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Identification of active chemical compounds and potentialantibacterial snail mucus (*Achatina fulica*) on bacteria *Enterococcus foecalis* causes of periodontitis

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ABSTRACT

Background: A chemical compound is a chemical compound present in a natural source that gives it the special properties and characteristics of the plant or animal source. Snail slime is one of the traditional medicines from animals that is used by the community as a traditional medicine for healing minor wounds and toothaches. The aim of this study was to identify the active chemical compounds and antibacterial potential of snail mucus against the bacteria *E. foecalis* that causes periodontitis.

Method: True experiment laboratory research method which is carried out by laboratory analysis of snail mucus to obtain chemical elements, the content of compounds contained in a test sample. The content of active compounds based on the GasChromatography-Mass Spectrometry (GCMS) test.

Results: The average content of heparan sulfate with four repetitions was 16.45 mg/100g, Acharan sulfate 21.33 mg/100g, protein achasin 102.22 mg/100g, Glycoconyugat 88.6 mg/100g, Ion Ca2+ 86.2 mg/100g, Beta agglutinins 85.22 mg/100g. Toxicity test was carried out with four repetitions with concentrations of 100%, 50%, 25%, and 12.5%. And the lowest results of the toxicity test were at a concentration of 100% with a value of 0.171, while at a concentration of 50% 0.302, 25% 0.453, and 12.5% 0.768, for bacteria *E. Foecalis* with an inhibitory power of 23.15 mm, the category was very strong.

Conclusion: The activechemical content in snail mucus is Acharan sulfate, achasin protein, Glycoconyugat, Ca2+ ion, Beta agglutinin, and the antibacterial potential of snail mucus against *E. foecalis* bacteria is very strong.

Keywords: identification of chemical compounds, antibacterial potential, snail mucus, *E. foecalis*.
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INTRODUCTION

Indonesia is a tropical country and many species of snails are found, including *Achatina fulica* or often referred to as land snails. *A. fulica* is found in many species of snails, and is considered one of the worst snail pests from the tropics and subtropics. These animals consume a lot of plants, modify their habitat and are able to compete with native snails.[1] A chemical compound is a chemical compound present in a natural source (either plant or animal) that gives it the special properties and characteristics of the plant or animal source.

The discovery of a chemical compound can encourage the achievement of advances in the science of chemical synthesis of natural materials due to the opportunity for the synthesis of certain synthetic compounds, to the demand for the supply of a form of pioneering compound for the benefit of an advanced synthesis. Snail mucus is one of the traditional medicines from animals that is used by the community as a traditional medicine for healing minor wounds and toothaches. Therefore, snail slime needs to be researched and developed. Scientific research has been carried out on snail mucus as an antibacterial for *Escherichia coli*, *Streptococcus mutans*, and *Propionibacterium acnes*.[3] The discovery of a chemical compound can encourage the achievement of the progress of the science of chemical synthesis of natural materials due to the opportunity for the synthesis of certain synthetic compounds, to the demand for the supply of a form of pioneering compound for the benefit of an advanced synthesis. Periodontitis is a multifactorial disease that causes inflammation of the periodontal tissue.[4] In general, periodontal disease is caused by

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terial plaque on the tooth surface, where plaque is a thin layer of biofilm containing a collection of pathogenic microorganisms such as *Porphyromonas gingivalis*, *Actinobacctinomycetemcomitans*, *Prevotela intermedia*, *Tannerella forsythia* and *Fusobacterium nucleatum* in bacteria *E. foecalis* which is a gram-positive bacterial species and is commonly found in periapical root canal lesions with apical periodontitis.

The main virulence factor released by *E. faecalis* is Lipoteichoic acid (LTA) which can induce an inflammatory process and evoke an immune response in the host's body. Periodontitis is a risk factor that plays a role in impaired masticatory function and tooth loss, disorders that are often encountered and occur in humans.[5] This study aimed to identify the active chemical compounds of snail mucus and its antibacterial potential against the bacteria *E. foec* s that causes periodontitis. This research was conducted at the Oral Biology Laboratory, Universitas Airlangga, Surabaya, Indonesia.

MATERIAL AND METHOD

The method used in this research is a true experiment laboratory research approach carried out by laboratory analysis of snail mucus for chemical elements and by using the diffusion method with four repetitions. The test was carried out using the antibacterial potential of *E. foecalis* bacteria. A total of 50 snails were taken from community plantations in Nyalian village, Banjarangkan district, Klungkung Regency, Bali, Indonesia to take 200 ml of mucus, to test the analysis of the active chemical and antibacterial compounds contained in it.

The tools used in this study were a set of 18 optical tools, a set of distillation tools, a set of column chromatography tools, oven, Buchi B-720 rotary evaporator, GC-MS Shimadzu QP-5000 and UV lamps 254 and UV 365 nm, snail slime. and methanol.

Preparation of snail mucus solution

A total of 10 ml of standard snail slime was measured using a measuring cup. The solution was then dissolved using pure pro-analysis methanol (98% with the Merck brand) in a 10 ml volumetric flask to the mark so that a standard solution of snail slime was obtained with a concentration of 100 ppm, standard solu n of 100 ppm snail mucus was pipetted 0.3 mL, 0.7 mL, 1 mL, 1.5 mL, and 2 mL respectively into a 10 mL flask, Point C was added to 98% methanol at each concentration of the solution so that was obtained standard solutions were 3 ppm, 7 ppm, 10 ppm, 15 ppm, 20 ppm, all standard solutions were derivatized before being injected into the GC-MS system.

Analysis of snail mucus solution using GCMS

The snail slime extract that had been obtained was then analyzed using GCMS with an Agiletn 5973 inert MSD detector, 2 l of snail slime extract sample solution was injected into the GCMS which has a lumn J&W scientific capillary, Helium carrier gas at a flow rate of 1 ml/min (constant) with a split ratio of 1:10, The oven temperature was programmed at 50°C and kept isothermal for 5 minutes, The rate of increase to 10°C/min and the temperature was increased to 280° C for 15 minutes, the temperature of injector port is 290 Celcius and the mass spectrometer interface = 230 Identification of phytochemical compounds contained in snail slime was carried out using the Willey database version 7.0 / W10N11 library database by comparing the mass spectrum pattern and the fragmentation pattern of the reference compound stored in Willey's library.

Antibacterial Potential Test of Snail ime Against *E. foecalis*

Planting the stock of germs using sterile osse on brain heart infusion (BHI) broth media then incubation for 48 hours, the turbidity of the germs was observed then standardized with the standard mc Farland 0.5, then planted the germs on the Hilton agar muller media with the spreading technique, for the next treatment the test sample on paper disk sterile 0.01 ml with a sterile micropipette, then paste it on the surface of the agar medium, then incubate for 48 hours, observe and measure the diameter of the clear zone. Bacterial stock is taken from ATCC 29212.

RESULT

The active compound content of snail slime (*Achatina fulica*)

GCMS tested the content of active chemical compounds from snail mucus to identify, the most was achasin protein with an average of 102.20 mg/100g, and the lowest was glycoconjugate which was 8.86. The average content of the compounds found in the GCMS test was the average Heparan sulfate 16.45 mg/100g, Acharan sulfate 21.33 mg/100g. Achatin 86.12 mg/100g, Beta agglutinin 58.22 mg/100g, protein achasin 102.22 mg/100g, glycoconjugate 8.86mg/100g (Table 1).

The results of the examination of the inhibition of snail mucus against the bacteria *E.foecalis* (four repetitions)

Table 2 show 4 repetitions of inhibitory in different concentration towards *E. foecalis*. Table 3 shows that the most extensive inhibition zone was in the treatment group with a concentration of 100%, namely 21.93 mm, while the lowest was at a concentration of 12.5%, there was no inhibition zone.

Table 4 shows a significant difference inhibition between treatment groups. There are significant differences in the antibacterial power of various concentrations of snail mucus against *E. foec* s in vitro (Figure 1). Post Hoc LSD test showed that there were differences in the clear zone around the well which was dripped with snail mucus with various concentrations. At all concentrations of snail mucus used showed significant differences inhibition.

DISCUSSION

The content of active chemical compounds from snail slime tested by GCMS (Gas Chromatography-Mass Spectrometry) is to identify, at most is achasin protein with an average of 102.20 mg/100g, and the lowest is glycoconjugate which is 8.86. average content of the compounds found in. The GCMS was the average Heparan sulfate 16.45 mg/100g, Acharan sulfate 21.33 mg/100g. Achatin 86.12 mg/100g, Beta agglutinin 58.22 mg/100g, protein achasin 102.22 mg/100g, glycoconjugate 8.86mg/100g. Achasin protein in snail

Table 1. Chemical compounds GCMS analysis test.

Repeat	Heparan Sulfate (mg/100g)	Acharan Sulfate (mg/100g)	Achatin Sulfate (mg/100g)	Ion Ca^{2+} (mg/100g)	Beta Agglutinin mg/100g	Protein achasin (mg/100g)	Glycoconyugat (mg/100g)
1.	16.60	21.35	36.10	86.15	58.21	102.20	8.90
2.	16.50	21.30	36.00	86.10	58.19	102.15	8.87
3.	16.30	21.37	36.08	86.13	58.25	102.25	8.82
4.	16.40	21.33	36.06	86.11	58.23	102.30	8.85
mean	16.45	21.33	36.06	86.12	58.22	102.2	8.86

Table 2. Bacterial Inhibitory *E. foecalis*.

Repeat	*E foecalis*					
	100%	50%	25%	12.5 %	control (+)	control(-)
1.	21.80	18.80	14.20	-	27.20	-
2.	21.75	18.20	14.40	-	27.40	-
3.	22.00	19.00	14.35	-	27.00	-
4.	22.20	18.75	14.80	-	27.20	-
mean	21.93	18.68	14.43	-	27.20	-

Table 3. Inhibitory Zone Diameter on *E. foecalis* Bacteria in the Treatment Group.

Subject Group	N	Mean ± *Fusobacterium nucleatum* Inhibition Zone (millimeters)	Bacteriacount (CFU/m l)	p
Control	4	27.20±0.81		0.001
Snail slime12.5%	4	0 ± 0.00		
Snail slime 25%	4	14.43± 0.12	0.5 Mc Farland	
Snail slime 50%	4	18.68 ± 0.73		
Snail slime100%	4	21.93± 0.10		

slime has important biological functions, including binding to proteins (enzymes) that present in bacteria and will interfere with the activity of these enzymes. When it infection happens, the bacteria that will carry out the replication process will fail to separate because prevented by the achasin protein, theseptum is not formed so it does not separately become daughter cells.[2] The protein content of achasin in Snail mucus is very high, so it is beneficial for healing periodontitis is indispensable.

The lowest glycoconjugate content in snail slime is carbohydrate molecules bound to other compounds, such as proteins and lipids. This molecule's shape serves various functions in connective tissue, including communication cell to cell and cross-links between proteins.[6] Achatina slime fulica which is secreted to the outer surface of the body as mucus material from granules internal contains glycosaminoglycans, and which are included in glycosaminoglycans are heparan sulfate, heparin, keratan sulfate and hyaluronic acid.[7] Snail mucus contains chemicals such as achatin isolate, and calcium. The content of achatin isolate is useful as an antibacterial and analgesic, whereas calcium plays a role in hemostasis.The effect of snail slime as an anti-agent Inflammation will accelerate the inflammatory phase so that the wound healing phase is also faster.[8]

Antibacterial Potential

Enterococcus faecalis is a Gram-positive bacterium in pairs, single or short chains. *Enterococcus faecalis* is oval or ovoid in shape. On blood agar, the colony's surface is circular, smooth and thorough. Enterococcus faecalis is a facultative anaerobic bacterium.[9] The ability of *E. faecalis* to live in an unsupportive environment and survive as a microorganism in root canals causes this bacterium to become a pathogen that can lead to failure of root canal treatment. The inhibition of snail mucus against *E. foecalis* bacteria is 21.93 mm, including the very strong category, according to the theory of Davis and Stout (1971), the antibacterial power is the diameter of the inhibition zone of 5 mm or less categorized as weak, 5-10 mm with medium category, 10-20 mm is categorized as strong, and 20 mm more with very strong category. So the snail slime's power against *E. foecalis* bacteria is very strong. Snail slime contains chemicals including achatin isolate, heparan sulfate, and calcium.

The content of achatin isolates is useful as an antibacterial and anti-pain, while calcium plays a role in hemostasis. Achasin attacks or inhibits the formation of common parts of bacterial strains such as peptidoglycan and the cytoplasmic membrane. The peptidoglycan layer is the layer that forms the cell wall, where the bacterial cell wall plays a very important role in resisting osmotic pressure from the outside. The achasin protein in snail slime has important biological functions, including as a bacterial protein binding receptor (enzyme). Achasin protein will bind to proteins (enzymes) that exist in

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Table 4. Differences in the inhibition of *E. foecalis* bacteria between treatment groups.

Variable	Study group		Mean difference (I-J)	p
Snail Slime	Control	12.5%	27.20	1.00
		25%	12.77	<0.001*
		50%	8.52	<0.001*
		100%	5.27	<0.001*
	12.5%	25%	-14.43	<0.001*
		50%	-18.68	<0.001*
		100%	-21.93	<0.001*
		control	-27.20	<0.001*
	25%	50%	-4.25	<0.001*
		100%	-7.5	<0.001*
		control	-12.77	<0.001*
		12.5%	14.43	<0.001*
	50%	100%	-3.25	<0.001*
		control	-8.52	<0.001*
		12.5%	18.68	<0.001*
		25%	4.25	<0.001*
	100%	control	-5.27	<0.001*
		12.5%	19.20	<0.001*
		25%	6.80	<0.001*
		50%	3.80	<0.001*

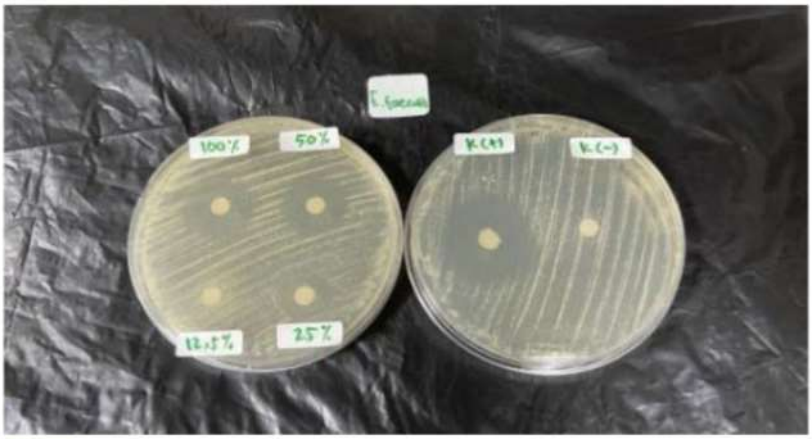

Figure 1. Inhibitory zone of snail slime bacteria against bacteria *E. foecalis*.

bacteria and will interfere with the activity of bacteria that will carry out replication will fail to carry out the replication process will fail to separate because it is prevented by achasin protein, the septum is not formed and separates into daughter cells.[2]

CONCLUSION

Snail slime has a variety of active compounds that can be used for the cell regeneration process and the healing process of inflammation, besides that, snail slime also has the ability as an antibacterial against *E. foecalis* bacteria as one of the causes of periodontitis with a very strong inhibitory power.

CONFLICT OF INTEREST

All author declares there is no conflict of interest.

ETHICAL CONSIDERATION

This study has been approved by ethical committee Health Polytechnic Denpasar, Bali, Indonesia with ethical clearance reference number: LB.02.03/EA/KEPK/0655/2021.

AUTHOR CONTRIBUTION

All authors has been contributed to manuscript writing and agreed for the final version of manuscript for publication.

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