, pH (4.00 to 5.3) (table 1). About five to ten minutes at room temperature, it will turn into a powder as flour is yellowish white. Needle injection of bee venom is present on the part of the abdomen called the sting. Sting is present on the end of the abdomen. Sting of *A. dorsata* Binghami is blackish at the base, with a venom sacs containing fresh venom (Figure 2). Isolation of venom from 100 individual of bees produce dry bee venom 0,038 grams on average.

Table 1: Characteristics of fresh and dry bee venom of *A. dorsata* Binghami

Characteristics	Fresh Bee venom	dry bee venom
pH	4,00 – 5,3	4,6 – 5,8
Form	Semisolid fluids	powder/crystals
Colours	Clear white	White yellowish
Solubility	Soluble in water, ethanol and ammonium sulfate	Soluble in water, ethanol and ammonium sulfate
taste on the tongue	Burning	Burning with the intensity of the smaller
The Phytochemicals compounds		
- Polifenol	+	+
- Flavonoid	+	+

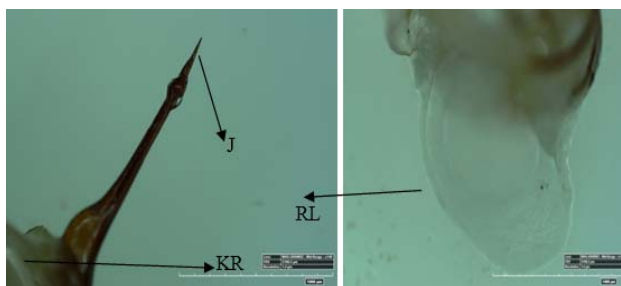


Fig 2: Bee venom *A. dorsata* observed Hirox KH-8700 microscope with Description : J = sting, KR (venom sach), RL = bee venom

3.2 The analysis of bee venom component using SDS-PAGE

Polyacrylamide gel concentrations used in this study was 17.5%. The results of the analysis of SDS-PAGE of bee venom obtained 5 a clear tape. Molecular weight in five consecutive AD1 Ribbon was 33.53 kDa, 21 kDa, 10 kDa, 6.1 kDa and 2.67 kDa, while for AD2, it was found that ordered consecutive was 33.52 kDa, 31.21 kDa, 21 kDa, 10 kDa, 6.1 kDa and 2.67 kDa (Figure 3 and table 2).

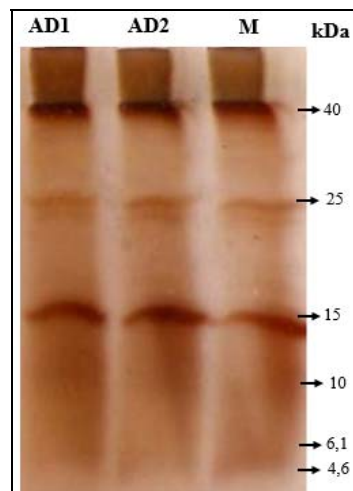


Fig 3: Chromatogram results of the SDS PAGE. Description: M = marker protein, AD1 (*A. dorsata* from Kaweruan Forests), AD2 (*A. dorsata* from Kombi forests)

Table 2: Molecular weight (kDa) bee venom after analyzed based on SDS-PAGE Chromatogram

No	Sample	Molecular weight (kDal)					
		Band 1	Band 2	Band 3	Band 4	Band 5	Band 6
1	AD1	33.53	31.21	21.51	10	6,1	2.67
		Hyaluronidase/ phospholipase A	Phospholipase	Phospholipase A or lysophospholipase pfangten 5	uk	Inhibitor protease	Mellitin
2	AD2	33.52	21.51	14.43	uk	6,14	2,43
		Hyaluronidase/ phospholipase A	Phospholipase A or lysophospholipase or antigen 5	Phospholipase A		Inhibitor protease	Mellitin
3	Marker	40	25	15	10	6,1	4.6

3.3 The activity of the free radical DPPH Scavenging

The activity of the free radical DPPH scavenging by bee venom was isolated from fresh *A. dorsata* Binghami on natural nesting in Kombi forests (AD2) was compared with the isolation of Kaweruan Forests (AD1). Inhibitory concentration 50 (IC₅₀) AD1 is 103,28 ppm (y = 16, 063ln (x)-24,492; R² = 0,9344) while in AD2 IC₅₀ is 139,13 ppm (y = 16, 241ln (x)-30,151, R² = 0,8515) (Figure 2). Compared to positive control i.e. synthetic antioxidants Butyl hydroxy toluene (BHT) antioxidant activity, crude venom *A. dorsata* Binghami is still stronger based on the IC₅₀ (Figure 4).

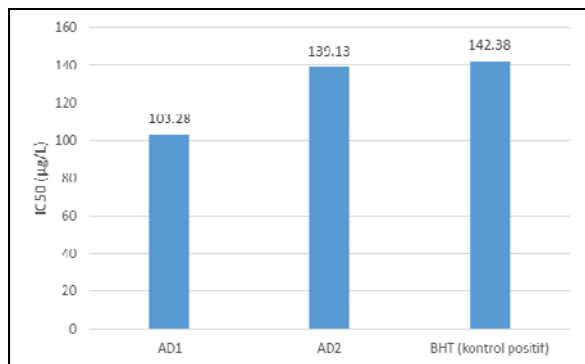


Fig 4: The activity of free radical scavenging *A. dorsata* bees venom derived from two natural nesting (AD1 and AD2) compared to positive control.

3.4 Cytotoxic Activity

Honey bee venom that is used to test the toxicity of the venom *A. dorsata* is crude bee venom. Cytotoxic test results showed that *A. dorsata* venom had a stronger cytotoxic activity in cell

murine leukemia p388. Inhibitory concentration 50 (IC₅₀) of AD1 and AD2 are 36,12 µg/l, and 48,59 µg/ml, respectively. Compared with the positive control, AD2 showed stronger cytotoxic effect (Figure 5).

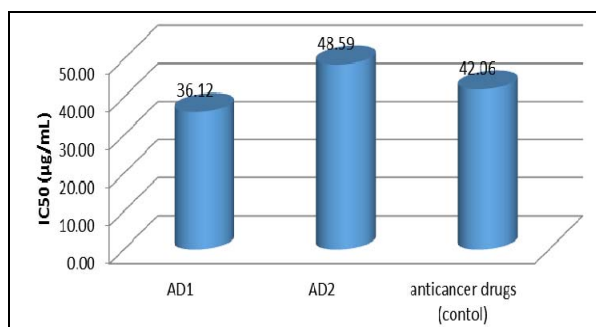


Fig 5: Bee venom LC₅₀ AD1 and AD2 compared with control

4. Discussion

Characteristic and composition of *A. dorsata* Binghami venom

Bee venom has been used in wound healing for centuries. From the results of the analysis of fresh venom of *A. Dorsata*, it was found that it contains polyphenols and flavonoids compounds. SDS-PAGE analysis based on molecular weight peptides revealed that fresh bee venom of *A. dorsata* Binghami contains hyaluronidase, phospholipase, phospholipase A, inhibitor protease and melitin. There are some bands with unknown types of peptides. The difference in molecular weight peptides were detected on the SDS-PAGE of the AD1 and AD2 indicates that the content of

bioactive materials on honey bees can be different depending on the variety and availability of plant as their food sources. Mellitin and phospholipase A was a constituent component of bee venom honey with a high toxicity compared to other components. Mellitin compiled a 30% up to 50% dry weight venom of *A. mellifera* while phospholipase A was approximately 10% to 12%. Our results of the SDS-PAGE with fresh bee venom *A. dorsata* indicate that the thickness of the band is high, not only on melitin and protease inhibitors but also on phospholipase and hyaluronidase. The thickness of the tape shows the number concentration of compounds that contained. Venom from other Apis species is similar, but even the venoms from the various races within each species are slightly different from each other. The toxicity of *Apis cerana* venom has been reported to be twice as high as that of *A. Mellifera* (Ali, 2012). With the existence of a band that has not been known, it is imperative to conduct further analysis with different methods to find out the bioactive substances.

4.1 Antioxidant Activity

Antioxidant activity of honey bee venom was analyzed through a process of reaction with using DPPH. Due to these reactions, bee venom stabilizing free radicals and reduces absorption as a consequence and DPPH radical DPPH decrease to DPPH-H. The degree of discoloration shows the potential silencing of free radical antioxidant substance or extract with hydrogen gives. The DPPH antioxidant reacts will show a change in color from orange to yellow, with the colour intensity depending on the ability of antioxidants (Benabadji *et al.* 2004). In this research, it was shown radical curbs AD1 DPPH with IC50 values of 103,28 µg/ml whereas AD2 IC50 of 139,13 µg/ml. Previous research with tiobarbiturat acid method (TBA) showed that at concentrations of 500 ppm poison of *Apis nigrocinta* is able to inhibit the oxidation of linoleic acid of 75,10%, whereas *A. dorsata* in a concentration of 200 ppm has been able to inhibit the oxidation of linoleic acid 80,78% (Mokosuli *et al.* 2013 [28, 44]).

Based on the analysis of the composition of the venom using SDS-PAGE, the content of peptide found in the venom of bees is melitin, phospholipase, phospholipase A or lysophospholipase or antigen 5, protease inhibitors and hyaluronidase. Bee venom was also shown to significantly decrease the level of Radical Oxygen Species (ROS)-induced oxidative damage to synovial fluid proteins in a rat model of rheumatoid arthritis (Frances *et al.*, 2014). DPPH free radical scavenging activity of the Bee Venom group was 2.8 times stronger than that of the Sweet Bee Venom group (Chull *et al.* 2006) [6]. The honey bee venom also possesses a considerable hydroxyl radical scavenging activity which was evaluated by its competition with dimethyl sulphoxide for OH-. Honeybee venom is found to significantly inhibit lipid peroxidation of non-enzymatic reactions. It also possesses a considerable hydroxyl radical scavenging activity, evaluated by its competition with dimethyl sulfoxide for HO (Rekka *et al.*, 1990) [34].

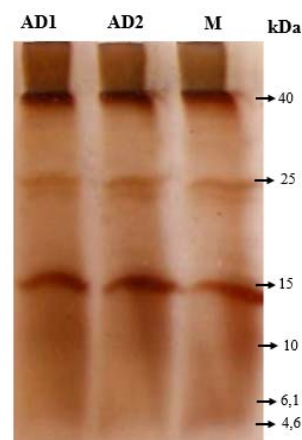
Analysis of the content of the phytochemicals are known to bee venom *A. dorsata* Binghami contains flavonoids and polyphenols. The antioxidant activity of polyphenols are powerful (Boukra and Sulaiman, 2009) [4]. Phenolic compounds exhibit a wide range of biological activities and contain one or more aromatic rings bearing one or more hydroxyl groups. They are categorized by the number of phenolic rings and the structural elements that link these rings (Fresco *et al.* 2006; Henshaw *et al.* 2014, Samie and Ali. 2012) [9, 11, 36].

4.2 Cytotoxic Activity

As with antioxidant activity, cytotoxic activity of AD1 (IC50: 36,12 µg/l) is more powerful than the AD2 (IC50: 48,59 µg/ml). Melitin are cytotoxic on cancerous cells with cell membrane damage. Bee venom may inhibit tumor cell growth (Orsolio *et al.*, 2009) [33]. Mellitin and phospholipase A can increase the synthesis of tumor cell necrosis factors, such as cytokines and interleukin-1, stimulates the release of arachidonat acid which is produced in the process of immune response. The bee venom was able to induce apoptosis in many types of cancer cell culture. It also induces morphological changes and a decrease in the percentage of viable cells on cell culture of cervical cancer. Analysis of flow-cytometry shows that bee venom can induce the production of ROS, improving the content of cytoplasmic Ca²⁺, reduces mitochondrial membrane potential, that would eventually causes the release of cytochrome promoting activation and caspase-3 and trigger apoptosis. Bee venom also induces an increase in Fas, p53, p21 and Bax but lowers Bcl-2 (Wan *et al.*, 2008) [38]. Bee venom significantly inhibits the growth of cancer cells in the lungs. Bee venom also inhibits vascular endothelial growth factor (VEGF) that induce proliferation. (Huha *et al.*, 2010) [16]. Recent studies reported that bee venom possesses antimutagenic (Varanda *et al.*, 1999) [45], proinflammatory (Surendra *et al.*, 2011 [46], antiinflammatory (Nam *et al.*, 2005) [47], and antinociceptive effect (Kim *et al.*, 2013) [48]. Bee venom has also anti-cancer activity and has the capacity to kill cancer cells. The promise of this remedy exists with living honeybees, which make tumors disappear by killing cancer cells (Liu *et al.*, 2002) [23]. The cytotoxic effect through the activation of PLA₂ by melittin is believed to be an important mechanism of anti-cancer activity of BV. Thus the content of bee venom *A. dorsata* Binghami can be cytotoxic on cancerous cells of murine leukemia cell line P388. Future research needs to be done for *in vivo* test of *A. dorsata* Binghami venom to develop dose and formulation curve of effectiveness against cancer cells.



Fig: *A. dorsata* Bingham (Sample and natural nesting) in Kaweruan Forest, North Minahasa



	S	M	V1	V1	V1	V2	V2	V2	Stacking gel (-) start protein ↓ Gel running (+)
	--		--	--	--	--	---	--	
40	--	--	--	--	--	-	-	-	
25	--	--							
			-	-	-	-	-	-	
15	--	--	--	--	--	-	-	-	
10	--	--							
4,6	--	--	-	-	-				
	-	-	-	-	-				

Fig. Kromatogram results of the SDS PAGE. Found 5 ribbons of proteins with molecular weights in a row: 40 kDa, 25 kDa, 15 kDa, 10 kDa and 4.6 kDa. Apitoksin standard (S), marker proteins (M), the toxin a. dorsata (BV1), toxins of a. nigrocincta (BV).

Table. Molecular weight (kDal) bee venom after analyzed based on SDS-PAGE Cromatogram

No	Sample	Molecular weight (kDal)					
		Band 1	Band 2	Band 3	Band 4	Band 5	Band 6
1	AD1	33.53	31.21	21.51	10	6,1	2.67
		Hyaluronidase/ phospholipase A	Phospholipase	Phospholipase A atau lysophospholipase atau antigen 5	uk	Inhibitor protease	Mellitin
2	AD2	33.52	21.51	14.43	uk	6,14	2,43
		Hyaluronidase/ phospholipase A	Phospholipase A atau lysophospholipase atau antigen 5	Phospholipase A		Inhibitor protease	Mellitin
3	Marker	40	25	15	10	6,1	4.6

4.3 Antioxidants test

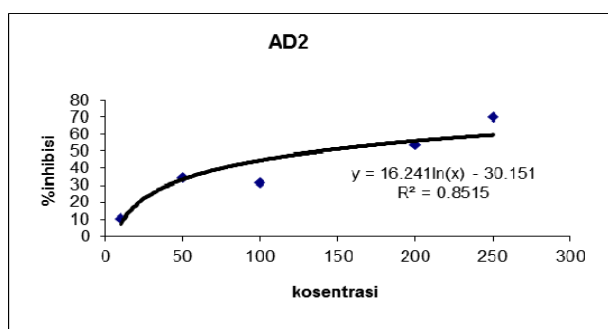
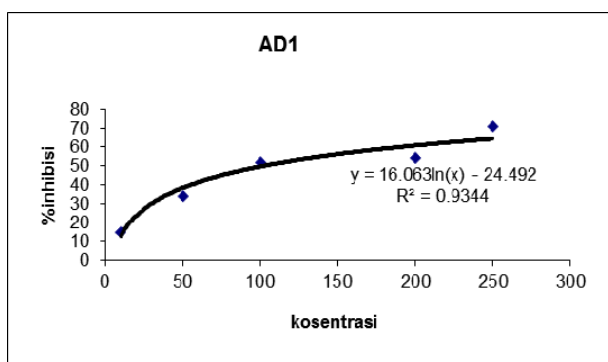


Fig. Regression analysis of the bee venom treatment against DPPH

5. Conclusion

Fresh crude venom of *A. dorsata* Binghami from Minahasa North Sulawesi contains hyaluronidase/phospholipase A; Phospholipase; lysophospholipase or protease inhibitor 5 antigens, and melitin. On fourth band, an unknown types of peptides was detected. Fresh crude venom also contains flavonoids and polyphenols. The antioxidant activity as well as cytotoxic activity, AD1 (*A. dorsata* Binghami from forest Kaweruan) is stronger than AD2 (*A. dorsata* Binghami from forest Kombi). Thus, in the future, the venom of the honey bee *A. dorsata* Binghami of Minahasa in North Sulawesi will have a potential to be developed as a source of antioxidant and anticancer bioactive substance.

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