MICROBIOLOGY
INDONESIA
ISSN 1978-3477, eISSN 2087-8575
Vol.13, No.3, June 2019, p 56-63

Available online at
http://jurnal.permi.or.id/index.php/mionline
DOI: 10.5454/mi.13.2.3

Antimicrobial Bioactive Compounds of Snail Seromucoid as Biological Response Modifier Immunostimulator

AGNES SRI HARTI[1*], NONY PUSPAWATI[2], AND RAHAJENG PUTRININGRUM[3]

[1]*Departemen of Nursing, STIKes Kusuma Husada Surakarta, Jalan Jaya Wijaya No. 11 Surakarta 57127, Indonesia;*
[2]*Faculty of Health Science, Universitas Setia Budi Surakarta, Jalan LetJen. Sutoyo, Surakarta 57127, Indonesia;*
[3]*Departemen of Midwery, STIKes Kusuma Husada Surakarta, Jalan Jaya Wijaya No. 11 Surakarta 57127, Indonesia.*

Anti-microbial bioactive compounds from snail (*Achatina fullica* Ferussac) contained in snail seromucoid. It contains bioactive compounds such as glycans, peptides, glycopeptides, and chondroitin sulfate which can function as biological response modifiers (BRM) immunostimulators. Immunostimulators are compounds that can increase cellular immune responses in various ways, namely increasing the number and activity of T cells, NK cells, and macrophages and releasing interferons and interleukin. Immunostimulators are compounds that can increase cellular immune responses in various ways, namely increasing the number and activity of T cells, NK cells, macrophages and releasing interferons and interleukins.The purpose of this study was to analyze antimicrobial bioactive seromucoid compound of snail (*Achatina fullica* Ferrusac) as biological response modifiers (BRM) immunostimulators. The research methods based on experimental laboratory results with research stages including snail seromucoid isolation; antimicrobial activity; characterization physicochemical and profile of snail seromucoid proteins. The results of antimicrobial activity showed that 100% seromucoid concentrations had MIC (Minimal Inhibition Concentration) in *Staphylococcus aureus, Candida albicans,* and *Pseudomonas aeruginosa.* The physicochemical examination results showed specific gravity of 1.010; pH 8, glucose 16 mg dL^{-1}; 9 mg dL^{-1} cholesterol; protein 2.8 mg dL^{-1} and negative heavy metals (Pb, Cu, Hg, Al). The results of the analysis of protein profiles showed that there were 3 subunits of proteins, range from 55 to 72 kDa and 1 specific protein sub unit of 43 kDa which was thought to be antimicrobial and biological response modifiers (BRM) immunostimulators.

Key words: antimicrobial, biological response modifier, immunostimulator, seromucoid, snail

Senyawa bioaktif anti mikrob bekicot (*Achatina fullica* Ferussac) terdapat dalam seromukoid bekicot. Seromucoid bekicot mengandung senyawa bioaktif seperti glycans, peptida, glikopeptida dan chondroitin sulfat yang dapat berfungsi sebagai *biological response modifiers* (BRM) immunostimulator. Imunostimulator merupakan senyawa yang dapat meningkatkan respon imun seluler dengan berbagai cara yaitu meningkatkan jumlah dan aktivitas sel T, sel NK dan makrofag serta melepaskan interferon dan interleukin. Tujuan penelitian adalah menganalisis senyawa bioaktif antimikrobial seromukoid bekicot (*Achatina fullica* Ferrusac) sebagai *biological response modifiers* (BRM) immunostimulator dan karakterisasi fisika kimiawi serta profil protein seromukoid bekicot. Metode penelitian berdasarkan hasil eksperimental laboratorium dengan tahapan penelitian meliputi isolasi seromukoid bekicot; karakterisasi fisika kimiawi, mikrobiologis, dan profil protein seromukoid bekicot. Hasil uji mikrobiologis menunjukkan seromukoid konsentrasi 100% bersifat MIC (*Minimal Inhibition Concentration*) terhadap *Staphylococcus aureus, Candida albicans,* dan *Pseudomonas aeruginosa.*. Hasil pemeriksaan fisika kimiawi seromukoid bekicot menunjukkan berat jenis 1.010; pH 8, glukosa 16 mg dL^{-1}; kolesterol 9 mg dL^{-1}; protein 2,8 mg dL^{-1} dan logam berat (Pb, Cu, Hg, Al) negatif. Hasil analisis profil protein menunjukkan adanya 3 sub unit protein yaitu kisaran 55 – 72 kDa dan 1 sub unit protein spesifik 43 kDa yang diduga bersifat antimikrob dan *biological response modifiers* (BRM) immunostimulator.

Kata kunci: antimikrob, bekicot, *biological response modifier*, imunostimulator, seromukoid

Immunostimulation can increase the body's specific and non-specific defense mechanisms as well as the occurrence of non-specific induction of both cellular and humoral defense mechanisms including inflammation. Immunostimulators are compounds that can increase the immune response in various ways, namely increasing the number and activity of T cells, NK cells and macrophages and releasing interferons and interleukins to increase cellular defense. Materials that can stimulate an increase in immune response are called immunostimulators (Levinson and Jawetz 2003). Groups of compounds that have potential immunostimulation activity are alkaloids, terpenoids,

*Corresponding author: Phone: +62-8179453831; Email: agnessriharti@yahoo.com

quinones; simple phenolic compounds; group of polysaccharides, peptides, glycoproteins and nucleotides. The use of bioactive compounds as immunostimulators aims to reduce intracellular microbial infections, overcome immunodeficiency, or to trigger the growth of body's defense cells in the immune system. Materials that can stimulate the immune system are called biological response modifiers (BRM) immunostimulators.

Snail hemolymph is the main fluid in the body of the snail as a seromucoid that functions for heart contractions and leg movements. Bioactive compounds in hemolymph are glycans, peptides, glycopeptides, and chondroitin sulfate. The snail chondroitin sulfate can function as an immunomodulation and immunosuppressant. Snail (*Achatina fulica*) mucus 100% concentration and snail mucus cream 5% have an effective effect on the duration of second degree (A) burn healing (Mandala and Agnes 2014). The content of bioactive compounds in seromucoid can stimulate the function of cellular immunity, namely lymphocyte proliferation and the production of reactive oxygen intermediated macrophages (Viera *et al*. 2004).

The results of the study by Harti *et al*. 2018 showed that snail mucus and chitosan were able to increase lymphocyte proliferation, but the characterization of protein profiles of bioactive compounds that play a role in the process was unknown. Immunostimulatory effects found in snail seromucoid can be used to increase body immunity against facultative intracellular bacterial infection. The increasing incidence of infectious and degenerative diseases and the absence of appropriate therapies for the use of natural bioactive compounds, research on the physical, chemical, microbiological, and biomolecular characteristics of snail seromucoid as biological response modifiers immunostimulators or effective anti-inflammatory drug candidates need to be conducted.

The purpose of this study was to analyze antimicrobial bioactive seromucoid compound of snail (*Achatina fullica* Ferrusac) as biological response modifiers (BRM) immunostimulators.

MATERIALS AND METHODS

Snail Sample Handling. Local snail samples (*Achantina fulica*) with an average weight of 19 g and a height/width of 25/43 mm were obtained from local cultures placed in plastic containers at room temperature of 20-22 °C and fed with cabbage (Fig 1).

Snail Seromucoid Isolation. Seromucoid samples were obtained from local *Achatina fulica* Ferussac snails, from 10 to 50 snails. The end of the shell was opened and using an injection syringe needle was inserted and turned so that the liquid that comes out was placed in a sterile conical tube. The liquid that comes out was clear to brownish yellow as seromucoid and then centrifuged 3000 rpm for 30 minutes.

Antimicrobial Activity. In vitro microbiological examination includes antimicrobial activity testing of diffusion and dilution methods to determine MIC (Minimal Inhibition Concentration) or MKC (Minimum Killing Concentration) (Soleha 2015). Antimicrobial activity test of the dilution method was performed to determine the MIC or MKC seromucoid concentration of 100% against *Staphylococcus aureus, Candida albicans* and *Pseudomonas aeruginosa* isolates obtained from the Microbiology Laboratory of the University of Setia Budi Surakarta. Seromucoid samples prior to diffusion and dilution tests were

Fig 1 Local snail samples (*Achatina fulica)*.

filtered aseptically using a disposible filter membrane.

Characterization of Chemical and Biochemical Physics. Physical examination, namely color, odor, consistency, and viscosity. Chemical tests were pH and heavy metals. Biochemical tests included measurements of glucose, cholesterol, and total protein.

Protein Profile of the SDS-PAGE Method. Snail seromucoid protein profile purification and characterization with sodium dodecyl sulphate polyacrilamide gel electrophoresis (SDS-PAGE) to obtain dominant bands with a certain molecular weight were done.Characterization was carried out using the SDS-PAGE technique (Wayan 1991) with a 10% separating gel (1.2 g acrylamid; 0.032 g bis-acrylamid; 3 mL 1.5 M Tris pH 8.8; 0.12 mL SDS 10%; 8.88 mL aquadest, 7 µl TEMED and 80 µl 10% APS) and 3% stacking gel (0.9 g acrylamid; 0.024 g bis-acrylamid; 2.52 mL 1.5 M Tris pH 6.8; 0.3 mL SDS 10%; 17.18 mL aquadest, 3.5 µl TEMED and 50 µl APS 10%).

Gel plate was added with a solution of separating gel 10% vertically, then the top was added to butanol and allowed to polymerize. The next process is the addition of 3% stacking gel put into the glass until it is full then installed a comb and left until polymerization occurs. The gel-filled plate was mounted on a Minigel Twin G-42 slab and poured with electrophoresis buffer (3g 0.0248 M Tris; 14.4 g glycine 0.19 M; 10 mL 0.1% SDS 10%) where the electrodes were poured. A total of 50 µl samples in ependorf plus 5 x SDS samples - buffer (red Prob-b) were 12.5 µl (2.5 mL 1.5 M Tris pH 6.8, 2 g SDS, 0.5 g Dithiothretol (DTT)) in 5 mL merchaptoethanol; 10 mg bromphenol blue; 10 mL of glycerin and 2.5 mL of aquadest). The sample is then boiled for 2 minutes removed and immediately put ice, poured little by little into each well of stacking gel. As a marker used protein with molecular weight in the range of 10 - 180 kDa, brand Vivantis. The power supply is turned on by the electric current used by 99.9 volts, 50 mA and 12 W. If the sample has reacted to the bottom, then the process is stopped. The plate is opened and separated and then washed with buffer and painted with methylene blue (Berniyanti and Suwarno 2007).

RESULTS

The recovery of snail seromucoid. The average seromucoid volume obtained for each snail varies from 1.0 to 4.0 mL when isolating the snail seromucoid. Samples were further treated, namely mucomucoid or mucus macerated with water for 24 hours at 40° C. The obtained supernatant is said to be WSF (water soluble fraction). The mucin fraction of WSF obtained then precipitated with a ratio of 1: 3 ethanol is a general method of isolation from mucus. WSF and the mixture were centrifuged at 2900 x g for 30 min. The precipitation obtained was reconstituted with Tris -Cl and mucin fraction was obtained. Seromucoid is centrifuged 3000 rpm for 30 minutes as a hemolymph fluid (Fig 2).

Antimicrobial Activity Test. The test results of snail seromucoid antimicrobial activity diffusion method on Muller Hilton medium were shown to be antimicrobial (Fig 3 and Table 1).

Physical-chemical and Biochemical Test. The results of physicochemical and biochemical test as show in Table 2.

Snail Seromucoid Profile with SDS-PAGE Method. The characterization of snail seromucoid protein profiles as Fig 4 was carried out with sodium dodecyl sulphate polyacrilamide gel electrophoresis (SDS-PAGE) to obtain dominant bands with certain molecular weights.

DISCUSSION

The results of antimicrobial seromucoid activity test of snail using diffusion method on Muller Hilton medium (Soleha 2015) did not show the existence of inhibition; this is likely due to seromucoid can not be diffused completely in the media so that it does not show any inhibitory power. Therefore continued dilution test with liquid culture and cell suspension according to 0.5 McFarland standard (1.5 x 10^8 cfu mL^{-1}) to determine MIC (Minimum Inhibition Concentration) or MKC (Minimum Killing Concentration).

There is a difference in the variation of antimicrobial activity compared to previous studies that show a difference in results. This is influenced by the different inoculum strains used which are related to the level of microorganism resistance as well as the type of antibacterial achasin protein that are the result of genetic expression of each snail strain that is different (Dolaskha *et al.* 2014). Various types of proteins or achasin proteins in snails have important biological functions as bacterial (enzyme) binding receptors (Dang *et al*. 2015). The results of the study by Berniyanti and Suwarno (2007) show that snail mucus is capable of being antibacterial to *Streptococcus mutans* and *Escherichia coli*. Anggraeni *et al.* (2018) stated that snail mucus was able to inhibit the growth of Methicillin Resistant Staphylococcus aureus (MRSA). The

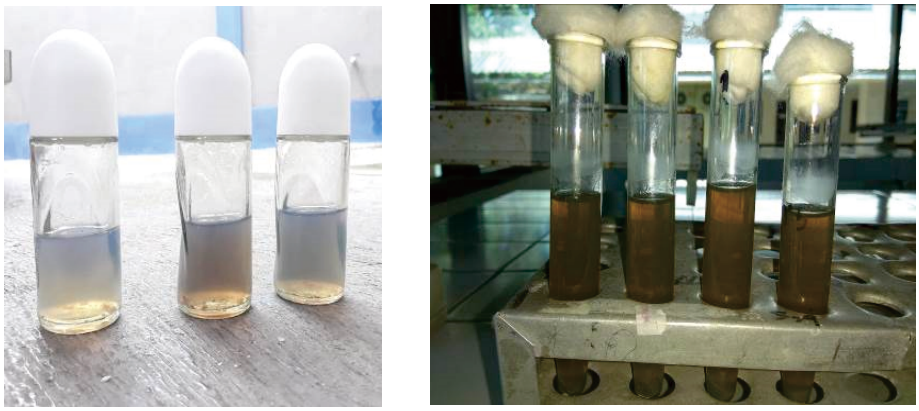

Fig 2 The results of seromucoid snail isolation.

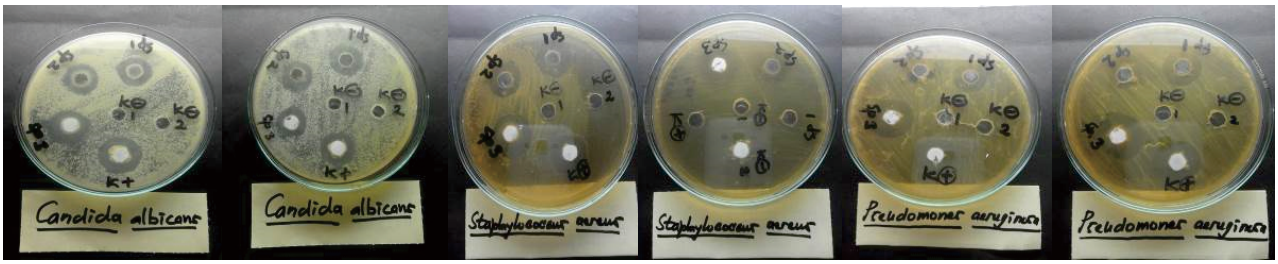

Fig 3 The result antimicrobial activity diffusion method of *Candida albicans, Staphylococcus aureus,* and *Pseudomonas aeruginosa.*

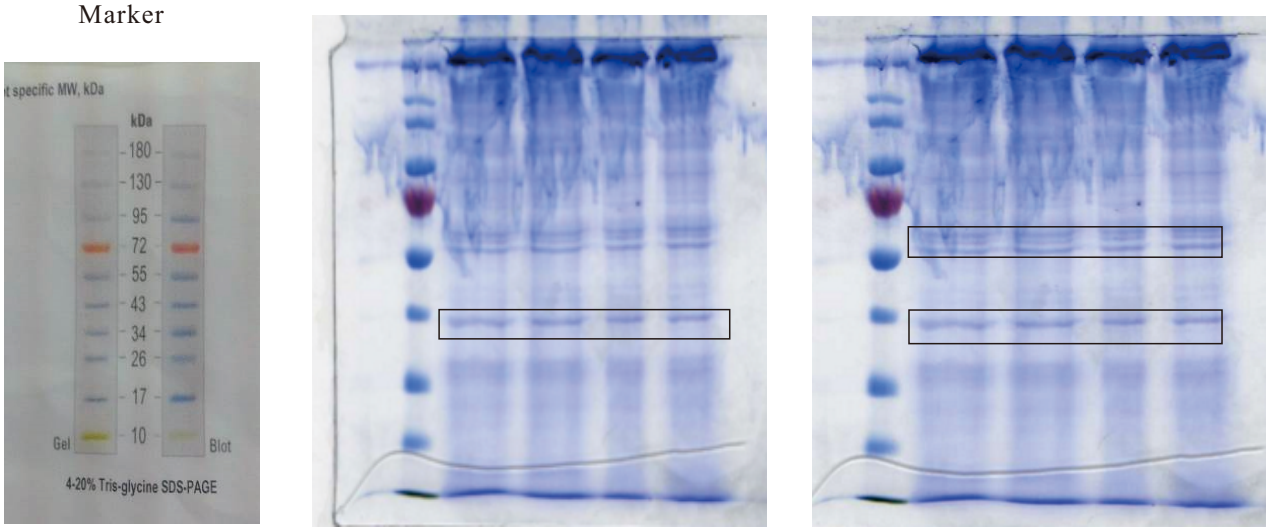

Fig 4 Profile of seromucoid snail protein with SDS-PAGE method.

concentration of 100% snail mucus effectively inhibits the growth of Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Salmonella typhosa*) (Huda and Marhamah 2016). The inhibitory potential and antibacterial snail mucus on the isolates of *Staphylococcus sp, Streptococcus sp* and *Pseudomonas sp* bacteria were varied (Etim *et al*. 2015). Antibacterial and antifungal test results from meat protein extracts of 7 different snail types showed variations in antibacterial and antifungal activity by diffusion and dilution methods, because it is influenced by the ecological conditions of snails (Ulagesan and Hak Jun Kim 2018).

The results of physicochemical and biochemical examinations as in Table 2 show that seromucoid or snail hemolymph has physical and chemical characteristics such as liquid consistency, transparent white to brownish yellow color, odorless or slightly fishy, specific gravity 1,010; pH 8; heavy metals (Pb,

Table 1 Results of examination of seromucoid antimicrobial activity test for diffusion method

No	Material	Diameter of resistance (mm)								
		Staphylococcus aureus			*Pseudomonas aeruginosa*			*Candida albicans*		
		1	2	average	1	2	average	1	2	average
1	Snail slime	18	16	17	18	18	18	19	19	19
2	Seromucoid	16	18	17	15	14	14.5	18	17	17.5
3	Snail slime cream	32	20	26	22	21	21.5	16	18	17
4	Positive control	20	20	20	21	21	21	19	20	19.5
5	Negative control	0	0	0	0	0	0	0	0	0

Table 2 Physicochemical and biochemicals test of seremucoid snail

	Examination Types	Results
Physicist	Consistency	Liquid
	Color	transparent white to brownish yellow
	Smell	odorless- a little fishy
	Density	1.010
Chemical	pH	8
	Heavy metals (Pb, Hg, Cu, Al)	negative
Biochemistry	Glucose	16 $mgdL^{-1}$
	Cholesterol	9 $mgdL^{-1}$
	Protein	2,8 $mgdL^{-1}$

Hg, Cu, Al) negative; glucose 16 mg dL^{-1}; cholesterol 9 mg dL^{-1} and protein 2.8 mg dL^{-1}.

Snails secrete mucus from the glands of the legs are used as a mechanical function, especially locomotor or adhesion movement for viscoelasticity on the substrate with a thickness of 10-20 um and containing water 99.7% by weight and in dry conditions in forming a solid and thin film layer. The content in mucus gel is composed of mucopolysaccharides and glycoproteins. The mucus composition of each snail species varies and is influenced by external or ecological factors, namely temperature, humidity, light intensity and food supply. The results of chemical analysis on mucus snails *Eobania vermiculata, Theba pisan* and *Monacha obstructa*, contain three main compounds namely oxime, methoxy-phenyl and cyclotrisiloaxane, hexamethyl (Zhuang *et al*. 2015).

Snails have a humoral and cellular immune system found in hemolymph. Hemolymph is composed of plasma and hemocytes. Snail plasma or mucomucoid contains a number of proteins that function to eliminate pathogens directly and hemocytes function as immune effector cells that play an important role in killing pathogenic microbes through various methods, namely phagocytosis, encapsulation and cytotoxic reactions.

Gastropod hemocytes play an important role in cell defensive reactions namely phagocytosis, encapsulation, nodulation and neutralization of parasites, the process of blood coagulation and wound healing. Snail hemolymph bioactive compounds as drug derivatives have been used in the medical field including skin smoothing, treatment of respiratory infections, burns (Bismili *et al*. 2013).

Snail seromucoid or hemolymph contain bioactive compounds such as glycans, peptides, glycopeptides and chondroitin sulfate. The snail chondroitin sulfate can function as an immunomodulation and immunosuppressant. The content of Glycoamino-glycans (GAGs), heparin, heparin sulfate, chondroitin sulfate, sulphate dermal and hyaluronic acid in

hemolymph and snail mucus function as primary biological modifiers that act as stabilizer cofactors, and or coreceptor for growth factors, cytokines, and chemokines; enzyme activity regulator; signifying or labeling molecules in response to cellular damage, such as wound healing, infection, and tumorigenesis; targets for bacterial, viral, parasitic virulence factors; and the immune system (Sallam *et al*. 2009). A number of protein lectins are known to be contained in snails, namely selectin, galectin, C-type lectins, and fibrinogen-related proteins (FREPs) secreted by snails after infection that plays a role in the process of pathogen agglutination (Dolaskha *et al.* 2015). Also the presence of aldolase and myosin are identified as proteins that play a role in the regulation of hemosite migration and have an impact on the process of killing pathogens by cytotoxic reactions and phagocytosis (Suwannatri *et al*. 2016).

Immunostimulatory effects found in snail and chitosan seromucoid can be used to increase body immunity against facultative intracellular pathogenic bacterial infections (Harti *et al*. 2018). This shows that the presence of bioactive compounds in snail hemolymph is very potential as an antibiotic candidate, namely the presence of bioactive compounds that are antioxidant, anticancer, antiviral and anti-inflammatory.

The content of bioactive compounds in snail seromucoid can stimulate the function of cellular immunity, namely lymphocyte proliferation and the production of reactive oxygen intermediated macrophages. The bioactive component of natural ingredients is to activate macrophage cells or work as an immunostimulant so that it is useful as adjunctive therapy for inflammation including cancer patients in relation to increasing the non-specific immune system of patients against infection, increasing the effectiveness of therapy through the tumorisidal effector system. Humoral immune response can be known from the antibody titer that is secreted. Cellular immune response is played by T lymphocytes and activated effector cells. This response can be known from the lymphocyte proliferation response. Humoral immune responses play a role in host defense against extracellular microbes, which are mediated by antibodies. Whereas defense against intracellular microbes requires an immunecellular response. Immunomodulatory activity can be carried out using the Haemagglutination test method in vivo by measuring the concentration of Tumor Necrosis Factor-α (TNF-α) as a proinflammatory cytokine and Interleukin -10 (IL-10) as an anti-inflammatory cytokine. An increase in IL-10 production can suppress the production of TNF-α production and affect the B lymphocyte cells to produce antibodies. Stimulated T lymphocytes will produce cytokines in the form of interferon-γ (IFN-γ) and interlukin-2 (IL-2). IFN will play a role in macrophage cell activation and can induce the expression of class II major histocompatibility complex (MHC) molecules in macrophage cells, thereby helping macrophage cell function in lymphoid follicles to recognize antigens. Macrophage cells can also release cytokines, IL-1, which play a role in stimulating the proliferation of Th and B cells. Whereas IL-2 does not only play a role in the expansion of T lymphocyte clones after being recognized as antigens, but also increases the proliferation and differentiation of other immune cells such as NK cells and B cells (Levinson and Jawetz 2003).

Based on Figure 4, that the results of the characterization of the snail seromucoid protein profile SDS-PAGE method showed that there were 3 sub-units of protein, namely the range 55 - 72 kDa and 1 specific protein sub-unit 43 kDa. From the results of this research that has been carried out shows that the 3 subunits of the protein are Achasin sulfate proteins that act as antimicrobials. While 1 sub-unit of spefysical protein with a molecular weight of 43 kDa is thought to be related to adhesin protein. The results of the analysis of total protein using the Bradford method with BSA (bovine serum albumin) as a standard and measured spectrophotometrically at ƛ 595 nm, the concentration of protein content was obtained 6.99 µg uL^{-1}. The difference in the protein profile of the results of research conducted with previous studies is due to variations in snail strains so that the type and amount of bioactive compounds in hemolymph is the result of gene expression of bioactive compounds.

The results of the research that have been carried out show that snail bioactive compounds are able to inhibit the growth of Methicillin Resistant Staphylococcus aureus (MRSA) and the results of protein analysis show that there are 4 subunits of protein, 87.59kDa; 77.66 kDa, 70.97 kDa and 49.46 kDa (Anggarini *et al.* 2018). Protein profiles of snail mucus obtained at basal and tip shells are different. *Helix aspersa* shows protein bands of 82.97 and 175 kDa (Greistorfer 2017), whereas in *Arion sub fuscus* snails there are 6 proteins in the range of 10 - 200 kDa with 2 specific proteins 15 kDa and 61 kDa in the mucous adhesive. In water snails *Lottia sp* showed 68

kDa protein in the upper mucus and 80 kDa and 118 kDa while in adhesive or lower mucus (Zhuang *et al.* 2015). In *Littoria sp* in the upper mucus showed protein profiles 59 and 65 kDa; and mucus under the presence of proteins 36 and 41 kDa (Etim *et al.* 2015).

To conclude, based on the results of the antimicrobial activity test the dilution method that has been carried out shows that the potential of the snail MIC seromucoid antibacterial at 100% concentration against *Staphylococcus aureus, Candida albicans* and *Pseudomonas aeruginosa*.

The physicochemical characterization of snail seromukoid shows specific gravity of 1,010; pH 8, glucose 16 mg dL^{-1}; cholesterol 9 mg dL^{-1}; protein 2,8 mg dL^{-1} and heavy metals (Pb, Cu, Hg, Al) are negative.

The protein profile of anti-microbial bioactive compounds in seromucoid or snail hemolymph (*Achatina fullica* Ferrusac) shows that there are 3 Achasin sulfate protein sub units with molecular weight in the range of 55 - 72 kDa and 1 specific protein sub unit of 43 kDa which is suspected as biological response modifiers (BRM).

ACKNOWLEDGEMENTS

Acknowledgments to the Directorate of Research and Community Service of the Ministry of Research and Technology of Indonesia and all parties who have assisted in this research. This article is part of the PDUPT (Higher Education Basic Research) DRPM program which is funded by Directorate of Research and Community Service of the Ministry of Research and Technology of Indonesia in 2019 with contract number 074 / L6 / AK / SP2H.I / Penelitian / 2019.

REFERENCES

Anggraini D, Sri Darmawati, Endang TWM. 2018. Protein profile and anti-microbial power of snail slime (*Achantina fulica*) against Methicillin Resistant *Staphylococcus aureus* (MRSA). Proceedings of the Unimus Student National Seminar. e-ISSN: 2654-766X vol. 1, 2018 hal 73 – 77.

Berniyanti T, Suwarno. 2007. Characterization of local isolate snail slime (achasin) protein as an antibacterial factor. J Veter Media. ISSN 2015-8930 , hal 139-144 Vol. 23, No. 3, September 2007.

Bislimi K. Behluli A, Halili J, Mazreku I, Osmani F, Halili F. 2013. Comparative analysis of some biochemical parameters in hemolymph of garden snail (*Helix Pomatia L.*) of the Kastriot and Ferizaj regions, Kosovo. Dec 2013. Vol. 4, No. 6 pp 11 - 18 ISSN 2305-8269; Int J Appl Sci Eng. © 2012 - 2013 EAAS & ARF www.eaas-journal.org.

Dang VT, Benkendorff K, Green T, Speck P. 2015. Marine snails and slugs: a great place to look for antiviral drugs. J Virol. 89(16):8114-8118. doi: 10.1128/JVI.00287-15. jvi.asm.org.

Dolashka P, Dolashki A, Voelter W, Van Beeumen J, Stevanovic S. 2015. Antimicrobial activity of peptides the hemolymph of *Helix lucorum* snails. Int J Curr Microbiol Appl Sci. 4(4):1061-1071.

Dolashka P, Dolashki A, Velkova L, Stevanovic S, Molin L, Traldi P, Velikova R, Voelter W. 2015. Bioactive compounds isolated from garden snails. J BioSci Biotechnol. SE/ONLINE: 147-155 SPECIAL EDITION / ONLINE Section "Biotechnologies & Applied Biology" Second National Youth Conference "Biological sciences for a better future", Plovdiv, October 30-31, 2015.

Etim LB, Chuku A, Godwin AO. 2015. Antibacterial properties of snail mucus on bacteria isolated from patient with wound infection. Br Microbiol Res J. 11(2): 1-9. doi: 10.9734/BMRJ/2016/21731.

Greistorfer S, Waltraud K, Norbert C, Andreas G, Livia R, Johannes S, Janek von B. 2017. Snail mucus - glandular origin and composition in *Helix pomatia*. Zoology. 122 :126-138. doi: 10.1016/j.zoo.2017.05.001

Harti AS, Murharyati A, Dwi Sulisetyawati S, Oktariani M. 2018. The effectiveness of snail mucus (*Achantina fulica*) and chitosan towards limfosit proliferation in vitro. Asian J Pharm Clin Res. 11(15): 85-88. doi: 10.22159/ajpcr.2018.v11s3.30041.

Huda M, Marhamah. 2016. Effect of snail mucus (*Achantina fulica*) on the growth of Gram-positive and Gram-negative bacteria. J Health Anal. 5(2):547-555. doi: 10.26630/jak.v5i2.461.

Levinson W, Jawetz E. 2003. Medical Microbiology and Immunology Examination and Board Review, McGraw-Hill, Singapore.

Mandala AP, Agnes SH. 2014. Effectiveness of 100% Snail Mucus (Achantina fulica) and 5% Cream Preparation for Duration of Second Degree Burn (A) In vivo. Thesis Bachelor Program Nursing STIKes Kusuma Husada Surakarta.

Sallam AAA, El-Massry SA, Nasr IN. 2009. Chemical analysis of mucus from certain land snails under Egyptian conditions. Arch Phytopathology Plant Protect. 42 (9): 874-881. doi: 10.1080/03235400701494448.

Sismindari AS, Handayani Yulia S, Candra E. 2002. Potent effect of protein extract containing ribosome-inactivating proteins (RIPs) isolated from *Erythrina fusca lour* on cancer cells. Indonesian J Biotechnol. 559–564.

Soleha TU. 2015. Sensitivity Test Against Antibiotics. Juke (Journal of Medicine) Lampung University. 5(9):119-123.

Suwannatri K, Aiporn S, Paitra T, Jariya UW, Sirikachorn T, Cinzia C, Jason M, Smarn T, Alex L, and Javier S. 2016. Differential protein expression in the hemolymph of *Bithynia siamensis goniomphalos* infected with *Opisthorchis viverrini*. Research article. PLOS Negl Trop Dis. 10(11):e0005104. doi: 10.1371/journal.pntd.0005104.

Ulagesan S, Kim HJ. 2018. Antibacterial and antifungal activities of proteins extracted from seven different snails. Appl Sci. 8(8):1362; doi: 10.3390/app8081362

Vieira TC, Costa-Filho A, Salgado NC, Allodi S, Valente AP, Nasciutti LE, Silva LC. 2004. Acharan sulfate, the new glycosaminoglycan from *Achatina fulica* Bowdich 1822. Structural heterogeneity, metabolic labeling and localization in the body, mucus and the organic shell matrix. Eur J Biochem. 271(4):845-854. doi: 10.1111/j.1432-1033.2004.03989.x.

Wayan TA. 1991. Genetic Engineering. Inter University Center for Biotechnology, Gajah Mada University, Jogjakarta.

Zhuang J, Coates CJ, Zhu H, Zhu P, Wu Z, Xie L. 2015 Identification of candidate antimicrobial peptides derived from abalone hemocyanin. Dev Comp Immunol. 49(1):96-102. doi: 10.1016/j.dci.2014.11.008.