Molekul, Vol. 10. No. 2. November 2015: 135 - 144

THE CHARACTERISTICS OF BIOACTIVE PEPTIDES AND ANTIBACTERIAL ACTIVITY OF HONEY BEE (*Apis nigrocincta*) SMITH VENOM, ENDEMIC TO SULAWESI

Mokosuli Yermia Semuel[1] dan Rudi Alexander Repi[1,2]

[1]Laboratory of Bioactivity and Molecular Biology, Department of Biology, Faculty of Mathematics and Natural Sciences, State University of Manado, Manado, North Sulawesi.
[2]Research Institutes, State University of Manado
The corresponding author : labbiomolunima@gmail.com

ABSTRACT

Apis nigrocincta Smith is a species of honey bee cavity nesting, endemic to Sulawesi. Research that aims to find the composition of the bioactive content of peptides and antibacterial activity of honey bee venom *A. nigrocincta* Smith has been conducted. Honey bee venom composition was analyzed using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) Method and Spectrophotometer UV-Vis Method. Analysis of antibacterial activity, was conducted using a modified agar diffusion method. The results showed that the venom of the honey bee *Apis nigrocincta* Smith has five bands of molecules with a molecular weight i.e. 33.54kDa; 21 kDa and 15.43 kDa. The peptide detected were hyaluronidase, fosfolipase A, mellitin, lysofosfolipase or antigen 5. Antibacterial activity was higher than the control ampisilin and antibiotic streptomycin.

Keywords: antibacterial, *Apis nigrocincta* Smith, bee venom, SDS PAGE.

INTRODUCTION

The venom of the honey bee (BV) has been used since the time of Ancient Egypt as a source of drug bioactive. Bee venom can serve modulator rheumatoid arthritis who dispose of radical oxygen species (ROS) (Murakami et. al., 1997; Oren and Shai, 1997; Hassanein and Hegab, 2010). BV has been known to induce cell or shutdown called by the process of apoptosis in many types of cancer cell culture. BV induces morphological changes and a decrease in the percentage of cells that viabel, in cell culture of cervical cancer. Analysis of flow sitometric shows that BV induces the production of ROS, improving the content of cytoplasmic Ca^{2+}, reduces mitochondrial membrane potential causes the release of cytochrome oxidase, and promoted activation of caspase-3 that directs cells on apoptosis. BV also induces an increase in the tumor suppressor gene namely p53, Fas, p21 and Bax, but lowers oncogenes include BCl-2 (Wan et. al., 2008). Bee venom honey significantly inhibits the growth of cancer cells in the lungs. BV also inhibits vascular endotheloal growth factor (VEGF) that induces cell proliferation (Huha, et. al., 2010). Research conducted by Han et. al., (2010), stating that the honey bee venom of *A. mellifera* as potential antibacterial agents are extremely especially bacteria isolated on the skin of the face, among others, *P. acnes*, *S. epidermidis* and *S. pyrogenes*. Until now, there has not been research reports the antibacterial activity of honey bee *Apis nigrocincta* venom as honeybees endemic to Sulawesi.

Potential pharmacological BV are caused by a biochemical composition of bee venom. Bee Sting venom overall consists of approximately 120 active chemical components, but the components of the 40s is already detected, including 11 peptides, enzymes, 53 amine, carbohydrates, fat and amino acids. Based on

current research, the most instrumental peptides is melittin, apamin, mast cell degranulating peptide (MCDP) and adolapin (NCBI, 2010). Honey bee venom is a complex mixture between the polypeptide enzymes, low molecular weight. A number of enzymes are reportedly contained in honey bee venom phospholipase A2 and hyaluronidase is, fosfomonoesterase acid esterase, and α-D-glucosidase, lysopfosfolipase, α-galaktosidase and α-asetilaminodeosi-glukosidase, arylamidase (Hassanein and Hegab, 2010).

Until now, it is still a little bit of research reports, about bioactivity of honey bee venom. Insect venom and toxin is a mixture of various types of active enzymes, cytotoxin, pheromones, chemical stimulants and neurotoxin. Some bee experts stated, that bee venom is a form of evolution of bees which caused this species occupies almost every area on planet Earth. This led to the study of the bioaktivity of bee venom into the research field of study still very broad (Zalat et. al., 2002). SDS-PAGE technique, most used to find out the composition of the honey bee venom peptide (Hassanein and Hegab, 2010; Zalat et. al., 2002). SDS PAGE is currently used by many researchers in conducting classification of insects based on the composition of their toxicity. With this technique the molecular composition is known to be contained in a sample of BV that will be analyzed. 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). Climate, habitat and the type of food greatly influences the composition of the honey bee venom (Chmielewska and Szczesna, 2004).

Previous research conducted on *Apis dorsata* Binghami bee venom showed strong anticancer activity in murine leukemia P388 cells line. In addition, *Apis dorsata* Binghami venom also showed strong the ability of the DPPH free radical scavengging (Mokosuli and Worang, 2015). *Apis dorsata* Binghami and *Apis nigrocincta* Smith are endemic to Sulawesi. However, both species have many differences. Both species of bees are very different in terms of foraging habitat or region, making the nest and nesting form. 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). Therefore the composition of secondary metabolites, produced honey bee namely honey, propolis, wax, venom and also heavily influenced by the kinds of plants that are available in its natural habitat. Bees that live in one area, it can have a different component of secondary metabolites. This research aims to characterize the composition of the venom and knows the antibacterial activity of honey bee venom *A. nigrocincta* Smith.

MATERIALS AND METHODS

Tools

Tools used one set of electrophoresis vertical model TV100YK-MODSYS, one set micropipet eppendorf and ultrasentrifuse eppendorf 5430R, autoclave SX series 700, laminar air flow, digital scales, eppendorf ultrasentrifuse 5430R, nanophotometerTM Pearl Version 1.0 Implen, vortex v-1 plus eppendorf, micropipette eppendirf and others.

Materials

The materials used, among others: Akrilamid, ddH_2O, bisakrilamid, a protein marker 4.6-100 kDal, TEMED, ethanol p.a., nutrient agar (NA), and nutrient broth (NB) (merck). Ammonium peroksodisulfat, akuades, crude bee venom of *A. nigrocincta*, obtained from several locations in the Regency of Minahasa and Regency North Minahasa, pure culture

isolates of bacterial infective wound (Microbiology Laboratory collection), *Escherecia coli* ATCC and *Staphylacocus aureus* ATCC.

Research methods

A sample of bees to test is collected on a sterile eppendorf vial 5 mL. Vial containing bee venom then stored on the box containing the ice so that the protein is not denaturated. Subsequently taken to the laboratory to test bioactivity.

SDS PAGE

SDS PAGE using a modification of the method of Laemmli (1970) and poliacrilamid concentrations of 17.5%. The composition of the gel separator prof 17.5% whereas the composition of the gel Gatherer prof 5%. After gel is created, the sample and the protein marker injected at existing wells. Running is done for 4 hours on a 60 volt, 20 mA. Protein gel will go down with the help of an electric current that moves from the negative to the positive pole pole. After running, the gel is passed on to the silver staining the gel is then soaked in a solution of fixation for more or less two hours, while diagitasi slowly. The gel is then washed with a solution of washing solution for 20 minutes (repeated three times) without agitation. Rinsed gel with aquades free ion (ddH_2O) for 10 seconds. The gel is then soaked in a solution of sensitize for 1 minute. Next gel with ddH_2O washed for 20 seconds (repeated three times). Gel incubated into the fridge with AgNO3 0.1% (silver nitrate) for 20 minutes. The gel is then washed or soaked with ddH_2O for 20 seconds and repeated three times. Next gel soaked with a developing solution, while diagitasi up to staining dye. Gel added stop solution for five minutes then rinsed with ddH_2O for five minutes. Scaning done and subsequent data analysis.

Antibacterial activity

Antibcaterial activity used diffusion agar method with modified (Irobi et al., 1996; Russel and Fur, 1997; Akarele, 2008; Mokosuli & Worang, 2009).

Bacterial isolates grown on media test NA for 18 hours Using a sterile lup, bacterial suspension taken and dissolved in a solution of NaCl on physiological reaction tubes. Measure the OD with the nanospektrofotometer. Using a sterile cotton bud, bacterial suspension is applied evenly on the surface of the media NA existed in the petri dish. Bee venom concentration i.e. 20 µ g/mL; 50 µ g/mL; 100 µ g/mL and 150 µ g/mL. Ampisilin and streptomycin as the control is created in a concentration of 50 µ g/mL. Disks created from Whatman filter paper and has been soaked in a solution of the test i.e. bee venom for 3 hours then put in aseptik on NA in the petri dish that has inoculated the bacterial isolates, respectively i.e. 4 isolates of bacterial infection, the wound *Escherecia coli* ATCC and *Staphylacocus aureus* ATCC. Incubated for 24 hours at room temperature the next measured the diameter of the zones of drag that is formed.

Determination Minimum Inhibitory Concentration (MIC)

Bee venom dissolved in buffer at a concentration of 40, 30, 20, 15, 10, 7.5 and 2.5 ppm (mg/L) by aseptic. The surface of the medium in the petri dish is dried before inoculated with bacteria cultures (mix of about 106 cfu/mL). After the inoculation, the petri dish is then incubated at a temperature of 32 °C aircraft as long as 72 hours after 30 minutes of inoculation is done. The lowest Concentration which prevent the growth of bacteria which look is the minimum concentration inhibitory grow. Each made 2 repeats. Bee venom dissolved in buffer at a concentration of 40, 30, 20, 15, 10, 7.5 and 2.5 ppm (mg/L) by aseptik. The surface of the medium in the petri dish is dried before inoculated with bacteria cultures (mix of about 106 cfu/mL). After the inoculation, the petri dish is then incubated at a temperature of

32 °C aircraft as long as 72 hours after 30 minutes of inoculation is done. The lowest Concentration which prevent the growth of bacteria which look is the minimum concentration inhibitory grow. Each made 2 repeats.

The Analysis Of Research Data

Isolation and characterization of the results Data of bee venom were analyzed qualitatively. Antibacterial activity. The inhibitory zone formed is measured in micrometers are then calculated average per test concentration. KHTM is determined based on the concentration of the test solution in which the smallest bee venom is still capable of inhibiting the growing bacteria.

RESULTS AND DISCUSSION

Characteristics of the Venom

The physical characteristics of the honey bee venom freshly taken from the worker bees are white, clear, odorless, tasteless as if burning on tongue, pH (3.70 to 5.7) (**Table 1**). About five to ten minutes at room temperature will be turned into a powder as flour is yellowish white. Needle injected bee venom is present on the part of the abdomen called the sting. Sting is present on the end of the abdomen. Sting *Apis nigrocincta* coloured blackish at the base there is a venom sacs containing fresh venom **(Figure 1**). Isolation of venom from 100 *Apis nigrocincta* bees produce dry bee venom 0.038 grams on average.

Table 1. Characteristics of fresh and dry bee venom of *Apis nigrocincta Smith*

Characteristic	Fresh Bee venom	Dry bee venom
pH	3,70 – 5,7	4,8 – 6,3
Form	semisolid fluids	Flour/Crystal
Colours	Clear white	White Brown
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 Analysis Of Phytochemicals :		
- Polifenol	++	+
- Flavonoid	++	+

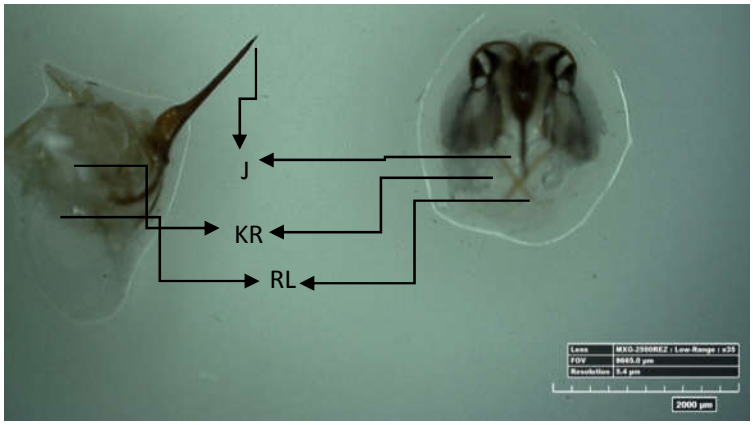

Figure 1. Bee venom *A. nigrocincta* observed by Hirox KH8700-Stereomicroscope 3D. Description : J = sting, KR (venom sach), RL = bee venom.

Wet weight of bee venom

Measurement of average wet weight of honey bee venom, conducted on 10 bees from each location for easy weighing on digital scales. Once the venom are removed and fresh fit on a sterile vial eppendorf, bee venom that weighed with digital scales which have been calibrated. The results showed that the weight of wet, bee venom varies according to the origin of the samples (**Figure 2**). From 150 bees *A. nigrocincta* obtained average weight of dry bee venom in powder form that is 0.0078 grams.

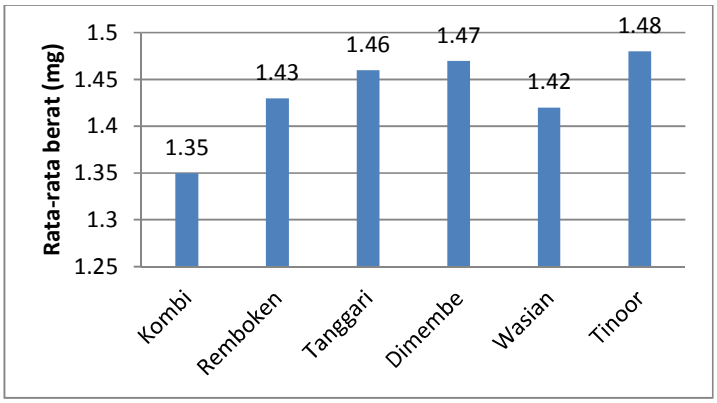

Figure 2. The average weight of fresh venom *A. nigrocincta* according to the origin of the samples.

Peptide Components of bee venom

Polyacrylamide gel concentrations used in this study was 17.5%. The results of the analysis of SDS-PAGE of *A. nigrocincta* venom only 3 bands detected clear and two bands detected unclear. Molecular weight 3 bands that appears clear is 33.54 kDa, 21 kDa, 15:43 kDa and 2 other bands not unidentified (**Figure 3** and **Table 1**).

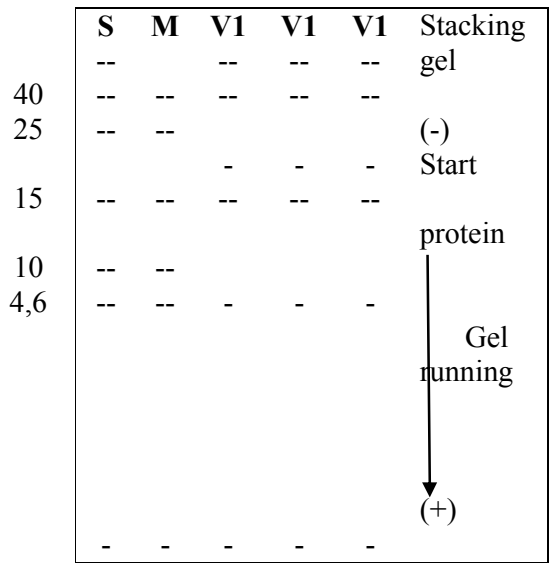

Figure 3. Kromatogram visualization results of SDS PAGE. Found 3 bands of proteins with molecular weights in a row: 33.54 kDA, 21.51 kDa, kDa, 15.43 kDa, 2 bands is not clear obvious. Apitoksin standard (S), marker proteins (M), venom *Apis nigrocincta* (V1).

Melitin and phospholipase A was a constituent component of bee venom honey have a high toxicity, activity compared to other components. Melitin compiled a 30% up to 50% dry weight toxins of *A. mellifera* while phospholipase A was approximately 10% to 12%. Melitin and phospholipase A, can increase the synthesis of tumor cell necrosis factors, such as Cytokines and interleukins 1, stimulates the release of aracidonat acid which is produced in the process of immune response. Bee venom analysis results *A. nigrocincta* indicates the thickness of the band is high not only on melitin and protease inhibitors also on phospholipase and hyaluronidase. The thickness of the bands shows the number concentration of compounds that contained (**Table 2**).

Table 2. Molecular weight (kDal) honey bee venom of *A. nigrocincta*

No	Sample	Molecular Weight				
		Band 1	**Band 2**	**Band 3**	**Band 4**	**Band 5**
1	BV2	33.54 Hyalorunidase/ fosfolipase A	21.51 Fosfolipase A and Lysofosfolipase and antigen 5	15.43 Fosfolipase A	td Mellitin	td -
3	Marker	40	25	15	10	4.6

Description : BV2 : *Apis nigrocincta* venom

Antibacterial activity

The venom of the honey bee after the isolated state in a fresh, centrifused on speed 12000 rpm for four minutes at a temperature of 40 °C, to isolate the venom with non venom components. The bacteria used are bacterial isolates, wound infection, which had been out in pure culture. Besides wound infections isolate, also used *Escherecia coli* ATTC and *Staphylacocus aureus* ATCC. Antibacterial activity assay performed on three test concentration i.e. 20 µg/mL, 30 µg/mL and 50 µg/mL of preliminary research in reference to the trials of antibacterial activity, conducted earlier by researchers. *Apis nigrocincta* venom showed strong antibacterial activity. In this study found the antibacterial activity increasing linearly with an increase in the concentration of the test solution. The spectrum of antibacterial activity of honey bee venom is shown by the inhibition zone or drag zone against some bacterial isolates the test used (**Table 3**). On concentration 50 µ g/mL BV, *A. nigrocincta* is capable of forming a diameter inhibitory zone average 14.50 mm (Figure 4).

Table 3. Average diameter drag zone (mm) bees *A. nigrocincta* against some bacterial isolates of wound infection

Konsentrasi (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

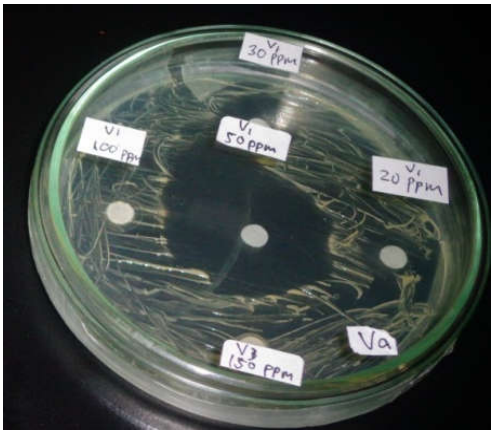

Figure 4. Formation of Inhibitory Zone of *Apis nigrocicnta* venom on wound infection bacteria isolate

The biggest inhibitory zone of *A. nigrocincta* venom found at concentrations of 50 µg/mL at wound infections bacterial isolates two. While the smallest diameter drag zone found at concentrations of 20 µ g/mL, i.e. 26.67 mm wound infections bacterial isolate one.

Overall inhibitory zones grow bacteria by BV *Apis nigrocincta* indicates strong bactericidal capability. Compared with ampisilin and streptomycin with each diameter inhibitory zone formed at a concentration of 30 µg/mL is 14.5 mm and 15 mm.

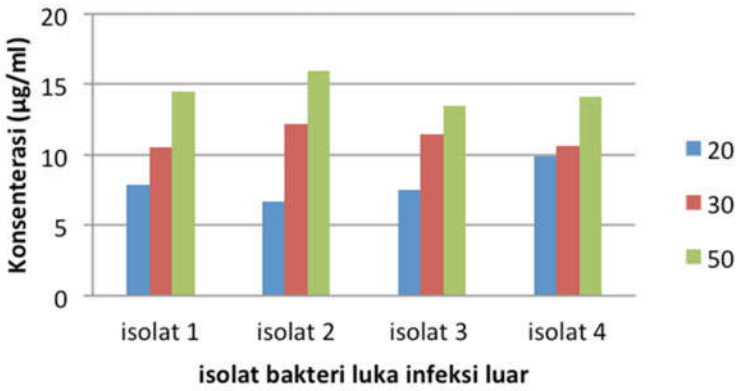

Figure 5. Diameter of Inhibitory Zone of *Apis nigrocincta* venom on some bacterial isolates from wound infection

Antibacterial activity of honey bee venom, two times stronger than Penisilin (Kim, et. al., 2009). Mellitin content on honey bee venom has antibacterial and antifungal activity are very strong. Mellitin has a strong affinity for lipid membrane component of bacterial cells or through the pore on the membrane of the cell (Bogdanov, 2011). Mellitin can destroy the bacterial cell membrane permiability (Klotz, et. al., 2007; Kim et. al., 2009) the results of the analysis of the content of honey bee venom contain significant mellitin. Some toxin contained in bee venom can trigger the destruction of the cell membrane, release of lisosim enzymes and granule cells and sitolisis (Lazarev, et. al., 2005). This activity allows the bee venom has strong antibacterial activity due to the shape of the main defense of the bacteria on his cell walls and membranes.

Table 4. The Minimum inhibitory concentration (MIC) of *Apis nigrocincta* venom on Bacterial Isolates

No	Bacterial test	Concentration (mg/mL)
1	Isolat 1	7,5
2	Isolat 2	2,5
3	Isolat 3	7,5
4	Isolat 4	7,5
5	*Escherecia coli* cc ATCC	7,5
6	*Staphylacocus aureus* cc ATCC.	7,5

The minimum inhibitory concentration of growing is the smallest concentration of a solution of honey bee venom test still able to inhibit bacterial grown power test significantly. From the results of the study found growing bacteria inhibitory power of the venom of the honey bee A. nigrocincta has stronger, compared to control. Of the six isolates of bacteria test used, bee venom *A. nigrocincta* found MIC 7.5 mg/mL, only 2 isolates found MIC 2.5 mg/mL (**Table 4**). The BV was isolated from honey bee *A. cerana*, *A. dorsata* and *A. florea* has influence very strong growth inhibition against bacteria and fungi that is *Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia. coli*, *Xanthomonas subtilis, Proteus vulgaris, Salmonella typhimurium* and *Candida albicans* with the diameter of highest inhibitory zones is 22.50 mm belongs to *A. cerana* venom (Surendra et. al., 2011). The research compared to the strong venom of antibacterial activity of honey bee *A. nigrocincta* against wound infections bacterial isolates and against *E. coli* and *S. aureus* belongs to the powerful.

CONCLUSION

From the results of this research it can be concluded that the Apis nigrocincta venom has 3 bands peptide was detected i.e. 33.54 KDa; 21 KDa and 15.43 KDa. The peptide is A hyaluronidase/fosfolipase, lysofosfolipase or antigen 5. Antibacterial activity was higher than the control antibiotic ampisilin and streptomycin.

AKNOWLEDGMENT

The Authors are gratefully thankful to the Directorate of Research and Community Service, The Ministry of Research, Technology and Higher Education, Republic of Indonesia, which has funded this research through Competitive Research Grant (Hibah Bersaing) Scheme in 2015.

REFFERENCES

Akarele, J.A. 2008. Antimicrobial activity of the ethanol extract and fractions of the seeds of *Garcinia kola* Heckel (Guttiferae). *Afr. J. Biotechnol*. Vol. 7 (2), pp. 169-172, 18 January, 2008

Bogdanov, S. 2011. Functional and Biological Properties of the Bee Products*: a Review*. www.bee-hexagon.net, Bee Product Science, 1.February 2011

Boukraa, L and S.A. Sulaiman, 2009. Rediscovering the Antibiotics of the Hive. *Recent Patents on Anti-Infective Drug Discovery,* 9, *4,* 206-213

Chmielewska H.R. and Szczêsna T. 2004. HPLC study of chemical composition of Honeybee (Apis mellifera L.) Venom. *Journal of Apicultural Science.* Vol. 48 No. 2 2004

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

_____ 2001. Keanekaragaman Spesies Lebah Madu Asli Indonesia. *Biodiversitas Volume 2 No. 1. Hal. 123-128.*

Hassanein N.M.A. and Hegab A.M. 2010. Bee Venom – Lead Acetate Toxicity Interaction. *Australian Journal of Basic and Applied Sciences* 4 (8) : 2206-2221.

Heldt and Heldt. 2005. *Plant biochemistry*. Elsevier, London.

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 Letters* Vol. 292,Issue 1, Pages 98-110.

Irobi O.N, Mon-Young and Anderson W.A. (1996). Antimicrobial activity of Annatto (*Bixa orellana* extract). *Int. J. Pharm*. 34: 87-90.

Klotz S.A, Gaur N.K, Rauceo J, Lake D.F, Park Y, Hahm K.S. and Lipke P.N. 2007. Inhibition of adherence and killing of Candida albicans with a 23-Mer peptide (Fn/23) with dual antifungal properties. *Antimicrobial Agents and Chemotherapy*. 2004 Nov;48(11):4337-41. PMID 15504862.

Lazarev V.N, Shkarupeta M.M, Titova G.A, Kostrjukova E.S, Akopian T.A, and Govorun VM. 2005. Effect of induced expression of an antimicrobial peptide melittin on Chlamydia trachomatis and Mycoplasma hominis infections in vivo. *Biochemical and Biophysical Research Commununications*. Dec 16;338(2):946-50.

Lazarev V.N, Stipkovits L, Biro J, Miklodi D, Shkarupeta M.M, Titova G.A, Akopian and T.A, Govorun V.M. 2004. Induced expression of the antimicrobial peptide melittin inhibits experimental infection by Mycoplasma gallisepticum in chickens. *Microbes and Infection*. May;6(6):536-41.

Lazarev V.N, Parfenova T.M, Gularyan S.K, Misyurina O.Y, Akopian T.A and Govorun V.M. 2002. Induced expression of melittin, an antimicrobial peptide, inhibits infection by Chlamydia trachomatis and Mycoplasma hominis in a HeLa cell line. *International Journal of Antimicrobial Agents*. Feb;19(2):133-7.

Liu, X. Chen, D.W., Xie, L.P. and Rongqing, Z. (2002): Effect of honey bee venom on proliferation of K1735M2 mouse melanoma cells *in-vitro* and growth of murine B 16 melanomas *in-vivo*. *J. Pharm. Pharmacol*., 54: 1083-1089

Murakami M, Nakatani Y, Atsumi GI, Inoue K , and Kudo I. 1997. *Regulatory functions of phospholipase A2. Crit Rev Immunol* 17: 225-283.

Mokosuli Y.S. 2008. Aktivitas antioksidasi dan antikanker ekstrak kulit batang Langsat (*Lansium domesticum L.*). [*Tesis*]. Sekolah Pascasarjana Institut Pertanian Bogor.

Mokosuli Y.S dan Worang R.L. 2009. *Pengantar Praktikum Mikrobiologi*. FMIPA Universitas Negeri Manado.

Mokosuli Y.S. dan Worang RL. 2015. Pengembangan Racun Lebah Madu Apis Dorsata Binghami Endemik Sulawesi Utara Sebagai Sumber Bioactive Obat Antioksidan Dan

Antikanker. *Laporan Penelitian Hibah Bersaing*, Lembaga Penelitian Universitas Negeri Manado.

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

Oren Z and Shai Y. 1997. Selective lysis of bacteria but not mammalian cells by diastereomers of melittin: Structure-function study. *Biochemistry* 1997; 36: 1826-1835.

Oršolić N, Josipović P and Bašić I. 2009. Direct Antitumor Activity Of Honey Bee Venom *In Vivo* And *In Vitro*. *Egyptian Journal of Natural Toxins*, Vol. 6(1): 1-5.

Son D.J., Lee J.W., Lee Y.H., Song H.S., Lee C.K., and Hong J.T. (2007) Therapeutic application of anti-arthritis, pain-releasing, and anti-cancer effects of bee venom and its constituent compounds. *Pharmacology & Therapeutics* 115, 246-270

Wan I.P.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*

Zalat S, Abouzeid A, Ibrahim A and Abd El-Aal A. 2002. Protein pattern of the honeybee venoms of Egypt. *Egyptian Journal of Biology*, Volume 4, 2002, pp. 42-46