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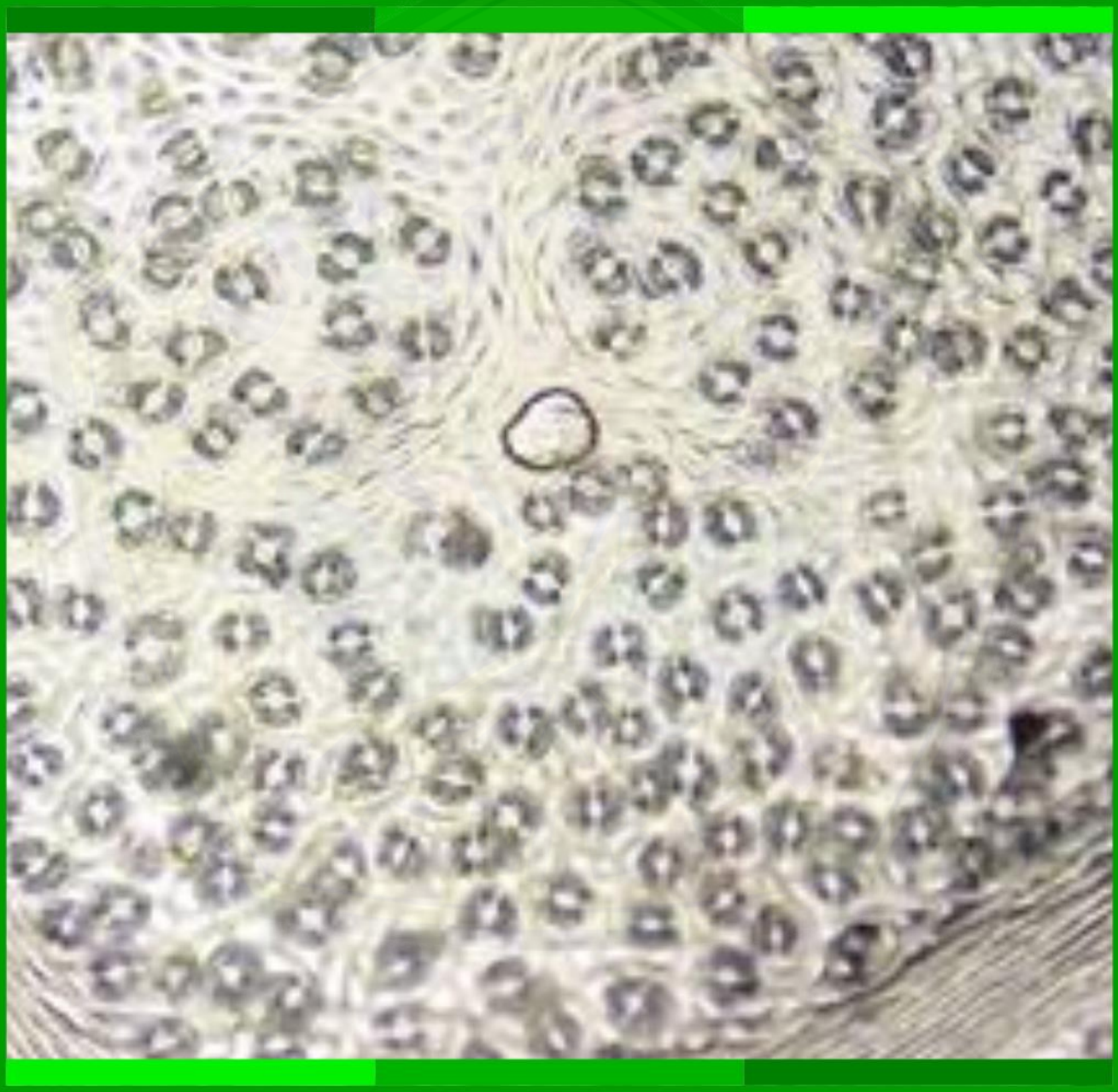

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PURIFICATION OF 31 AND 67 kDa PROTEIN FRACTION FROM SALIVARY GLAND OF *Aedes Albopictus* (SKUSE) (DIPTERA: CULLICIDAE)

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Abstract

Aedes albopictus mosquito is a potential vector for Dengue Haemorrhagic Fever (DHF) which transmits Dengue virus during blood feeding. The success of the blood feeding process is aided by the biological activity of proteins in the salivary glands of Aedes albopictus. There are 30 types of proteins from the salivary glands of Aedes albopictus which are carried along blood feeding process. Proteins in the salivary glands act as vasodilator and immunomodulator. Previous studies have identified two immunogenic proteins from the salivary glands of Aedes albopictus with molecular weight of 31 and 67 kDa. Further research on the biological function of these proteins requires its purified protein. The objective of this study was to obtain 31 and 67 kDa purified proteins by implementation of electroelution and dialysis purification. The 31 and 67 kDa protein was successfully purrified by this methods. This has been confirmed by a single band visualization after SDS-PAGE analysis.

Keywords: *Aedes albopictus, Purification, Dialysis, Electroelution, Saliva*

1. INTRODUCTION

Dengue Hemorrhagic Fever is an endemic disease in tropical and subtropical regions (Zhang and Lui, 2020), which is mainly caused by dengue virus infection (DENV) (Brown et al., 2019). Dengue virus has four serotypes, namely DENV-1, DENV-2, DENV-3 and DENV-4 (Hardani et al., 2018). Dengue virus is transmitted by arthropod vectors namely *Aedes* (*Ae.*) *aegypti* as primary vectors and *Aedes* (*Ae.*) *albopictus* as secondary vectors (Hussain et al., 2018). Transmission of dengue virus to the host (human) occurs when *Ae. Albopictus* does blood feeding. The success of the mosquito blood feeding process is aided by the biological activity of proteins in the salivary glands of *Ae. albopictus* (Sri-in, 2019).

Arthropod salivary gland proteins play an important role in the process of blood feeding because they contain vasodilator and immunomodulatory components (Titus et al., 2006; Juhn, 2011; Sri in, 2019). The vasodilator component serves to inhibit hemostasis (anti-platelet aggregation and anti-coagulant) and vasoconstriction so that it can prevent the process of blood clotting at the host when the artopods do blood feeding. The immunomodulatory component plays essential role in modulating the immune response in the form of anti-inflammatory, allergic and itchy responses in the host (Fontaine et al., 2011), so that it can help the vector do blood feeding (Titus et al., 2006). The vasodilator and immunomodulatory components found in salivary gland proteins have the potential to develop as vector-based vaccine candidates (Manning et al., 2018).

The results of a western blot analysis show that there are immunogenic proteins from the salivary glands of *Ae. albopictus*, with molecular weights of 31 and 67 kDa (Khasanah, 2019). The analysis of Laser Desorption/Ionization-Time of Flight (MALDI-TOF) shows that the protein in the salivary glands *Ae. albopictus* with a molecular weight of 18-39 kDa contains the D7 family protein which plays a role in the modulation of the immune response and anti-hemostasis. The proteins with molecular weights of 61-63 kDa are apyrases that play a role in inhibiting platelet aggregation (Docoure et al., 2013). Both of these proteins have the potential to develop vector-based vaccines, so research on their biological functions needs to be carried out.

Further research on these proteins requires pure protein preparations.

Pure protein is obtained from protein purification. There are several protein purification methods, and the selection of the right purification method is one of the keys to getting pure protein preparations (Sattayasi, 2012). An easy and widely used purification technique for proteins that are reparated through SDS-PAGE method is by electroelution and dialysis (Shoji et al., 1995). However, in carrying out the protein purification process, each target protein sample requires different electroelution and dialysis conditions (Reddy, 2014), so that it is necessary to purify proteins 31 and 67 kDa from *Ae*. *albopictus*s salivary gland in the right conditions.

2. MATERIALS AND METHODS

Research Site and Setting

This research was carried out at the Biotechnology Laboratory, Mathematics and Natural Sciences Faculty, University of Jember from November 2019 to July 2020.

The Isolation of *Ae. Albopictus* Salivary Gland

The isolation of salivary glands was done using microdissection, a technique aimed to take salivary glands from mosquito body components by using a needle under a microscope. The isolation began by dripping PBS solution on the slide (Schmid et al., 2017). The isolation of the salivary glands of female *Ae. albopictus* was performed under a stereo microscope (NIKON SMZ745, Japan). The salivary glands were then stored in a 1 mM solution of Phenylmethylsulfonyl Fluoride (PMSF) in Phosphate Buffered Saline (PBS) pH 7.4 (Sigma-Aldrict, USA) and stored at temperature -20ºC.

Salivary Gland Protein Electrophoresis

The protein electrophoresis was conducted using the Sodium Dodecyl Sulfate Polyacrilamide Gel Electrophoresis (SDS-PAGE) method. Salivary gland tissue samples were added with another buffer (1:1 (v/v)) and then heated for 3-5 minutes to get the salivary gland protein extract (Wathon et al. 2020). The protein extract was put into a polyacrylamide gel consisting of 12% separating gel and 4% stacking gel. Protein electrophoresis was carried out at a voltage of 100 V (constant volts) for 100 minutes at room temperature and a pH electrode buffer of 8.3.

The coloration of polyacrylamide gel was done using Coomassie Brilliant Blue (CBB) solution (Sigma-Aldrict, USA) for 60 minutes and continued with the dyeing process 3 times every 30 minutes by using a staining solution (destaining) to remove the CBB coloring on the CBB polyacrylamide gel which was not bound to protein. Part of the polyacrylamide gel containing 31 and 67 kDa target protein bands was separated aseptically and stored in a pH 8.3 electrode buffer at 4° C as a sample for protein purification.

Purification of Protein Fractions 31 and 56 kDa

Proteins 31 and 67 kDa from the SDS-PAGE analysis were purified using the electroelution to remove proteins from the polyacrylamide gel (Vázquez-Iglesias et al. 2017). Pieces of gel containing protein were inserted into the cellophane membrane (Merck, USA) which contained an electrode buffer of pH 8.3. The cellophane membrane was tightly closed and inserted in a horizontal electrophoresis tank (Bio-Rad, 62 USA) which contained an electrode buffer of pH 8.3. The electroelution process was carried out at room temperature at a voltage of 25 V (constant volts) for 150 minutes until the gel pieces became clear.

Electroelution resulted in the cellophane membrane, which was then transferred to the new cellophane membrane to proceed to dialysis stage. Dialysis aimed to remove contaminants and other compounds which were not needed during the protein purification (Sattayasai 2012). The cellophane membrane which contained electroelution solution was then tightly closed and put into a beaker containing PBS solution pH 7.4. The dialysis process was carried out at 4ºC on the magnetic stirrer for 24 hours and the PBS solution was replaced once every 12 hours. The results of dialysis were concentrated using a microcentrifuge tube with a 10 kDa Molecular Weigh Cut-Off (MWCO) (Corning, USA). Purification

results were confirmed by the SDS-PAGE method. Pure protein samples 31 and 67 kDa were added with buffer buffer (1:1 (v/v)). Electrophoresis was carried out at a 150 V (constant volts) for 1 hour at room temperature using an electrode buffer of pH 8.3. Next, the coloration of polyacrylamide gel was performed using CBB solution for 60 minutes and continued with the dyeing process 3 times every 15 minutes.

3. RESULTS AND DISCUSSION

Salivary Gland Isolation

Male and female adult mosquitoes obtain energy from sugar through sugar feeding. In addition to sugar, female mosquitoes also need blood from the host for the maturation of their eggs through the process of blood feeding (Prasadini et al., 2019). The process of blood feeding in female mosquitoes is often constrained by homeostasis; inflammation and host immunity, so we needed components to stimulate the blood feeding. Salivary glands are known to have anti-homeostasis and anti-inflammatory components that help mosquitoes in sucking blood from the host through the process of blood feeding (Titus and Ribeiro, 1988).

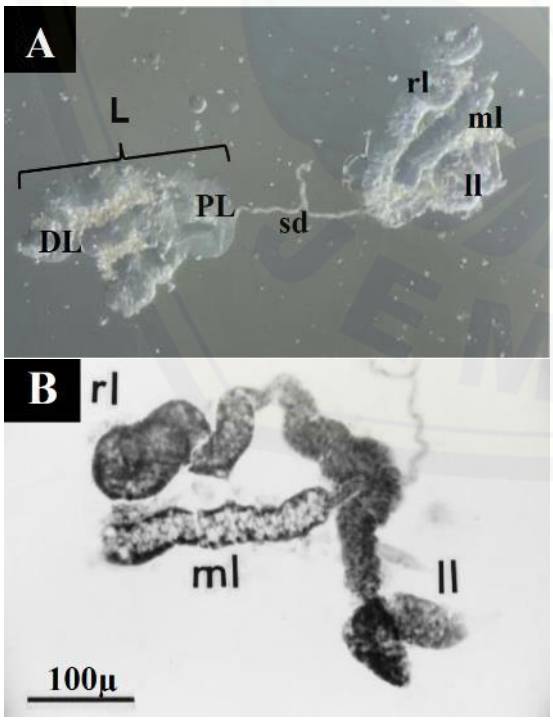

Figure 1. Salivary Gland Morphological Structure of Female *Ae. albopictus*. (A) composed of Salivary Duct (sd), Lateral Lobe (L), Proximal-Lateral (PL), Distal-Lateral (DL), Medial Lobe (ml), Right Lateral Lobe (rl), Left Lateral Lobe (ll) (NIKON SMZ745 Stereo Microscope with 50x magnification (Image: Personal Documentation). (B) The salivary gland of female *Ae. albopictus* according to Bowers et al., (2003).

The saliva glands of female *Ae. albopictus* is located in the thorax and is taken using the microdissection technique (Schmid et al., 2014). Salivary glands have a structure consisting of 2 parts connected by salivary ducts. Each section has 3 lobes, i.e. two lateral lobes (L) and one medial lobe (M). Lateral lobes consist of right lateral lobe (RL), medial lateal lobe (ML) and left lateral lobe (LL). Each lobe has two regions commonly referred to as the Proximal Lateral (PL) and Distal Lateral (DL) lobes (Juhn et al., 2011; Vega-Rúa et al., 2015; Bowers et al., 2003). The morphological structure of female Ae. *albopictus* salivary gland can be seen in Figure 1. Each lobe in the salivary glands of *Aedes albopictus* secretes a different protein in each part of the lobe (Juhn et al., 2011). Protein secretion from the salivary gland plays an important role in the success of the blood feeding process (Tittus et al., 2006). The dominant protein secreted in the lateral and medial lobe is the D7 family protein and *apyrase* (Docoure et al., 2013).

Salivary Gland Protein Electrophoresis

The SDS-PAGE analysis results were separated based on the molecular weight of *Ae. albopictus* salivary gland protein successively. The analysis identified 19 protein bands with molecular weights: ~ 324, 183, 111, 101, 97, 73, 70, 76, 60, 51, 43, 40, 31, 29, 27, 26, 24, 15, and 14 kDa. The salivary gland protein profile of *Ae.*

Albopictus can be seen in Figure 2. Proteins with molecular weights of 31 and 67 kDa from the salivary glands of *Ae. albopictus* is immunogenic or able to induce the host immune response (Khasanah, 2019). Immunogenic protein plays a role in the process of blood feeding so that the host does not coagulate blood. In addition, it prevents platelet aggregation, dilation of blood vessels, itching and pain (Doucoure et al., 2013; Oktarianti et al., 2015; Fong et al., 2018; Calvo et al., 2009).

Proteins from the salivary glands of *Ae. albopictus* with a molecular weight of 31 kDa contained at least three types of proteins namely: antigen-5 family allergens, 30-kDa allergens, D7 family and Angiopoietin. The protein has the ability to stimulate the immune system in the host or immunogenic body. (Wasinpiyamongkol et al., 2012; Arca et al., 2007; Calvo et al., 2006; Almeras et al., 2010; Cantillo et al., 2016; Doucoure et al., 2013).

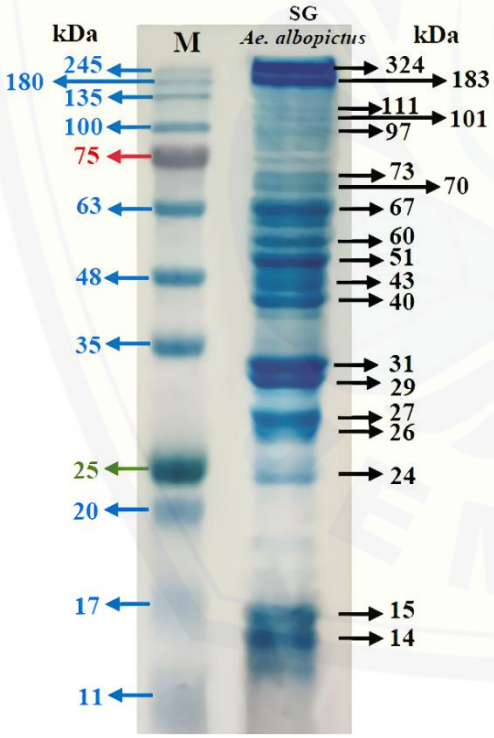

Figure 2. Salivary gland protein profile of *Ae. albopictus*, Marker Jena Bioscience BlueEye Prestained Protein Marker 104 (M) using the SDS-PAGE method (Image: Personal Documentation).

Proteins which belonged to the 30-kDa family were able to act as immunomodulators. This protein type will increase the IFN-γ factor so that Th2 cell proliferation is increasing. Th2 cells will induce B cells to form immunoglobulins (Opasawatchai et al., 2020). Amilli antigen-5 protein is responsible for degranulation of mast cells which will eventually secrete histamine (Fontaine et al., 2011). Pharmacologically D7 protein facilitates blood uptake by counteracting host hemostatic, inflammatory, and immunological defenses (Wichit et al., 2016). Angiopoietin possesses a certain mechanism to suppress plasma leakage, inhibit blood vessel inflammation, and prevent endothelial death when mosquitoes do blood feeding (Brindle et al. 2006).

A molecular weight of 67 kDa in the salivary glands of *Ae. albopictus* was identified to contain apyrase (ATP-diphosphohydrolase) which is able to cause an immune response in humans through the mechanism of inhibition of platelet aggregation (Wasinpiyamongkol et al., 2012; Carvalho et al., 2011; James and Rossignol, 1991). Apyrase will act as ADP hydrolyator released by cells that have been damaged by blood feeding so that active platelets become AMP and inorganic phosphate. Secondary homeostasis or blood clotting requires a trigger factor in the form of ADP so that blood can clot (Fontaine et al., 2011).

Protein Purification

Purification of protein fractions with molecular weights of 31 and 67 kDa was carried out by electroelution and dialysis. Electroelution is a protein purification technique with the principle of removing protein from the SDS-PAGE gel with the aid of electric voltage (Wathon et al, 2020; Iglesias et al., 2017). Electroelution is relatively easy and affordable to perform (Reddy, 2014). However, each protein sample requires different optimal conditions in the electroelution process. Factors influencing the success of electroelution are germane to (1) concentration and type of buffer system (Ion, pH) on SDS-PAGE, (2) electroelution buffer system and voltage,

electroelution time and (3) biochemical characteristics of target protein (Shoji et al., 1995). The buffer system used in the electroelution process is similar to that used in the SDS-PAGE process (Shoji et al., 1995) with the electrode buffer pH 8.3 (Wathon et al., 2020). The higher the voltage applied at the time of electroelution, the faster the protein will migrate out of the polyacrylamide gel (Harrington, 1990). However, the increase in voltage causes an increase in heat in the buffer system. The biological nature of the targeted protein is not resistant to temperature changes, so the voltage used cannot be significantly increased (Hunkapiller et al., 1983). Based on the electroelution process of proteins 31 and 67 kDa carried out at room temperature with a voltage of 50 Volts for 1 hour, at the end of the electroelution process a reverse current was carried out for 2 minutes. It aimed to release proteins attached to the membrane side (Chen et al., 2013). The position of the membrane when the electroelution was placed perpendicular to the direction of the electric current. It is important to make the electric current pass through the membrane better (Li et al., 2012). Electroelution was followed by a dialysis stage aimed at removing contaminants attached to proteins, namely salt, dye and SDS (Lei et al., 2007).

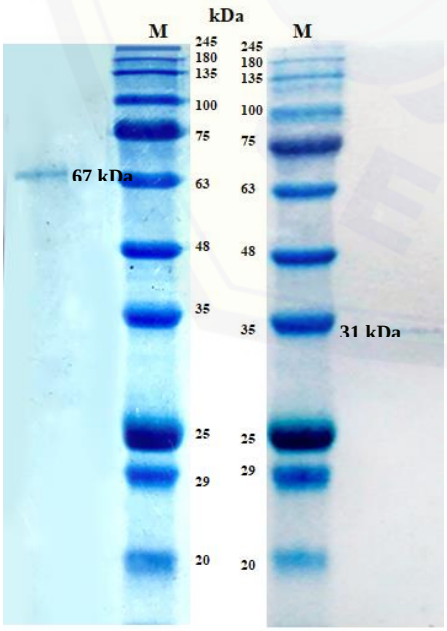

Figure 3. Confirmation of the purification results of immunogenic protein 67 kDa (A) and 31 kDa (C) and total extract of salivary gland of *Ae. albopictus* (B) through analysis using SDS-PAGE, Marker *Gangnam stain Protein Ladder* (M) (Image: Personal Documentation).

Dialysis was done using a buffer without SDS namely Phosphate Buffer Saline (PBS) which was stirred continuously using a magnetic stirrer during the dialysis process. This aimed to prevent bubbles from sticking to the bottom of the membrane and inhibiting the dialysis (Gromov and Celis, 2001). The dialysis is based on the principle of osmosis, namely the transfer of molecules from high concentration to low concentration (Himmelfarb and Ilkizer, 2014). SDS components attached to the electrolyte sample will diffuse into PBS solution that does not contain SDS through the cellophane membrane (Iglesias et al., 2017). Dialysis pure protein samples were concentrated using a microcetrifuge tube of Molecular Weight Cut Off (MWCO) measuring 10 kDa. It aimed to separate samples with salt contaminants below 10 kDa. Target proteins measuring 31 and 67 kDa will remain in the membrane (Yussof et al., 2017).

The success of the purification process was confirmed visually using SDS-PAGE analysis which was marked by the emergence of a single band (Mohammadian et al., 2010). The results of protein purification were confirmed by the SDS-PAGE method, which showed that proteins 31 and 67 kDa were successfully purified. This was evidenced by the visualization of SDS-PAGE in the form of a single band parallel to the protein marker Gangnam Stain Protein Ladder. The purification results of SDS-PAGE protein are shown in Figure 3.

4. CONCLUSION

Proteins 31 and 67 kDa have been successfully purified by electroelution and dialysis methods. Purification results have been confirmed through SDS-PAGE

analysis which showed the single band visualization of these 31 and 67 kDa.

5. REFERENCES

Almeras, L., A. Fontaine, M. Belghazi, S. Bourdon, E. Boucomont-Chapeaublanc, E. Orlandi-Pradines, M. Baragatti, N. Corre-Catelin, P. Reiter, B. Pradines, T. Fusai, and C. Rogier. 2010. Salivary gland protein repertoire from *Aedes aegypti* mosquitoes. *Vector-Borne and Zoonotic Diseases*. 10(4): 391-402.

Arca`, B., F. Lombardo, I. M. B. Francischetti, V. M. Pham, M. Mestres-Simon, J. F. Andersen, J. M. C. Ribeiro. 2007. An insight into the sialome of the adult female mosquito *Aedes albopictus*. *Insect Biochemistry and Molecular Biology*. 37: 107–127.

Brindle, N. P., P. Saharinen and K. Alitalo. 2006. Signaling and functions of angiopoietin-1 in vascular protection. *Circulation research*. 98(8): 1014-1023.

Brown, J. A., G. Singh, J. A. Acklin, S. Lee, J. E. Duehr, A. N. Chokola, J. J. Frere, K. W. Hoffman, G. A. Foster, D. Krysztof, R. Cadagan, A. R. Jacobs, S. L. Stramer, F. Krammer, A. García-Sastre, and J. K. Lim. 2019. Dengue virus immunity increases zika virus-induced damage during pregnancy. *Immunity*. 50(3): 751-762.

Calvo, E., B. J. Mans, J. F. Andersen, and J. M. Ribeiro. 2006. Function and evolution of a mosquito salivary protein family. *Journal of Biological Chemistry*. 281(4): 1935-1942.

Calvo, E., B. J. Mans, J. M. Ribeiro, and J. F. Andersen. 2009. Multifunctionality and mechanism of ligand binding in a mosquito anti-inflammatory protein. *Proceedings of the National Academy of Sciences*. 106(10): 3728-3733.

Cantillo, J. F., L. Puerta, S. Lafosse-Marin, J. L. Subiza, L. Caraballo, and E. Fernández-Caldas. 2016. Identification and characterization of IgE-Binding tropomyosins in *Aedes aegypti*. *International archives of allergy and immunology*. 170(1): 46-56.

Carvalho, I. D. L., D.K. Rocha and A. P. G. Almeida. 2011. Immune reactivity to dengue and *Aedes albopictus* mosquitoes in the population from Macao, China, before dengue occurrence. *In Vivo*. 25: 625-632.

Chen, W. Q., E. Karnaukhova, and G. Lubec . 2013. The use of native gels for the concomitant determination of protein sequences and modifications by mass spectrometry with subsequent conformational and functional analysis of native proteins following electro-elution. *Amino Acids.* 44(5): 1381-1389.

Doucoure, S., S. Cornelie, S. Patramool, F. Mouchet, E. Demettre, M. Seveno, J. S. Dehecq, H. Rutee, J. P. Herve, F. Favier, D. Missé, P. Gasque and F. Remoue. 2013. First screening of *Aedes albopictus* immunogenic salivary proteins. *Insect Molecular Biology*. 22(4): 411-423.

Fic, E., S. Kedracka-Krok, U. Jankowska, A. Pirog, and M. Dziedzicka-Wasylewska. 2010. Comparison of protein precipitation methods for various rat brain structures prior to proteomic analysis. *Electrophoresis*. 31(21): 3573–3579.

Fong, S. W., R. M. Kini and L. F. P. Ng. 2018. Mosquito saliva reshapes alphavirus infection and immunopathogenesis. *Journal of Virology*. 92(12): 1-10.

Fontaine, A., I. Diouf, N. Bakkali, D. Missé, F. Pagès, T. Fusai, C. Rogier and L. Almeras. 2011. Implication of haematophagous arthropod salivary proteins in host-vector interactions. *Parasites & Vectors*. 4(187): 1-17.

Gratz, N. G. 2004. Critical review of the vector status of *Aedes albopictus*. *Medical and Veterinary Entomology* 18: 215–227.

Gromov, P.S and J.E. Celis. 2004. Electroblotting of proteins from polyacrylamide gels. *Methods in Molecular Biology (Clifton, N.J.)*. 244: 345–352.

Harandi, M., M. R. Ramadhian, and R. Wahyudo. 2018. DENV-5: ancaman serotipe baru virus dengue. *Majority*.

7(2): 1–6.

Harrington, M.G. 1990. Elution of Protein from gels. *Methods in enzymology.* 182 : 487-498.

Hunkapiller, M.W.E., F. Lujan, F. Ostrander and L.E. Hood. 1983. *Methods Enzymol.* 91: 227

Himmelfarb, J. and T. A. Ikizler. 2010. Medical progress: hemodialysis. *New England Journal of Medicine*. 363(19): 1833–1845.

Hussain, M., S. Munir, K. Rahim, N. H. Bashir, A. Basit, and B. Khattak. 2018. Characterization of dengue virus in *Aedes aegypti* and *Aedes albopictus* spp. of mosquitoes: a study in Khyber Pakhtunkhwa, Pakistan. *Molecular Biology Research Communications*. 7(2): 77–82.

James, A. A. and Rossignol, P.A. 1991. Mosquito salivary glands: parasitological and molecular aspects. *Parasitology*. 7(10): 267-269

Janzen, H.G. and K.A. Wright. 1971. Salivary Glands of *Aedes aegypti* (L.): *An Electron Microscope Study*. *Canadian Journal of Zoology*. (49): 1-2.

Juhn, J., U. Naeem-Ullah, B. A. M. Guedes, A. Majid, J. Coleman, P. F. P. Pimenta, W. Akram., A. A. James. and O. Marinotti. 2011. Spatial mapping of gene expression in the salivary glands of the dengue vector mosquito, *Aedes aegypti. Parasites & vectors*. (4)1: 1.

Khasanah, R.N. 2019. Protein Imunogenik Kelenjar Saliva *Aedes albopictus* Vektor Potensial Demam Berdarah Dengue (DBD) di Wilayah Endemik Kabupaten Jember. *Skripsi* Jember : Fakultas Matematika and Ilmu Pengetahuan Alam Universitas Jember.

Lei, Z., A. Anand, K.S. Mysore and L.W. Sumner. 2007. Electroelution of intact proteins from SDS-PAGE gels and their subsequent MALDI-TOF MS analysis. *Plant Proteomics*: 353-363.

Li, G. Q., J. Shao, C.G. Guo, J.Y. Dong, L.Y., Fan, and C. X. Cao. 2012. A simple monolithic column electroelution for protein recovery from gel electrophoresis. *Analytical biochemistry.* 430(1): 24-31.

Manning, J. E., D. M. Morens, S. Kamhawi, J. G. Valenzuela, and M. Memoli. 2018. Mosquito saliva: the hope for a universal arbovirus vaccine?. *The Journal of infectious diseases.* 218(1): 7-15.

Medlock J.M., D. Avenell, I. Barrass and S. Leach. 2006. Analysis of the potential for survival and seasonal sctivity of *Aedes albopictus* (Diptera: Culicidae) in the United Kingdom. *J Vector Ecol.* 31: 292–304.

Mohammadian, T., M. Doosti, M. Paknejad, F. Siavoshi, and S. Massarrat. 2010. Preparative SDS-PAGE electroelution for rapid purification of alkyl hydroperoxide reductase from *Helicobacter pylori*. *Iranian Journal of Public Health*. 39(1):85–91.

Monteiro, C.C., Laura, R.B. José and D. Souza, M.R. Cleide and D. Albuquerque. 2007. Eclosion rate, development and survivorship of *Aedes albopictus* (Skuse) (Diptera: Culicidae) under different water temperatures. *Neotropical Entomology*. 36(6).

Oktarianti, R., K. Senjarini, T. Hayano, F. Fatchiyah, and Aulanni'am. 2015. Proteomic analysis of immunogenicproteins from salivary glands of *Aedes aegypti*. *Journal of Infection and Public Health*. 8(6): 575-582.

Opasawatchai, A., W. Yolwong, W. Thuncharoen, N. Inrueangsri, S. Itsaradisaikul, C. Sasisakulporn, W. Jotikasthira, O. Matangkasombut, O. Reamtong, W. Manuyakorn, W. Songnuan and P. Matangkasombut. 2020. Novel salivary gland allergens from tropical mosquito species and IgE reactivity in allergic patients. *World Allergy Organization Journal*. 13(2): 1-15.

Paupy, C., H. Delatte, L. Bagny, V. Corbel, D. Fontenille. 2009. *Aedes albopictus*, an arbovirus vector: from the darkness to the light. *Microbes and Infection.* 11: 1177-1185.

Prasadini, M., D. Dayananda, S. Fernando, I. Harischandra, and N. De Silva, N. 2019. Blood feeding preference of female *Aedes aegypti* mosquitoes for

human blood group types and its impact on their fecundity: implications for vector control. *American Journal of Entomology*. 3(2): 43-48.

Reddy, G. S. 2014. Cost-effective and efficient apparatus for electroelution of micro-and macrogram quantities of proteins from polyacrylamide gels. *Preparative Biochemistry and Biotechnology*. 44(8): 805–810.

Ribeiro, J. M. and Francischetti, I. M. 2003. Role of arthropod saliva in blood feeding: sialome and post-sialome perspectives. *Entomol*. 48: 73–88.

Rosa, E. 2007. Studi Tempat Perindukan Nyamuk Vektor Demam Berdarah Dengue di Dalam and di Luar Rumah Di Rajabasa Bandar Lampung. *Jurnal Sains MIPA*. 13(1).

Rueda, L. M. 2004. Pictorial keys for the identification of mosquitoes (Diptera: Culicidae) associated with dengue virus transmission. *Zootaxa*. 589: 1–60

Sattayasai, N. 2012. *Protein purification*. Dalam chemical biology. Editor P. D. Ekinci and ISBN. Shanghai: In Tech.

Schmid, M. A., E. Kauffman., A. Payne., E. Harris., L. D. Kramer. 2017. Preparation of mosquito salivary gland extract and intradermal inoculation of mice. *Bio-Protocol*. 7 (14) : 13-16

Shoji, M., M. Kato, and S. Hashizume. 1995. Electrophoretic recovery of proteins from polyacrylamide gel. *Journal of Chromatography*. 698(2):145–162.

Sri-in, C., S. C. Weng, W. Y. Chen, B. A. Wu-Hsieh, W. C. Tu, and S. H. Shiao. 2019. A salivary protein of *Aedes aegypti* promotes dengue-2 virus replication and transmission. *Insect Biochemistry and Molecular Biology*. 111.

Titus R.G. and Ribeiro J.M. 1988. Salivary gland lysates from the sand fly Lutzomyia longipalpis enhance Leishmania infectivity. *Science*. 239(4845): 1306-1308.

Titus, R. G., J. V. Bishop, and J. S. Mejia. 2006. The immunomodulatory factors of arthropod saliva and the potential for these factors to serve as vaccine targets to prevent pathogen transmission. *Parasite Immunology*. 28(4):131–141.

Vega-Rúa, A. Schmitt, C. Bonne, I. Krijnse J. Locker and A. B. Failloux. 2015. Chikungunya virus replication in salivary glands of the mosquito *Aedes albopictus*. *Viruses*. 7(11): 5902-5907.

Wasinpiyamongkol, L., S. Patramool, S. Thongrungkiat, P. Maneekan, S. Sangmukandan, D. Missé, and N. Luplertlop. 2012. Protein expression in the salivary glands of dengue-infected *Aedes aegypti* mosquitoes and blood-feeding success. *Southeast Asian Journal of Tropical Medicine and Public Health*. 43(6): 1346-1357.

Wathon, S., F. Muti'ah., R. Oktarianti, K. Senjarini. 2020. Purifikasi protein imunogenik 31 and 56 kda dari kelenjar saliva Aedes aegypti. *Jurnal Bioteknologi & Biosains Indonesia (JBBI)*. 7(1): 59–71.

Wichit, S., P. Ferraris, V. Choumet and D. Misse. 2016. The effects of mosquito saliva on dengue virus infectivity in humans. *Current Opinion in Virology*. 21:139–145.

Vázquez-Iglesias, L. B. Estefanell-Ucha, L. Barcia-Castro, M. P. de la Cadena, P. Álvarez-Chaver, D. Ayude-Vázquez and F. J. Rodríguez-Berrocal. 2017. A simple electroelution method for rapid protein purification: isolation and antibody production of alpha toxin from Clostridium septicum. *PeerJ*. (5).

Wright, K.A. 1969. The Anatomy of Salivary Glands of *Anopheles stephensi* Liston. *Canadian Journal Zoology*. 47: 579-588.

Yusoff, I.I., R. Rohani. and A.B. Mohammad. 2017. Molecular weight cut-off determination of pressure filtration membranes via colorimetric detection method. *Malaysian Journal of Analytical Science*. 21(2):484–495.

Zhang, H. and R. Lui. 2020. Releasing *Wolbachia*-infected *Aedes aegypti* to prevent the spread of dengue virus: a mathematical study. *Infectious Disease Modelling*. 5:142–160.