International Journal of
Science and Engineering Investigations vol. 2, issue 18, July 2013
ISSN: 2251-8843

Pharmacological Bioactivity Honey Bee Venom Apis Nigrocincta Smith and Apis Dorsata Binghami Endemic to North Sulawesi

Mokosuli Y. Semuel[1], Jantje Pelealu[2], Max Tulung[3], Lucia C. Mandey[4]
[1]Postgraduate Student, Department of Entomology, Sam Ratulangi University, Manado, Indonesia
[2, 3, 4]Department of Entomology, Sam Ratulangi University, Manado, Indonesia
([1]ymokosuli@yahoo.com)

Abstract- The aims of this research are to find the venom composition and pharmacologic bioactivity of *A. nigrocincta* Smithand *A. dorsata* Binghami honey bee which are endemic of Sulawesi, including antibacterial, antioxidant, and anticancer activity. To identify the venom composition, the SDS PAGE method was applied. Antibacterial activity was assessed using agar diffusion method. Antioxidant activity was assessed by TBA method, while anticancer activity was examined using cytotoxic method in leukemia P388 cell line. The results showed that the BV1 (*Apis dorsata* Bighami) has 4 molecular bands of 33.54 kDa, 32.21 kDa, 21.51 kDa, 6.7 kDa, and 2.88 kDa, while DV2 (*Apis nigrocincta* Smith) has 4 peptide bands detected as follows: 33.54 kDa, 21.51 kDa, and 15.43 kDa. It was suspected that BV1 contains hyaluronidase/phospholipase A, phospolipase, lysophos-pholipase or antigen 5, protease inhibitor, and melitin. BV2 was suspected to contain hyaluronidase / phospholipase A, lysophospholipase or antigen 5. BV1 had a stronger antibacterial activity compared to BV2 towards E. cole cc and S. aureus cc isolated from infected wounds. The average BV1 antibacterial activity was higher than the activity of 50 ug/mL Ampicilin as positive control. BV1 antioxidant activity was stronger than BV2, in terms of the inhibition of MDA formation at concentration 500 ug/mL (80.78% vs. 75.10%, respectively). The anticancer activity of BV1 was also better than BV2 in term of IC_{50} (46.77 ug/mL and 66.2 ug/mL, respectively).

Keywords- *bee venom, antibacterial, antioxidants, anticancer*

I. INTRODUCTION

Honeybees are one of the species in the class of insects that are beneficial to human life. Honey has long been known and used in the food and pharmaceutical fields as well as bee venom has been used in the treatment of rheumatoid disease in Egypt and Europe since the ancient time. Bees venom are used for the treatment of Chinese medicine and acupuncture in Korea today. Some studies reported that bee venom (BV) with pharmacological potential broad spectrum.

BV honey bees have been used since long time ago in diseases that has relationship with immune response. Imunoterapi by using bee venom is very effective. Bee venom can serve modulator rheumatoid arthritis who throws radical oxygen species (ROS) [1]. BV has known to induce cell shutdown called by the process of apoptosis in many types of cell culture of cancer. BV induces morphological changes and decreased the percentage of viable cells in cultured cervical cancer cells. Sitometric flow analysis showed that BV induces ROS production, increased content of cytoplasmic Ca_2 +, reducing the potential mitochondrial membrane causes the release of cytochrome oxidase and promote activation of caspase-3 which directs cells to apoptosis. BV also induced an increase in tumor suppressor genes, namely Fas, p53, p21 and Bax but lower content of oncogenes such as BCl-2 [2]. Honey bee venom significantly inhibited cell growth of lung cancer. BV honeybee endotheloal also inhibit vascular growth factor (VEGF), which induces cell proliferation [3].

BV is caused by the pharmacological potential biochemical composition of bee venom is unique. Overall bee sting venom consists of approximately 120 active chemical components, but only the 40s component is detected, including 11 peptides, 5 enzymes, 3 amines, carbohydrates, fats and amino acids. Based on current research reports, most peptides are melittin role, apamin, mast cell degranulating peptide (MCDP) and adolapin [4]. Honey bee venom is a complex mixture between enzymes, polypeptides with low molecular weight. Reported a number of enzymes contained in honey bee venom is *phospholipase* A2 and *hyaluronidase*, and *fosfomonoesterase acid esterase*, *α-D-glucosidase*, *lysopfosfolipase,* *α-galactosidase* and *α-asetilamino-deosiglukosidase*, *arylamidase* [1].

Until now there are few reports of research on the bioactivity of honey bee venom in the field of insects, especially bees farmakologi. BV honeybee is a mixture of different types of active enzymes, cytotoxins, pheromones, chemical stimulants and neurotoksin. Some of bee experts claim that evolution is a form of bee venom that causes this bee species occupies nearly every area in the earth.This bioactivity study of bee venom causes a field of research studies that are still very broad.

SDS-PAGE techniques, the most widely used to determine the composition of honey bee venom peptide [1]. SDS PAGE to currently use by many researchers in the classification of insects based on the composition of the poison. With this

technique it can be seen that the molecular composition contained in a sample to be analyzed insect venom.

Biochemical composition of bee venom is strongly influenced by food source, namely the kinds of plants as a source of nectar and pollen available in the habitat where the bees live. Season, location and type of food greatly affect the composition of honeybee venom [5].

North Sulawesi has many endemic species of plants as a source of nectar and pollen for the establishment and development of the honey bee. Each plant has its own characteristics of secondary metabolites, including the process and composition of nectar and pollen are formed and subsequently became the feed honeybees. Therefore the composition of secondary metabolites produced honey bees is honey, propolis, wax and toxins are also greatly influenced by the types of plants that are available in the natural habitat. Bees that live in one area can have different components of the secondary metabolites. Hadisoesilo [6] states that the worker bees *Apis cerena* and *Apis nigrocincta* living in Sulawesi have different foraging activities and different types of plants. In foraging bees *A. cerana* fly 2 hours earlier than *A. nigrocincta*. This study aimed to characterize the composition of honeybee venom *A. nigrocincta* and *A. dorsata* and obtain scientific data of pharmacological bioactivity antibacterial, antioxidant and anticancer *in vitro.*

II. MATERIALS AND METHODS

A. Equipment and Materials

1) SDS-PAGE

Materials used include: acrylamide (Merck), dH2O, bisakrilamid, ammonium peroxodisulfate, protein marker 4.6 - 100kDal (Merck), TEMED, ethanol pa (Merck), distilled water, fresh honey bee venom of *A. dorsata* Binghami and A. *nigrocincta* Smith obtained from several locations in the district of Minahasa Induk and North Minahasa regency. 1 set of tools used electrophoresis-MODSYS TV100YK vertical models, 1 set Eppendorf micropipette and Eppendorf centrifuge and microscope KH-8700 3D hirox.

2) Antibacterial activity

Materials used include: nutrient agar medium Merck, pure cultures of bacterial isolates infective wound outside collection Microbiology Laboratory Faculty UNIMA, *Escherecia coli cc* ATCC and *Staphylacocus aureus cc* ATCC etc.. The tools used include: autoclave 700 SX series, laminar air flow, digital scales AND HR250A, ultrasentrifuse Eppendorf 5430R, nanophotometerTM Pearl Version 1.0 Implen, Vortex V-1 plus, Eppendorf micropipette etc….

3) Antioxidative activity

Materials used include Trifluoroacetic acid, membrane filters 0:45 lm, 10% NaOH, acetic acid anhydride, filter paper, deionized water, 75% ethanol, absolute ethanol, linoleic acid (Sigma Aldrich), 0.1 M phosphate buffer pH 7, Fe CL2.4H2O, HCL, ammonium thiocyanate, α-tocopherol (Sigma Aldrich), 1,1,3,3-tetrametoksipropana (TMP) 6 M, tiobarbiturat acid (TBA), acetic acid and 50% trichloroacetic acid (TCA) 20% . The tools used include: UV-Vis Spectrophotometer Perkin Elmer Lamda 35, ultrasentrifuse eppendof 5430R, mammert waterbath, incubator mammert, digital scales AND HR250A, 1 set Eppendorf micropipette, hot plate, etc.

4) Anticancer activity

Materials used include: cell cancer of the P388 murine leukemia Natural Products Chemistry Laboratory ITB, *media Rosewell Park Memorial Institute* (RPMI) 1640, *fetal bovine* serum, kanamycin, dye reagent [3 - (4,5-dimethyl thiazol-2-yl) -2,5 diphenyl tetrazolium bromide], a solution of 10% SDS-0, 01 N HCL. The tools used include: nanospektrofotometer, microplate reader, CO2 incubators, glassware etc.

B. Research Methods

1) Electrophoresis

SDS PAGE electrophoresis using a modified method of Laemmli (1970) with a concentration poliakrilamid17, 5%. Ge composition; separator composition prof 17.5% while 5% gel collectors prof. Once the gel is made, sample and marker proteins injected in existing wells. Running carried out for 4 h at 60 voltm, 20 mA. Protein gel will go down with the help of electric current that moves from the negative pole to the positive pole.

After running, the gel was then forwarded to the silver staining gel immersed in fixation solution for approximately 2 hours while slowly agitated. Gel is then washed with a solution of washing solution for 20 minutes (repeat 3x) without agitation. Gel was rinsed with deionized distilled water (ddH2O) for 10 seconds. Then the gel was soaked in a solution sensitize for 1 minute. Subsequently the gel was washed with ddH2O for 20 seconds (repeated 3 times). Gel was incubated in a refrigerator with 0.1% AgNO3 (silver nitrate) for 20 minutes. Gel is then washed or soaked with ddH2O for 20 seconds and repeated 3 times. Subsequently the gel was soaked in developing solution, while agitated until staining dye. Gel was added stop solution for 5 minutes and then rinsed with ddH2O for 5 minutes. Then performed scanning and data analysis.

2) Pharmacological studies bioactivity

a) Antioxidative activity of TBA method

Antioxidative activity using tiobarbiturat acid (TBA) is BV of honeybee solution was made in 50, 100, 200, 500 and 1000 ppm concentration. Each sample was taken as 1 mL and then dissolved in 2 mL of 0.1 M phosphate buffer pH 7.0 and 2 mL of 50 mM linolenic acid in ethanol 98.8%. Positive control solution (control antioxidant) used α-tocopherol 1 mL, 2 mL of 0.1 M phosphate buffer pH 7.0 and 2 mL of 50 mM linolenic acid in ethanol 99.8%. Negative control solution consisting of 1 mL of deionized water, 2 mL of 0.1 M phosphate buffer pH 7.0 and 2 mL of 50 mM linolenic acid in ethanol 98.8%. All mixture placed in dark bottles threaded lid and incubated at 400C. One day after the maximum incubation time of methods *ferric thiocyanate* (FTC) conducted measurements of *thiobarbituric acid reactive substances* (TBARS) by the method TBA [7] by taking as much as 1 mL of each test solution. Then added 2 mL of 20% TCA and 2 mL of 1% TBA in 50% acetic acid. The reaction mixture was shaken and placed in a 1000C water bath for 10 minutes. After

chilling the solution centrifuged at 3000 rpm for 15 minutes. Then the absorbance was measured at a wavelength of 532 nm with 3 replications.

Manufacture of standard curves using a solution of 1,1,3,3-tetrametoksipropana (TMP) with konsenterasi 0:15, 0:30, 0.60, 0.75. 1.50 and 3.0 lm. Each solution of various konsenterasi respectively pipetted 1 mL and added 2 mL of 20% TCA and 2 mL of 1% TBA in 50% acetic acid solvent. The reaction mixture was shaken and placed in water bath 1000 C for 10 minutes. Once cool, the solution was centrifuged at 3000 rpm for 15 minutes. It's absorbent then measured at a wavelength of 532 nm with two replications [8].

b) Antibacterial bioactivity

Analysis of antibacterial bioactivity using agar diffusion method modified [9], namely: test bacterial isolates were grown on NA medium for 18 hours. By using a sterile loop, bacterial suspension was taken and dissolved in physiological saline solution in a test tube. Measure OD with a spectrophotometer. Furthermore, by using a sterile swab, bacterial suspension applied evenly on the surface of the existing NA medium in a petri dish. Bee venom were tested both *A. nigrocicnta* Smith either *A. Dorsata* Binghami made each in the distribution of the test concentration 20 ug / ml, 50 ug / ml, 100 ug / ml and 150 mg / ml. Ampicillin and streptomycin as antibiotic control made in concentration 50 ug / ml. Disks are made of Whatman filter paper and was immersed in a test solution that bee venom for 3 hours and then placed aspetik on NA medium in a petri dish that had been inoculated with each of the four isolates of wound infection and bacterial isolates, *Escherecia coli* ATCC and *Staphylacocus aureus* ATCC. Experimental unit is the amount of total isolates x 2 is 12. Inkubasikan for 24 hours at room temperature subsequently measured inhibition zone diameter formed.

Determination of GMIC (Growing Minimum Inhibitory Concentration)

Bee venom, dissolved in buffer at concentration 40, 30, 20, 15, 10, 7.5 and 2.5 ppm (mg / L) aseptically. Surface of the medium in a petri dish is dried before inoculated with cultured bacteria (mix approximately 106 cfu / ml). After inoculation, the petri dish and then incubated at 320C for 72 hours after 30 minutes of inoculation is done. Lowest Concentration that prevents visible growth of bacteria is growing concentration minimum inhibitory (KHTM). Each made 2 replications.

c) Anticancer bioactivity

Analysis of *in vitro* anticancer activity in murine leukemia P388 cells using methods developed by the *Tokyo University of Pharmacy and Life Science Hachioji Japan* and ITB. P388 cells cultured in RPMI 1640 medium (Appendix 10) is equipped with 5% FBS (*Fetal Bovin Serum*) and kanamycin (100 ug / ml). Cells (3 x 103 cells per well) in 100 mL mikroplate contain culture in growth media per well and incubated at 370C for 24 hours in 95% humidity water and 5% CO2 atmosphere. Cell culture are used to test the anticancer activity have ± 95% viability. Experiment by using 10µL solution with various concentration added to the cell culture transplantasi.Pada day after the third day was added 20 mL solution of dye 3 - (4,5-dimethyl thiazol-2-yl) -2,5-diphenyl tetrazolium bromide) 5 mg / mlper wells. After 4 hours of incubation solution was added 100 mL of 10% SDS-0, 01N HCl was added into each well. Further formazan crystals in each well, dissolve with stirring using a micropipette. Optical density measurements performed using the microplate reader at two wavelength regions (550 and 700 nm). All stages performed triplo.

Data Analysis Research

1. Data resulting from the isolation and characterization of bee venom were analyzed qualitatively.

2. Percent inhibition of the oxidation of linoleic acid derived from linoleic average MDA formed divided by the average MDA formed each treatment multiplied by 100%.

3. Antibacterial activity. Body of bacteria inhibition zone was measured with a micrometer and then calculated the average per konsenterasi test. KHTM determined by the smallest concentration where bee venom tests solution is still able to inhibit bacterial growth.

4. Anticancer activity. IC50 values are concentration extract necessary for cancer cell growth inhibition of murine leukemia P388 by 50%. Data were analyzed by linear regression equations.

III. RESULTS AND DISCUSSION

A. Characterization Poison Honey Bees

Honey bee venom was isolated directly from the bees and stored in Eppendorf vials. To *A. dorsata*, bee venom collection of manually drawn directly from the sampling sites bees. While for *A. nigrocinta* due to its smaller size made the decision poison laboratory. Bee venom is taken only in the worker bees.

1) Physical characteristics

Physical characteristics of honey bee venom freshly taken from the worker bees translucent white, odorless, bitter sharp (Figure 1 and Figure 2). Fresh bee venom pH ranged from 6.3 to 6.7.

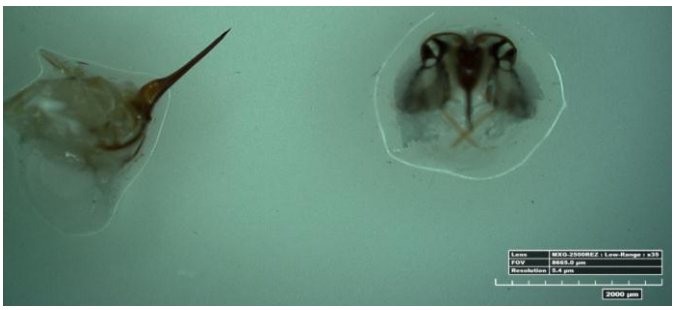

Fig.1. Bee venom *A. nigrocincta* observed by 3D microscopy hirox KH-8700

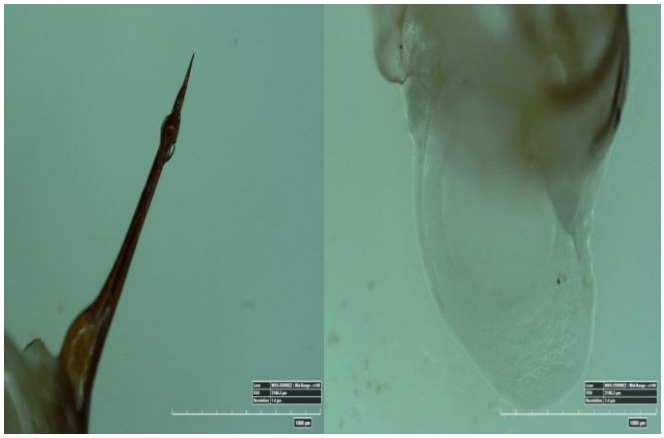

Fig.2. Bee venom *A. dorsata* Binghami observed by 3D microscopy hirox KH-8700

2) Wet weight of bee venom

Measurement of the average wet weight of bee venom honey bee done on 10 animals of each location to facilitate weighing on digital scales. Once toxins are removed and stored wet in sterile Eppendorf vials, bee venom was weighed with a digital scale that has been calibrated. The results showed that the wet weight of bee venom varies according to the origin of the samples. The average wet weight of *A. dorsata* larger than the average wet weight of *A. nigrocincta*.

The average weight of honey bee venom *A. nigrocincta* Smith from different locations in North Minahasa regency colonies greater than the average weight of honey bee venom coming from the District Minahasa. But found the average weight of honey bee venom *A. nigrocincta* with sample origin Tanggari-Tomohon larger than the North Minahasa and Minahasa. Also on *A. dorsata*, the average weight of honey bee venom that comes from multiple locations in North Minahasa greater than the average weight of honey bee venom derived from Minahasa (Figure 3 and Figure 4).

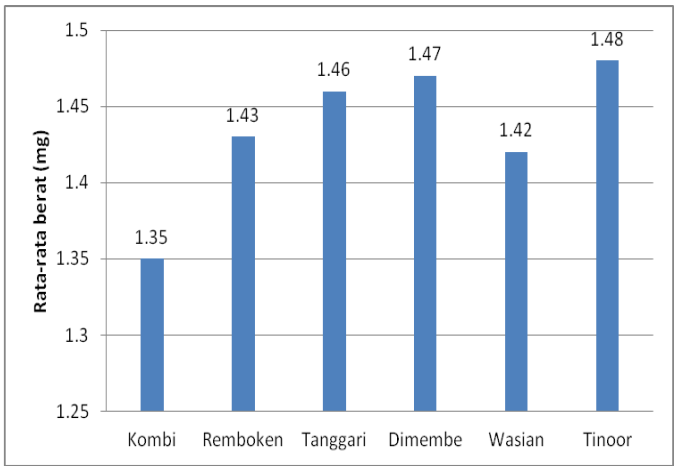

Fig.3. Average weight of bee venom 10 *A. nigrocincta* Smith according to sample origin.

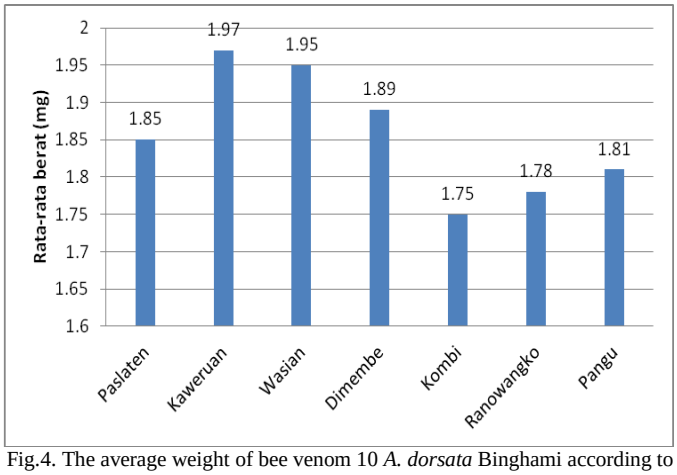

Fig.4. The average weight of bee venom 10 *A. dorsata* Binghami according to sample origin

Approximately 150 bees *A. dorsata* can be isolated dry bee venom on average 0,045 g, while for *A. nigrocincta* just the same amount on average only 0.0078 g of dried bee venom. In the 1000 the bee *Apis meliffera* can produce about 1 gram of dry bee venom .The prize of 1 gram of dried bee venom around $ U.S. 81 or the current exchange rate of Rp. 729 000 per gram. Thus the economic potential of honey bee venom is very prospective.

B. Bee venom Component Analysis Using SDS-PAGE

Concentration polyacrylamide gel used in this study was 17.5%. Results of SDS-PAGE analysis of venom *Apis dorsata* there are 5 clear tapes while to poison *A. nigrocincta* only 3 bands were detected clearly. Molecular weight toxins ribbon at 5 *A. dorsata* in a row is 33.54 kDa, 32.21 kDa, 21:51 kDa, 6.7 kDa and 2.88 kDa. 3 molecular weight bands is evident is 33.54 kDa, 21:51 kDa, 15:43 2 kDa and other bands are not detected (Figure 5 and Table 1).

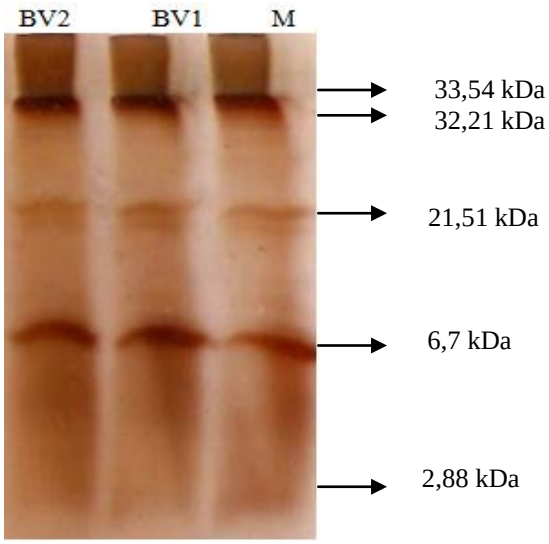

Fig.5. SDS-PAGE results of honey bee venom. Row from right to left Lanes 1 protein marker (M), lane 2 bee venom *A. dorsata* Binghami (BV1) and Lanes 3 Bee toxin *A. nigorcincta* (BV2).

TABLE 1. MOLECULAR WEIGHT (KDAL) HONEY BEE TOXIN *A. NIGROCINCTA* AND *A. DORSATA*

No	Sample	Molecular weight				
		Tape 1	Tape 2	Tape 3	Tape 4	Tape 5
1	BV1	33.54 Hyalorunidase/ fosfolipase A	32.21 Fosfolipase	21.51 Fosfolipase A or lysofosfolipase or antigen 5	6,7 Inhibitor protease	2.88 Melitin
2	BV2	33.54 Hyalorunidase/ fosfolipase A	21.51 Fosfolipase A or lysofosfolipase or antigen 5	15.43 Fosfolipase A	td -	Td -
3	Marker	40	25	15	10	4.6

Description:
BV1: bee toxin A. dorsata
BV2: bee toxin A. nigrocincta

C. Antibacterial activity

Honey bee venom (BV) after isolated in a fresh state centrifuged at a speed of 12,000 rpm for 4 min at 40C, to separate the components of non toxic poison. Bee venom better than *A. nigrocincta* and *A. dorsata* directly used to test the antibacterial activity. The bacteria used were wound infection by bacterial isolates that have been in pure culture. Besides wound infection isolates also used *Escherecia coli* ATTC dan*Staphylacocus aureus* ATCC. Antibacterial activity test performed on 3 concentration test is 20 ug / ml, 30 ug / ml and 50 ug / ml refers to the antibacterial activity test preliminary study conducted previously by researchers.

Both BV *A. nigrocincta* and *A. dorsata* showed antibacterial strong activity .In this study found that the antibacterial activity increased linearly with increasing solution concentration uji.Spektrum antibacterial activity of honey bee venom is indicated by a zone of inhibition or inhibition zone test against several bacterial isolates were used (Tables 2 and 3).

Antibacterial activity of toxin *A. dorsata* stronger than *A. nigrocincta*. At concentration 50 ug / ml *A. dorsata* capable of forming inhibition zone diameter of 18.17 mm on average at 1 isolates konsenterasi while at the same and the same isolates BV *A. nigrocincta* only able to form inhibition zone diameter of 14.50 mm on average.

For BV *A. dorsata*, the largest inhibition zone diameter shown in isolates 2 concentration 50 ug / ml at 2 isolates with an average inhibition zone formed is 18.33 mm. While the smallest inhibition zone diameter on concentration 20 ug / ml, 1 yaitu11 isolates, 17 mm (Table 2 and Table 3). Largest inhibition zone diameter at BV *A. nigrocincta* found on concentration 50 ug / ml in 2 isolates. While the smallest inhibition zone diameter was found on concentration 20 ug / ml, ie 6,67 mm 2 isolates (Figure 6 and Figure 7).

TABLE 2. AVERAGE INHIBITION ZONE DIAMETER (MM) BEES *A. NIGROCINCTA* AGAINST SOME WOUND INFECTION BACTERIAL ISOLATES

Konsenterasi (mg/ml)	Isolat 1	Isolat 2	Isolat 3	Isolat 4
20	7.83 ± 0.76	6.67 ± 1.53	7.50 ± 0.50	9.33 ± 0.58
30	10.50 ± 0.50	12.17 ± 0.29	11.50 ± 0.87	10.67 ± 1.15
50	14.50 ± 0.50	16.00 ± 1.00	13.50 ± 0.87	14.17 ± 0.29

TABLE 3. AVERAGE INHIBITION ZONE DIAMETER (MM) BEES *A. DORSATA* AGAINST SOME ISOLATES BACTERIAL WOUND INFECTIONS

Konsenterasi (mg/ml)	Isolat 1	Isolat 2	Isolat 3	Isolat 4
20	11.17± 0.76	12.17± 0.29	11.33 ± 1.15	13.00± 0.29
30	13.83 ± 0.29	15.17± 0.29	13.33 ± 1.15	13.00 ± 1.00
50	18.17 ± 0.76	18.33 ± 0.58	16.33 ± 1.04	14.83 ± 0.29

Overall inhibitory activity zones rough BV isolated from both types of honey bees that have strong activity compared to the positive comparator antibiotics used were ampicillin and streptomycin with each inhibition zone diameter formed on concentration 30 ug / ml was 14.5 mm and 15 mm.

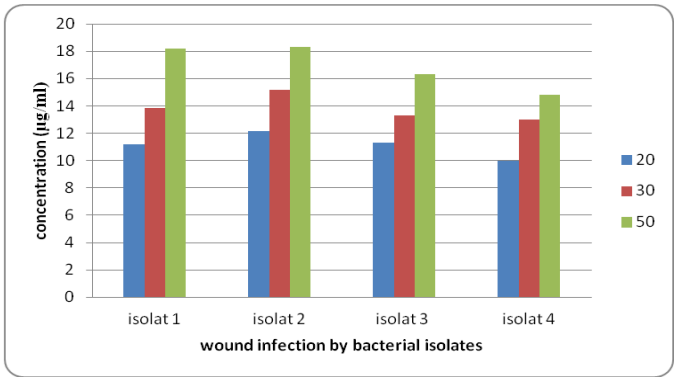

Fig.6. Zone inhibitory toxin *A. dorsata* in some isolates of wound infection

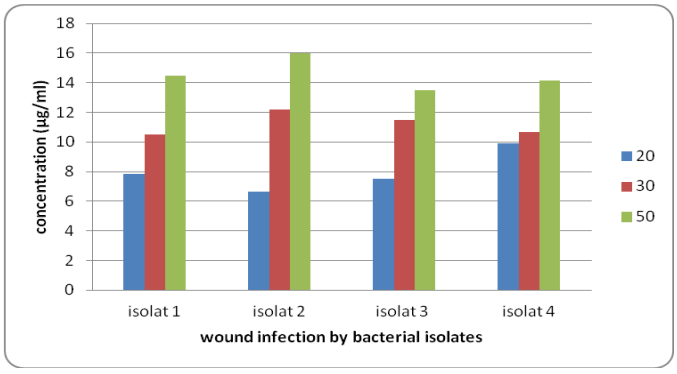

Fig.7. Zone inhibitory toxin *A. nigrocincta* in some isolates of wound infection

Antibacterial activity of bee venom honey two times stronger than penicillin [10] . Gynecology melitin on honey bee venom has antibacterial and antifungal activities were very strong. Melitin have a strong affinity to the lipid component of bacterial cell membrane or through pores in the cell membrane. Melitin can damage bacterial cell membrane permeability. Results of the content analysis of honey bee venom both BV1 and B2 have content that signifikan.Some melitin contained peptide in bee venom can trigger the destruction of the cell membrane, the release of the enzymes lysozyme and granule cells and cytolysis. This activity allowing bee venom has strong antibacterial activity as the main form of defense in the bacterial cell wall and membrane.

Concentration minimum growth inhibitory test solution is the smallest concentration honeybee venom still able to inhibited power grow bacteria in signifikan.From the results test found the inhibition of bacterial growth honeybee toxin *A. dorsata* Binghami stronger than the inhibition of bacterial growth honeybee venom *A. nigrocincta*. From 6 test bacterial isolates used, bee venom *A. nigrocincta* Smith found KHTM 7.5 mg / ml, only 2 isolates found KHTM 2.5 mg / ml. Compared with bee venom *A. dorsata* Binghami of the same number of test isolates only 4 isolates KHTM 7.5 mg / ml while the other isolates to the conseneration 2.5 mg / ml still showed bacterial growth inhibition activity (Table 4).

TABLE 4. CONCENTRATION OF GROWING POWER MINIMUM INHIBITORY HONEYBEE TOXIN *A. NIGROCINCTA* SMITH AND *A. DORSATA* BINGHAMI

No	bacterial isolates	concentration (mg/ml)	
		***A. nigrocicnta* Smith**	***A. dorsata* Binghami**
1	Isolat 1	7,5	2,5
2	Isolat 2	2,5	2,5
3	Isolat 3	7,5	2,5
4	Isolat 4	7,5	7,5
5	*Escherecia coli* cc ATCC	7,5	2,5
6	*Staphylacocus aureus* cc ATCC.	7,5	2,5

Research conducted stated that the honey bee toxin *A. mellifera* potential as antibacterial agents, especially bacteria isolated in the skin such *as P. acnes, S. epidermidis* and *S. pyrogenes*. Toxins isolated from honey bee *Apis cerana, Apis dorsata* and *Apis Florea* has the effect of very strong growth inhibition against bacteria and fungi *Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Xanthomonas subtilis, Proteus vulgaris, Salmonella typhimurium* and *Candida albicans* with highest inhibition zone diameter was 22.50 mm is the poison of *A. cerana* [11]. Compared with the study strong antibacterial activity of honey bee venom *A. nigrocincta* Smith and *A. dorsata* Binghami against 4 bacterial isolates outside and against wound infections *Escherecia Staphylacocus coli* and *Staphylococcus* aureus ATTC ATTC quite strong.

D. Antioxidant activity

Fresh toxins isolated from worker bee *A. dorsata* Binghami and *A. nigrocincta* Smith tested the antioxidative activity using Tiobarbiturat acid method. Toxic poison used is rough (*crude venom*) were taken directly from the worker bees are housed in Eppendorf vials. Antioxidative test begins with a standard curve analysis ferotiosianat (FTC). Oxidation of linoleic acid by FTC method aims to determine the maximum incubation time concentration MDA (malondiadelhida). In this study linoleic acid is placed in a dark bottle threaded then incubated for approximately 8 days at 40^{o}C incubator temperature; hidrogenperoksida formed where the analysis is done every day to achieve the maximum absorbance. During incubation of linoleic acid is oxidized by air. In the early stages of oxidation of linoleic acid (phase lag) will be formed hidrogenperoksida. Propagation phase followed. At this stage hidrogenperoksida levels will increase until it reaches the maximum level, indicated by the maximum absorbance peak occurred on day 5 after going through a stage that hydroperoxide decomposition to form MDA (Figure 8).

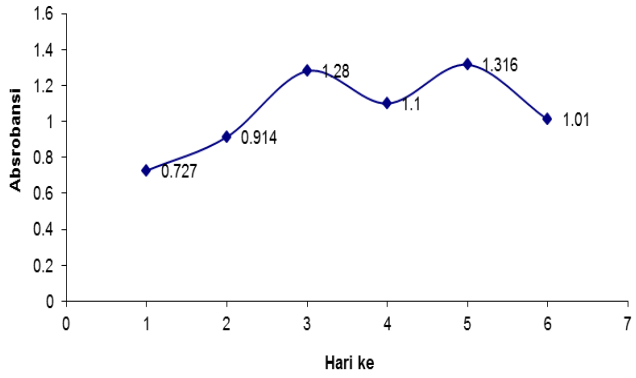

Fig.8. Value absrobansi hydroperoxide of linoleic acid oxidation

Measurement of MDA Concentration

Based on the analysis of hydroperoxide with the FTC method, MDA concentration measurements performed on the 7th day in the hope of all hydroperoxide formed as a result of oxidation of linoleic acid has decayed into MDA. The intensity of the color formed on the samples showed antioxidative potential. The more faded red color means the better formed possessed antioxidative potential (Figure 9 and Figure 10)

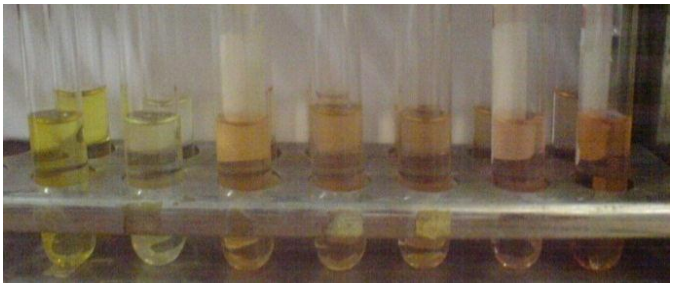

Fig.9. Formation of color in the TBA test BV1 from right to left: linoleic acid, BV1 50 ug / ml, BV1 100 ug / ml, BV1 200 ug / ml, BV1 500 ug / ml, BV1 1000 ug / ml and α-tocopherol.

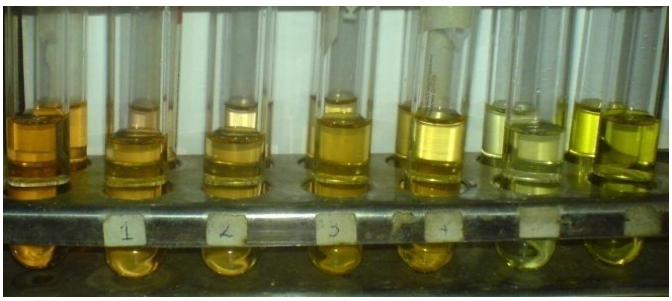

Fig.10. Formation of color in the TBA test BV2 from left to right: linoleic acid, BV2 50 ug / ml, BV2 100 ug / ml, BV2 200 ug / ml, BV2 500 ug / ml, BV2 1000 ug / ml and α-tocopherol.

In the control (without the addition of linoleic acid extract) has a more concentrated color intensity of the tube 2 (BV1 50 ug / ml) 3 (BV1 100 ug / ml), 4 (BV1 150 ug / ml), 5 (BV 200 ug / ml), 6 (BV 500 ug / ml), 7 (BV 1000 ug / ml). BV1 As in the control tube (without the addition of linoleic acid extract) has a more concentrated color intensity of the tube 2 (BV2 50 ug / ml) 3 (BV2 100 ug / ml), 4 (BV2 150 ug / ml), 5 (BV2 200 ug / ml), 6 (BV2 500 ug / ml), 7 (BV2 1000 ug / ml).

From the results obtained with the incubation without the substrate antioxidants oxidized linoleic acid without the addition of bee venom both BV1 and BV2 konsenterasi MDA has a high end that is 25,580 lm (Table 5).

TABLE 5. CONCENTRATIONS OF MDA AND TBA POWER INHIBITORY OXIDATION METHOD

Treatment	Average MDA (μM)	Inhibition oksidasi (%)
Asam linoleat	25.58	0.00
α-tokoferol 200	3.57	85.57
BV1 50	17.18	31.08
BV1 100	9.62	58.53
BV1 200	5.41	73.97
BV1 500	5.12	75.10
BV1 1000	7.05	67.90
BV2 50	7.28	72.65
BV2 100	5.58	71.30
BV2 200	3.57	80.78
BV2 500	4.82	75.68
BV2 1000	5.08	76.33

Oxidation Inhibitory power

The inhibition of the oxidation of α-tocopherol as positive control was 85.57%, while the inhibition of oxidation BV1 and BV2 shown in Table 5 above. At BV1 concentration who has the best oxidation inhibition was 500 ug / ml was 75.10%, while for the inhibition of oxidation BV2 best concentration at 200 ppm which is 80.78%. Thus compared to the inhibition of the positive control α-tocopherol (vitamin E) which have been used and are known to have antioxidant activity poison rough (crude venom) BV1 and BV2 on concentration 500 ug / ml and 200 ppm have antioxidant activity inhibition better. Comparison of the inhibition of BV1 and BV2 are shown in Figure 11 and 12 as follows:

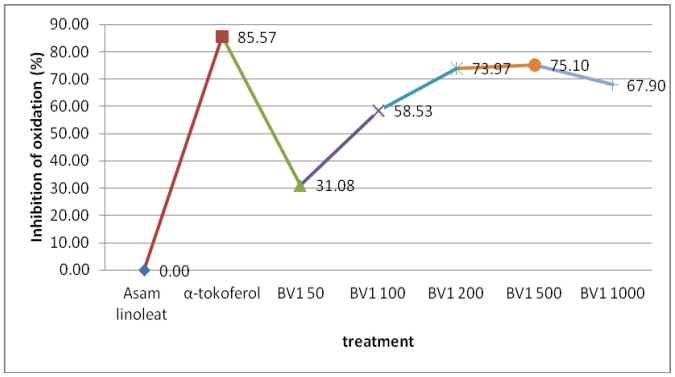

Fig.11. Power inhibition of linoleic acid oxidation by BV1 on each concentration compared with α-tocopherol

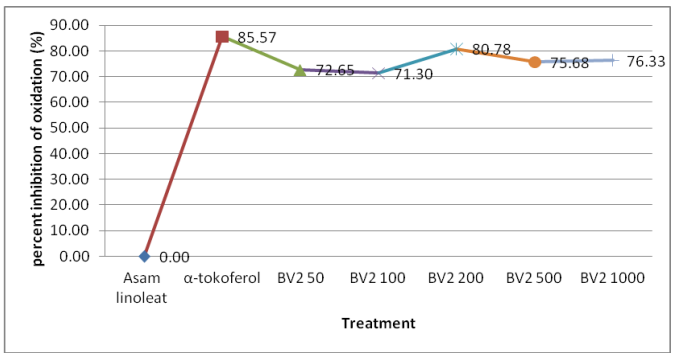

Fig.12. Power inhibition of linoleic acid oxidation by BV2 on Each concentration compared with α-tocopherol

From the results of the study proved that honey bee venom *A. dorsata* Binghami and *A. nigrocincta* Smith potentially inhibit the formation of MDA in linoleic acid. The antioxidant activity due to the content of bioactive components contained in the honey bee venom. Results of SDS-PAGE analysis of protein and peptide components contained in the honey bee venom is hyaluronidase, phospholipase A, melitin, lysofosfolipase or antigen 5 and protease inhibitors.

One of the products of lipid peroxidation is MDA. Lipid peroxidation apt to occur in long-chain fatty acids with more than one double bond such as linoleic, linolenic and arachidonic. The fatty acids are constituents of cell membrane-bound phospholipids, glycolipids and cholesterol. Membrane peroxidation in animal cells causes loss of membrane permeability, being reactive and nonfunctional. Lipid peroxidation can produce singlet oxygen, lipid hydroperoxide and epoxide. Aldaheida which can be formed on lipid peroxidation is malondialdehida (MDA) and 4-hydroxynonenal (4-HNE). MDA is a major metabolite of arachidonic no fatty acid (20:4). Test MDA (TBARS) was used to measure the peroxidation of membrane lipids that occur in. 4-HNE generated by arachidonic through autooksidasi. 4-HNE reacts with cellular components is stronger than the MDA. Therefore 4-HNE is more toxic than MDA but not reactive with TBA [12].

Oxygen radical species attack the nitrogenous bases in nucleic acids, amino acids in the protein, the double bond in the fatty acid chain length in which the hydroxyl group is the most powerful attacker. The ROS attack causes oxidative stress. In addition to lipid peroxidation, free radicals are also produced by a number of cellular reactions associated with the work lipooksigenase enzyme system, NADPH oxidase and xanthine oxidase.

One method used to determine the occurrence of lipid peroxidation is to measure the secondary product malondialdehyde. MDA is a three-carbon molecule with a low molecular weight which results in the peroxidase activity of unsaturated fatty acid chain length. Analysis of MDA with TBA method has been widely applied in knowing the lipid peroxidation in biological systems. The principle is the reaction of MDA and TBA under acidic conditions after heating [13]. MDA binds with TBA to form a red solution which can be measured at a wavelength of 532 nm [12].

Activity of free radicals and lipid peroxidation results oxygenase enzyme systems continuously attacking others when cell membrane fatty acids will cause a lot of damage pathological. Accumulation of free radical damage in tissues in vivo, among others, to cause cancer, inflammation, atherosclerosis, etc.... Many studies have reported that the enzymatic antioxidant activity in the body is insufficient to neutralize free radicals in the body.

The inhibition of linoleic acid oxidation indicated by honey bee venom (BV1 and BV2) is quite good compared to α-tocopherol. It is proved that the bioactive content available on the BV1 and BV2 potential as exogenous antioxidants. Oxidation inhibition activity BV1 and BV2 konsenterasi best on more than equal to 200 indicates that the antioxidative power of honey bee venom rough best tested above 200 ppm.

E. Anticancer activity in vitro

To determine the cytotoxic activity of the honey bee toxin *A. dorsata* and *A. nigrocincta* cytotoxic test using murine leukemia cells P388.Hasil cytotoxic test showed crude venom toxin *A. dorsata* has a stronger cytotoxic activity compared to *A. nigrocicnta*. Inhibitroy concentration (IC50) of toxin *A. dorsata* was 46.77 mg / l whereas *A. nigrocincta* was 66.2 ug / ml (fig. 13). Compared with positive controls used both the cytotoxic activity of honey bee venom is very good. Honey bee venom toxin used is still a rough (crude bee venom).

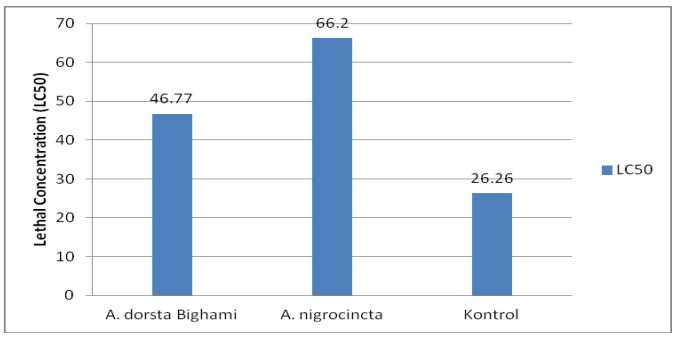

Fig.13. LC50 bee toxin *A. dorsata* and *A. nigrocincta* compared with the positive control

SDS PAGE analysis of the results of well known toxicities of *Apis dorsata* (BV1) and *Apis nigrocincta* (BV2) include phospholipase, phospholipase A or lysofosfolipase or antigen, protease inhibitors and melitin. Melitin is cytotoxic to cancer cells by damaging the cell membrane. BV can induce apoptosis in many types of cancer cell cultures, bee venom induces morphological changes and decreased the percentage of viable cells in cultured cervical cancer cells. Sitometrik flow analysis showed that bee venom can induce ROS production, increased content of cytoplasmic Ca2 +, reduced mitochondrial membrane potential causes the release of cytochrome and promote activation of caspase-3 which directs cells to apoptosis. Bee venom also induces an increase in Fas, p53, p21 and Bax but lower content of BCl-2 [2]. Bee venom significantly inhibited cell growth of lung cancer. Bee venom endotheloal also inhibits vascular growth factor (VEGF), which induces proliferation [3]. Furthermore, BV has a radio-protective activity against oxidative DNA damage in lymphocytes Wistar rats. BV genotoxic and does not produce oxidative damage in a small concentration [14]. Acupuncture uses BV lowering levels of ROS-induced damage to synovial fluid proteins [15]. BV also suppress the formation of nitric oxide in mice and synoviosit makropfag selpsel obtained from aethritis arthritis patients [16]. Thus the content of bee venom can be cytotoxic to cancer cells P388 murine leukemia.

IV. CONCLUSION

From these results it can be concluded:

1. BV1 honey bee venom (*Apis dorsata* Bighami) has 4 bands of molecules with a molecular weight of 33.54 kDa namely; 32.21 kDa; 21.51 kDa; 6.7 kDa and 2.88 kDa whereas BV2 (*Apis nigrocincta* Smith) has 4 peptide bands were detected, namely 33.54 kDa; 21:51 kDa and 15.43 kDa.
2. BV1 allegedly containing enzyme Hyaluronidase / phospholipase A, phospholipase; lysofosfolipase or antigen 5, protease inhibitors and melitin.
3. BV2 allegedly containing hyaluronidase / phospholipase A, lysofosfolipase or antigen 5.
4. Stronger antibacterial activity than the BV1 BV2 on wound infection and bacterial isolates of *E. coli* cc and *S. aureus* cc.
5. Antibacterial activity BV1 average stronger than the positive control test consetration ampicillin at 50 ug / ml.
6. Stronger antioxidative activity than the BV1 BV2 where 80.78% BV2 able to inhibit the formation of MDA in concentration 500 ug / ml and at 75.10% BV1 able to inhibit the formation of MDA.
7. BV1 anticancer activity is still better than the BV1 BV2 where IC50 was 46.77 ug / ml whereas BV2 is 66.2 ug / ml.

REFERENCE

[1] Hassanein NMA and Hegab Am. 2010. Bee Venom – Lead Acetate Toxicity Interaction.Australian Journal of Basic and Applied Sciences 4 (8) : 2206-2221.)

[2] Wan IP S, Wei Chuan H, Lin Pin J, Kuo Man H, Liu Ching K, Hsu Chun S, Yang Sing J, Yang Due M, Chiu Hung T, Han Mi, S and Chung Gung J. 2008. Bee Venom Induced Cell Cycle Arrest and Apoptosis in Human Cervical Epidermoid Carcinoma Ca Ski Cells. Anticancer Research March-April 2008 vol. 28 no. 2A 833-842

[3] Huha Eun J, Hyeon Baekb Y, Hoo Leec, M, Young Choic, D, Suk Parkb D and Doong Leec, J. 2010. Bee venom inhibits tumor angiogenesis and metastasis by inhibiting tyrosine phosphorylation of VEGFR-2 in LLC-tumor-bearing mice.Cancer LettersVol. 292,Issue 1, Pages 98-110.

[4] NCBI, 2010. Melitten Compound Summary PubChem. http://www.pubchem.ncbi.nlm.nih.gov[di akses 7 april April 2011].

[5] Chmielewska HR and Szczêsna T. 2004. HPLC study of chemical composition of Honeybee (Apis mellifera L.) Venom. Journal of Apicultural Science. Vol. 48 No. 2 2004

[6] Hadisoesilo S. 1997. A comparative study of two spesies of cavity-nesting honey bees of Sulawesi, Indonesia. [Dissertation] The University of Guelph, Canada.

[7] Kikuzaki H, Nakatani N. 1993. Antioxidant effects of some ginger constituents. J Food Sci 58:1407-10

[8] Mokosuli YS. , 2008. Antioxidative and anticancer activity of the stem bark extract Langsat (Lansium domesticum L.) [thesis] Graduate School of Bogor Agricultural University.

[9] Mokosuli YS, Worang RL dan Sumampouw H. 2010 Panduan Praktikum Mikrobiologi. Jurusan Biologi FMIPA Universitas Negeri Manado.

[10] Kim Te S, Hwang JY, Sung Suk M, Je Yang. S. bae Rok D. Mi Han S and Lee Hae S. 2009. The minimum inhibitory concenteration (MIC) of bee venom against bacteria isolatd from Pigs and Chikens. Korean J. Vet. Serv. 29(1) : 19-26.

[11] Surendra NS, Jarayam GN and Reddy MS. 2011. Antimicrobial activity of crude venom extracts in honeybees (Apis cerana, Apis dorsata, Apis florea) tested against selected pathogens. African Journal of Microbiology Research. Vol. 5 (18) pp 2765-2772.

[12] Behbahani M, Ali AM, Muse R, Mohd NB. 2007. Anti-oxidant and anti-inflamatory activities of leaves of Barringtonia racemosa. J Med Plant Res 96-102

[13] Tukozkan N, Erdamar H, Seven I. 2006. Measurement of total malondialdehydein plasma and tissues by High-Performance Liquid Chromatography and Thiobarbituric acid assay. Fırat Tıp Dergisi. 11(2): 88-92

[14] Gajski, G. and V. Garaj-Vrhovac, 2009. Radioprotective effects of honeybee venom (Apis mellifera) against 915-MHz microwave radiation-induced DNA damage in Wistar rat lymphocytes: in vitro study. Int.J Toxicol., 28(2): 88-98.

[15] Suh, S.J., K.S. Kim, M.J. Kim, Y.C. Cang, S.D. Lee, M.S. Kim, D.Y. Kwon and C.H. Kim, 2006. Effects of bee venom on protease activities and free radical damages in synovial fluid from type II collagen-induced rheumatoid arthritis rats. Toxicol In Vitro., 20(8): 1465-1471

[16] Park, H.J., D.J. Son, C.W. Lee, M.S. Choi, U.S. Lee, H.S. Song, J.M. Lee and J.T. Hong, 2007. Melittin inhibits inflammatory target gene expression and mediator generation via interaction with Ikappa B kinase. Biochem. Pharmacol., 73: 237-247.