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Research Report

Antimicrobial proteins of Snail mucus (*Achatina fulica*) against *Streptococcus mutans* and *Aggregatibacter actinomycetemcomitans*

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ABSTRACT

Background: *Achasin and mytimacin-AF are proteins of snail mucus (Achatina fulica) which have antimicrobial activity. Snail mucus is suspected to have other proteins which have antimicrobial activity against Streptococcus mutans and Aggregatibacter actinomycetemcomitans the oral pathologic bacteria.* ***Purpose:*** *The study were aimed to characterize the proteins of snail mucus (Achatina fulica) that have antimicrobial activities to Streptococcus mutans and Actinobacillus actinomycetemcomitans, and to compared the antimicrobial effect of achasin and mytimacin-AF.* ***Methods:*** *The sample of study was the mucus of snails which were taken from Yogyakarta Province. The isolation and characterization of protein were conducted by using SDS-PAGE method, electro-elution, and dialysis. Nano drop test was conducted to determine protein concentration. The sensitivity test was conducted by using dilution test, and followed by spectrophotometry and paper disc diffusion tests.* ***Results:*** *The study showed that proteins successfully characterized from snail mucus (Achatina fulica) were proteins with molecular weights of 83.67 kDa (achasin), 50.81 kDa, 15 kDa, 11.45 kDa (full amino acid sequence of mytimacin-AF) and 9.7 kDa (mytimacin-AF). Based on the dilution test, Achasin had better antimicrobial activities against Streptococcus mutans, while mytimacin-AF had better antimicrobial activities against Aggregatibacter actinomycetemcomitans. But the paper disc diffusion test result showed that Achasin had antimicrobial activities against Streptococcus mutans and Aggregatibacter actinomycetemcomitans, while mytimacin-AF had no antimicrobial activities.* ***Conclusion:*** *The proteins with molecular weights of 50.81 kDa, 15 kDa, 11.45 kDa were considered as new antimicrobial proteins isolated from snail mucus. Achasin, had better antimicrobial activities against Streptococcus mutans, while mytimacin-AF had better antimicrobial activities against Aggregatibacter actinomycetemcomitans.*

Key words: *Achatina fulica, achasin, mytimacin-AF, antimicrobial*

ABSTRAK

Latar belakang: *Achasin dan mytimacin-AF adalah protein lendir bekicot (Achatina fulica) yang memiliki aktivitas antimikroba. Lendir bekicot diduga memiliki protein lain yang memiliki aktivitas antimikroba terhadap Streptococcus mutans dan Actinobacillus actinomycetemcomitans bakteri patologis oral.* ***Tujuan:*** *Penelitian ini bertujuan untuk mengkarakterisasi protein lendir bekicot (Achatina fulica) yang memiliki aktivitas antimikroba terhadap Streptococcus mutans dan Aggretibacter actinomycetemcomitans, dan membandingkan efek antimikroba protein achasin dan mytimacin-AF.* ***Metode:*** *Sampel penelitian adalah lendir bekicot yang diambil dari Provinsi Yogyakarta. Isolasi dan karakterisasi protein dilakukan dengan metode SDS-PAGE, elektro-elusi, dan dialisis. Nano drop test dilakukan untuk menentukan konsentrasi protein. Uji sensitivitas dilakukan dengan menggunakan uji dilusi, dan diikuti oleh spektrofotometri dan tes difusi kertas cakram.* ***Hasil:*** *Protein dari lendir bekicot (Achatina fulica) yang ditemukan adalah protein dengan berat molekul 83,67 kDa (achasin), 50,81 kDa, 15 kDa, 11,45 kDa (urutan asam amino penuh mytimacin-AF) dan 9,7 kDa (mytimacin- AF). Berdasarkan uji dilusi, Achasin memiliki aktivitas antimikroba yang lebih baik terhadap Streptococcus mutans, sedangkan mytimacin-AF memiliki aktivitas antimikroba yang lebih baik terhadap Aggregatibacter actinomycetemcomitans. Namun hasil uji difusi cakram kertas menunjukkan bahwa Achasin memiliki aktivitas antimikroba terhadap Streptococcus mutans dan*

Aggegatibacter actinomycetemcomitans, sementara mytimacin-AF tidak memiliki kegiatan antimikroba. ***Simpulan:*** *Protein dengan berat molekul 50,81 kDa, 15 kDa, 11,45 kDa merupakan protein antimikroba baru diisolasi dari lendir bekicot. Achasin, memiliki aktivitas antimikroba yang lebih baik terhadap Streptococcus mutans, sedangkan mytimacin-AF memiliki aktivitas antimikroba yang lebih baik terhadap Actinobacillus actinomycetemcomitans.*

Kata kunci: *Achatina fulica, achasin, mytimacin-AF, antimikroba*

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INTRODUCTION

Antimicrobial materials derived from nature either from animals and plants have commonly been used since they are easily obtained and have low side effects.[1] Antimicrobial materials derived from phenolic group actually have been considered as a solution. However, some researchers suggest that phenol still has some disadvantages.

Traditionally, Snail mucus has been used by Javanese to heal wound.[2] Achasin and Mytimacin AF have been extracted from Snail mucus and it is reported to have anti microbial effect. Achasin contained in snail mucus is known as an effective antimicrobial protein to Gram-positive and-negative bacteria that have been successfully extracted since 1982 by Iguchi *et al*.[3] Besides that, mytimacin-AF is also known as a broad-spectrum antimicrobial protein.[4] Achasin has been identified as a protein with molecular weight of 59.086-150 kDa.[5] Meanwhile, mytimacin-AF protein has been identified as a protein with molecular weight of 9.7 kDa.[4]

Streptococcus mutans is a Gram-positive bacteria in cluster known more as opportunistic pathogenic bacteria in oral cavity, that has an ability to form plaque and cause primary caries in oral cavity.[6] Moreover, *Streptococcus mutans* are in symbiosis with other pathogenic bacteria causing caries in oral cavity.[7] On the other hand, *Aggregatibacter actinomycetemcomitans* is a Gram-negative bacterium that also has the properties of opportunistic pathogens in oral cavity. It has virulence factors that can cut IgG, immunosuppressive factors, and strong leukotoxin, as well as produce collagenase enzyme, which is harmful to the health of periodontal tissues. Thus, *Aggregatibacter actinomycetemcomitans* are usually associated with aggressive periodontitis diseases and other periodontal diseases. Aggressive periodontitis disease is caused by the lack of the body's defense system, and often occurs during pubertal period.[8]

The study were aimed to characterize the proteins of Snail mucus (Achatina fulica) that have antimicrobial activities to *Steptococcus mutans* and *Aggregatibacter actinonycetemcomitans,* and to compared its effectiveness with adhesin and mytimacin AF.

MATERIALS AND METHODS

This experiment can be considered as a laboratory experiment with post test-only control group design. The samples of this research were obtained by using simple random sampling technique. The samples were snail mucus (*Achatina fulica*) obtained from 36 snails taken from Cangkringan village, Sleman. However, the Snails used as the samples of this experiment had to meet certain requirement, had 4.5 to 10 cm shell length and they could move actively. They had to be quarantined for two days in a plastic box. And, they had to have fasting for 12 hours before their mucus was taken into coconut wood box.

Snail mucus was taken by using a method with electrical shock from 4.5 to 12 volts at 1.5 amperes for 60 seconds. In the protein isolation phase the mucus obtained was mixed with twice the early volume of water, and then stirred in the stirrer overnight. It was centrifuged at 11,000 g by using Beckman Model J-21 for 30 minutes, and then supernatant called Water Soluble Fraction (WSF) was produced. The WSF was added with three times the early volume of the WSF, and then centrifuged at 2900 g with Yuan Kr 422 for 30 minutes. As a result, precipitation known as ethanol precipitated fraction (ETP) was obtained. Then ETP was diluted with 50 mM Tris-HCl (pH 8.0) before electrophoresis was performed.[9]

In the electrophoresis phase, the proteins derived from the snail mucus was characterized by using SDS-PAGE technique with both Thermo Scientific Spectra Multicolor Low Range Protein Ladder, about 4.6-40 kDa and Thermo Scientific Spectra Multicolor Broad Range Protein Ladder, about 10-260 kDa. In other words, SDS-PAGE technique used in this phase was divided into two sections. The first running by using the low range marker and the second running by using the broad range marker. Material used for the low range marker was 16% separating gel, consisting of 5.3 ml of Bis-Acrylamide, 2.1 ml of DDI H_2O, 2.5 ml of buffer gel, and 0.1 ml of 10% SDS. Material used as the broad range marker was 10% separating gel, consisting of 3.3 ml of Bis-Acrylamide, 4.1 ml of DDI H_2O, 2.5 ml of buffer gel, and 0.1 ml of 10% SDS. Besides that, 5% stacking gel, consisting of 1.7 ml of Bis-Acrylamide, 5.7

ml of DDI H_2O, 2.5 ml of buffer gel, and 0.1 ml of 10% SDS was also used in this phase.[10]

The separating gels mixed with 0.05 ml of 10% APS and 0.01 ml of TEMED were used as catalyst, and then put into plates in vertical position reaching the bottom of the comb. After polymerization, the stacking gel was fully inserted and the comb was installed. The plates were set into electrophoresis chamber and poured with electrophoresis buffer (3.03 g Tris Base, 14.4 g Glycine, and 1 g SDS). Then 50 mL of ETP diluted was mixed with the buffer sample (3.55 mL of deionized water, 1.25 ml of 0.5 M Tris-HCl at pH 6.8, 2.5 ml glycerol, 2 ml of 10% SDS, and 0.2 ml of 0.5% Bromophenol Blue). Meanwhile, 950 mL of the other buffer sample was mixed with 50 mL of 2-merchaptoethanol, and boiled for 4 minutes at 95^0 C.

After the samples were in cold condition, they were loaded into wells using micropipette.[11] Electrophoresis was then conducted at 200 Volts in 39 minutes for the low range marker and 30 minutes for the broad range marker. Once the process was completed, the plates were opened, and the gels were stained with Comassie Brilliant Blue staining, and then destained. After the bands were clearly visible, the molecular weight of proteins were calculated by comparing both markers and then determined by using the formula Retardation factor (Rf) with logarithm derived from the molecular weight of protein markers known. Then the value of Rf obtained was put into a linear regression equation with the formula: y = ax + b, with y = logarithm of molecular weight and x = Rf values. To simplify the calculation, the calculation was conducted by using the Microsoft-Excel 2007 program.

Protein production was analogous to proteins that have been identified through previous researches, such as proteins with a molecular weight of 9.7 kDa (mytimacin-AF) and 11.45 kDa which were analogous to amino acid sequence of the full mytimacin-AF since they have closest molecular weight to mytimacin-AF with a molecular weight of 12 kDa. Protein with a molecular weight of 15 kDa was analogous to protein contained in *Ictalarus punctatus* mucus since they had same antimicrobial activities and same molecular weight, about 15 kDa. Protein with a molecular weight of 50.81 kDa was analogous to protein contained in tinca tinca mucus with a molecular weight of 49 kDa since they have almost the same molecular weight, and also analogous to achasin with a molecular weight of 59.086 kDa and 83.67 kDa as found by Achasin Ebran *et al*.[4,5]

Those proteins were characterized by using SDS-PAGE technique followed by electro-elution and dialysis techniques. The SDS-PAGE technique actually consisted of the same steps as the profiling process, requiring two different wells and four gels for each electrophoresis. Those four first gels were used for low range protein marker, and the other four gels were used for broad range protein markers. The first well was used for both low range and broad range markers, while the second well was filled with isolated protein.

After the electrophoresis process was completed, staining was conducted until the protein bands could be seen quite clearly. Destaining was not necessary until the gel was completely clear. Next, the process of protein production was followed by electro-elution process. After the protein bands with certain molecular weight were cut, destaining

Table 1. The order of the eppendorf tube in dilution test

Tube number / Weight of protein molecule	The number of proteins in the tube (ml)/ protein number								
	1	2	3	4	5	6	7	8	9
83.67	0,5	0,25	0,125	0,0625	0,0312	0,0156	0,0078	KP	KN
50.81	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	KP	KN
15	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	KP	KN
11.45	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	KP	KN
9.7	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	KP	KN

Tabel 2. The concentration of protein in nanodrop test

No. Protein / The number of protein (ml)	Absorbance Values													
	Streptococcus mutans							Actinobacillus actinomycetemcomitans						
	1	2	3	4	5	6	7	1	2	3	4	5	6	7
	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078
1 (83.67 kDa)	1,504	1.243	1.119	1.292	1.095	0.728	0.832	0.525	0.104	0.597	0.250	0.803	-	0.332
2 (50.81 kDa)	-	0.934	0.586	1.056	0.815	1.043	-	1.466	0.636	0.543	0140	0.311	0.713	0.277
3 (15 kDa)	-	0.563	1.063	0.412	-0.355	0.495	0.762	-	0.454	0.482	0.479	-0.322	0.467	0.156
4 (11.45 kDa)	-	1.459	0.997	1.203	1.504	1.006	0.941	0.726	1.007	-0.284	-0.062	0.384	0.797	0.759
5 (9.7 kDa)	-	0.819	1.106	0.521	1.060	1.040	1.134	0.711	0.410	0.771	0.600	0.770	0.232	-0.045

Table 3. The results of dilution test on spectrophotometric absorbance values

Protein Labels	Inhibitory factor against *S. mutans* (cm)					Inhibitory factor against *A. actinomycetemcomitans* (cm)				
	1	2	3	4	Mean	1	2	3	4	Mean
1 (83.67 kDa)	1.640	1.860	1.855	1.950	1.826	1.200	0.990	1.115	1.200	1.126
2 (50.81 kDa)	1.840	1.980	1.780	1.905	1.876	1.410	1.295	1.255	1.515	1.369
3 (15 kDa)	1.525	1.390	1.665	1.625	1.551	1.365	1.225	1.205	1.140	1.234
4 (11.45 kDa)	0	0	0	0	0	1.180	1.065	1.125	1.180	1.138
5 (9.7 kDa)	0	0	0	0	0	0	0	0	0	0

Table 4. The results of paper disc diffusion test on inhibitory factor

Protein labels	Inhibitory factor against *S. mutans* (cm)					Inhibitory factor against *A. actinomycetemcomitans* (cm)				
	1	2	3	4	Mean	1	2	3	4	Mean
1 (83. kDa)	1.640	1.860	1.855	1.950	1.826	1.200	0.990	1.115	1.200	1.126
2 (50.81 kDa)	1.840	1.980	1.780	1.905	1.876	1.410	1.295	1.255	1.515	1.369
3 (15 kDa)	1.525	1.390	1.665	1.625	1.551	1.365	1.225	1.205	1.140	1.234
4 (11.45 kDa)	0	0	0	0	0	1.180	1.065	1.125	1.180	1.138
5 (9.7 kDa)	0	0	0	0	0	0	0	0	0	0

was re-done in a certain time until the gel appeared slightly clear.[12] Then dialysis process was conducted to purify the protein content. The pellets formed were measured with Ohauss scales and dissolved in a solution of 0.5 M Tris-Cl at pH 7.3. Hereafter, the concentration of each protein was measured by using NanoDrop test.

Dilution test was conducted by using Brain Heart Infusion medium (BHI) in eppendorf tubes. The first tube was filled with protein 0.5 ml and BHI 0.5 ml. The number of protein continued was then reduced 50% by dilution from tube 2 to tube 6 as seen in Table 1. The preparation then was conducted twice, and made into two groups. The first group was inoculated with *Streptococcus mutans,* and the second group was inoculated with *Actinobacillus actinomycetemcomitans*. The results of the preparations then were incubated under anaerobic conditions at a temperature of 37^0 C for 24 hours.

After 24 hours, the incubation result of *Streptococcus mutans* then was grown in TYC medium, while that of *Actinobacillus actinomycetemcomitans* was grown in Luria Berthani medium in order to determine the maximum inhibitory factor of proteins against both bacteria. Incubation result grown in BHI medium was analyzed for its absorbance values by using spectrophotometric at a wavelength (λ) of 600 nm to determine the level of turbidity of BHI media. Thus, the more turbid BHI medium is, the higher the growth of bacteria. Absorbance values that appear in the tool indicate the reduction result of the absorbance values of treatment and the absorbance values of negative control. The negative result of the reading can indicate that the turbidity value in the negative control is higher than that in the treatment. It means that there is no growth of bacteria. Meanwhile, the positive result of the reading can indicate the presence of bacterial growth.

Finally, the media in petri dish was then divided into five parts, on each of which bacterial swab was conducted evenly. Paper disc that has given protein was placed on each medium. These steps were repeatedly conducted four times. Petri dishes were incubated in anaerobic condition at a temperature of 37^0 C for 24 hours, and then measured for their inhibitory diameter by using calipers.

RESULTS

The samples of this experiments were thirty-six healthy and active snails. The total of snail mucus collected was 150 ml with the average of snail mucus taken from each sample about 3-5 ml. The molecular weight of proteins contained in the snail mucus was measured by using the formula Rf. Based on the results shown in the low range marker, it is known that there were proteins contained in snail mucus with a molecular weight of 28.59 kDa, 15 kDa, and 13.3 kDa. Meanwhile, based on the results shown in the broad range marker, it is known that there were proteins contained in snail mucus with a molecular weight of 83.67 kDa, 50.81 kDa, 48.1 kDa, 35 kDa, 28.88 kDa, 11.45 kDa, and 9.7 kDa.

The result of reading the value concentration of the isolated crude protein was 10.152 µg/ml, while the results of reading the value concentration of those proteins contained in snail mucus by using Nano Drop test were different among each other as seen in Table 2. It is also known that there was bacterial growth as shown in the result of inoculated subculture on TYC medium. The presence of bacterial growth was expected because of contamination occurred in one treatment to another one due to poor sterilization of spreader.

Based on the reading results of spectrophotometer, it was known that a protein with a molecular weight of 15 kDa has antimicrobial activities against *Streptococcus mutans* with absorbance value about -0.355 at the number of protein about 0.0312. The results also show that a protein with a molecular weight of 9.7 kDa (mytimacin-AF) had antimicrobial activities against *Actinobacillus actinomycetemcomitans* with absorbance value about 0.045 at the number of protein about 0.0078 ml. It was also known that a protein with a molecular weight of 11.45 kDa hadantimicrobial activities against *Streptococcus mutans* with absorbance value about -0.284 at the number of protein about 0.0625 ml and against *Actinobacillus actinomycetemcomitans* with absorbance values about -0.062 at the number of protein about 0.125 ml. Meanwhile, a protein with a molecular weight of 83.67 kDa (achasin) had antimicrobial activities against *Streptococcus mutans* with the lowest absorbance value about 0.0156 at the number of protein about 0.728 ml and against *Actinobacillus actinomycetemcomitans* with absorbance value about 0.104 at the number of protein about 0.25 mL. This suggests that Achasin found in this research did not have a maximum inhibitory factor against *Streptococcus mutans* and *Actinobacillus actinomycetemcomitans* as seen on the results of the dilution test in Table 3.

Based on the results of paper disc diffusion test, it was known that a protein with a molecular weight of 50.81 kDa had the greatest inhibitory factor against *Streptococcus mutans* with a mean inhibition of 1.876 cm and *Actinobacillus actinomycetemcomitans* with a mean inhibition of 1.369 cm. It was also known that a protein with a molecular weight of 83.67 kDa (achasin) had inhibitory factor against *Streptococcus mutans* and *Actinobacillus actinomycetemcomitans*. Proteins with a molecular weight of 11.45 kDa and 9.7 kDa (mytimacin-AF) had no antimicrobial activities against *Streptococcus mutans*. However, although a protein with a molecular weight of 11.45 kDa had no antimicrobial activities against *Streptococcus mutans,* but it still had inhibitory factor on the growth of *Actinobacillus actinomycetemcomitans*. In contrary, a protein with a molecular weight of 9.7 kDa (mytimacin-AF) had no inhibitory factor and antimicrobial activities against *Streptococcus mutans* and *Actinobacillus actinomycetemcomitans*. Finally, it was known that a protein with a molecular weight of 15 kDa had inhibitory factor about 1.551 cm against *Streptococcus mutans*, and about 1.234 cm against *Actinobacillus actinomycetemcomitans* (Table 4).

DISCUSSION

Achasin found in this research had a molecular weight of 83.67 kDa. This corresponds to a molecular weight range of achasin protein, from 59.086 to 150 kDa, ever found in previous researches conducted by Venugopal R[9] and Jyh-Yih.[13] These various molecular weights can be caused by the differences of snail subspecies and geographical location.

Based on the results of the protein characterization phase, it was known that achasin had many differences of its band thickness. These differences of its band thickness can affect on its concentration value. Thus, the thicker the band of a protein is, the greater the concentration value of the protein is.[14] It is because band thickness indicates that there are many levels of protein in the sample. Crude protein, for example, as the sample of protein isolated has a concentration of 10.152 µg/ml indicating that there is a great potential source of proteins contained in Snail mucus.[2,13]

Furthermore, based on the results of subculture conducted after the inoculation of bacteria in those eppendorfes, it was known that the number of bacteria was increased. It may be caused by bacterial contamination during the inoculation process of bacteria into TYC medium since inoculum contaminated from one treatment to other treatments had caused the increasing of bacterial growth. Better reading was found from the reading of the absorbance value. When bacteria lives and breeds, it will produce matrix from planting medium. As a result, the matrix made the planting medium become turbid. Thus, it can be said that if the culture medium becomes turbid, there must be bacteria grown. Nonetheless, there were still some samples that cannot be read (Table 3) because the number of samples did not meet the minimum standard volume (5 ml) specified by spectrophotometer so that the tool could not read their absorbance values.

Based on the results of dilution test, it was known that only protein with a molecular weight of 15 kDa has antibacterial activities against *Streptococcus mutans* and *Actinobacillus actinomycetemcomitans*. Similarly, in previous researches, it is known that a protein with a molecular weight of 15 kDa, namely HLP-1, taken from catfish mucus (*Ictalarus punctatus*) has been identified as an effective antimicrobial to Gram-positive and Gram-negative bacteria. This is because the protein works with two mechanisms: forming classic transmembrane channels to become the targeted cell membrane and making the cell membrane become more soluble with carpet like mechanism. These mechanisms have been considered to be effective for both Gram-positive and Gram-negative bacteria.

Besides that, it is also known that proteins with a molecular weight of 11,45 kDa and 9,7 kDa (mytimacin-AF) are only effective against *Actinobacillus actinomycetemcomitans.* Unlike the results of previous researches show that mytimacin-AF as a broadspectrum antimicrobial protein is more effective on Gram-positive bacteria, the result of this research showed that mytimacin-AF also had antimicrobial activity against Gram-negative bacteria. This is because the mechanism of mytimacin-AF as an antimicrobial protein basically consists of damaging cell membrane, interfering cell metabolism, and destroying

cytoplasmic cell components.[4] Therefore, this mechanism is considered as an effective mechanism for Gram-positive and negative bacteria.

Actually, the main reason of this lack of antimicrobial activity in these proteins with a molecular weight of 11.45 kDa and 9.7 kDa can be caused by the use of SDS-PAGE method during protein production for several reasons. The use of denaturing and dissociating agents in SDS-PAGE method can impair protein chain, then causing the loss of some or all its caracterization.[14] Besides that residual staining materials of Comassie Brilliant Blue can interfere the nature of proteins. Nevertheless, the use of SDS-PAGE method for protein production is not entirely disadvantageous since the properties owned by the protein did not disappear entirely, but its sensitivity will be not as good when using ion chromatography method.[12,15] Thus, ion chromatography method is necessary to be conducted in researches related to protein characterization and production.

Based on the results of the paper disc diffusion test, it was known that a protein with a molecular weight of 15 kDa was effective for *Streptococcus mutans* and *Actinobacillus actinomycetemcomitans*, while a protein with a molecular weight of 9.7 kDa (mytimacin-AF) hadno antibacterial activities. It is also known that a protein with a molecular weight of 11.45 kDa is only effective for *Actinobacillus actinomycetemcomitans*. It means that this protein with a molecular weight of 11.45 kDa is analogous to the amino acid sequence of the full mytimacin-AF, which is effective on Gram-negative bacteria as the same as a protein with a molecular weight of 9.7 kDa.

A protein with a molecular weight of 83.67 kDa (achasin) has a better inhibitory factor against *Streptococcus mutans*. Nevertheless, based on the results of this experiment, it is known that *Achasin* also has inhibitory factor against *Actinobacillus actinomycetemcomitans*. Similarly, previous researches also show that *Achasin* has antimicrobial activities that can inhibit the growth of Gram-positive and negative bacteria, but it gives a better inhibition on Gram-positive one.[3] This is because the mechanism of achasin is aimed to inhibit the formation of peptidoglycan and cytoplasmic membrane. Like achasin, a protein with a molecular weight of 50.81 kDa also has a greater inhibitory factor against *Streptococcus mutans* than against *Actinobacillus actinomycetemcomitans*. A protein with a molecular weight of 50.81 kDa is the closest analogue of the antimicrobial proteins derived from tinca tinca mucus, which has a molecular weight of 49 kDa and also has the best inhibitory factor against Gram-positive bacteria.[5]

Finally, the study showed that proteins successfully characterized from snail mucus (*Achatina fulica*) were proteins with molecular weights of 83.67 kDa (achasin), 50.81 kDa, 15 kDa, 11.45 kDa (full amino acid sequence of mytimacin-AF) and 9.7 kDa (mytimacin-AF). Achasin, based on the results of the dilution test, has better antimicrobial activities against *Streptococcus mutans*, while mytimacin-AF has better antimicrobial activities against *Actinobacillus actinomycetemcomitans*. Achasin, based on the results of the paper disc diffusion test, actually has antimicrobial activities against *Streptococcus mutans* and *Actinobacillus actinomycetemcomitans*, while mytimacin-AF has no antimicrobial activities. Those proteins with molecular weights of 50.81 kDa, 15 kDa, 11.45 kDa are considered as new antimicrobial proteins isolated from snail mucus. However, their effectiveness need to be studied further.

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PAGE 1

PAGE 2

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