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RESEARCH ARTICLE

Antimicrobial Properties of Snail Slime (Achatina Fulica) on the Growth of *Actinobacilus Actinomycetemcomitans* Bacteria Causing Periodontitis

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Abstract

Aim: The purpose of this study was to calculate the inhibition zones of the growth of *A. actinomycetemcomitans* by snail slime at concentrations of 12.5%, 25%, 50%, 100%. Methods: The study was an experimental randomized posttest-only control group design. This research was conducted in the oral biology laboratory of the Faculty of Dentistry, Airlangga University, Surabaya Indonesia. Variables in this study snail slime were made with concentrations of 12.5%, 25%, 50% and 100%. Take germ stock with osse, then plant on BHI broth media, then incubate 37C for 24 hours. Plant germs on BHI media so that with the swab technique to evenly spread on the surface, then contact the paper disc that has been given each concentration of 12,%, 25%, 50%, 100%, with tweezers on the surface as much as 10 micromillimeters, then incubate 37°C for 2x 24 hours, eight repetitions are carried out. Results: The mean diameter of the inhibitory zone snail slime on the growth of *A. actinomycetemcomitans* bacteria in the treatment groups had significant differences $p<0.05$. The mean inhibition zone between controls and LB treatment group (LB 12.5%, 25%, 50%, 100%), were statistically different ($p<0.05$).In contrast with LB 12.5% ($p>0.05$) which significant difference with the control group. Conclusion: The concentration of 100% snail slime has the highest inhibitory power, against the growth of the *A. actinomycetemcomitans* bacteria, The 12.5% concentration snail slime showed no antibacterial activity against the growth *A. actinomycetemcomitans.*

Keywords: *Snail slime, Actinobacilus actinomycetemcomitans bacteria, Inhibition zone diameter.*

Introduction

Periodontal disease is a dental and oral disease that has multifactorial causes, namely systemic factors, malnutrition, drugs and mainly caused by bacteria that accumulate in dental plaque. One of the gram-negative bacteria that cause periodontitis is the actinobacilus actinomycetemcomitans bacteria [1].

Periopathogenic bacteria as the main factor causing periodontitis, mainly the bacterium *Actinobacillus actinomycetemcomitans* which is one of the bacteria that has a high virulence due to the release of toxins that can inhibit the components of the immune system such as polymorphonuclear (PMN), immunoglobulins and complementary activity. The bacterial ability of *Actinobacillus actinomycetemcomitans* as a destructive etiology agent due to bacteria has a number of virulence determinants namely bacteriocin, leukotoxin, collagenase, endotoxin, fibroblast inhibitor factor [2], Bone resorption inducing factor, induces cytokine production from macrophages, modifies neutrophil function, and invasively degrades immunoglobulin who invaded epithelial cells.

Other alternative treatments use natural ingredients that are efficacious as antimicrobial ingredients, one of which is snail slime. Snail slime contains chemicals such as achatin isolates, heparan sulfate, and calcium, hyaluronic acid. The content of achatin isolates is useful as an antibacterial and pain reliever, while calcium plays a role in hemostasis [3].

The effect of snail mucus as an antibacterial and anti-inflammatory will further accelerate the inflammatory phase so that the proliferation phase will be faster in healing wounds [4]. The content of snail mucus which is thought to have the most influence on proliferation of fibroblasts is heparan sulfate which is useful in accelerating the wound healing process by helping the blood clotting process and fibroblast cell proliferation [5].

Heparan sulfate also functions for angiogenesis, inhibiting vascular endothelial growth factor or decreasing mitogen activity from fibroblast growth factor (FGF) [6, 7]. The purpose of this study was to determine the antimicrobial properties of snail mucus with a concentration of 12.5%, 25%, 50% and 100% of the inhibitory power of *Actinobacilus actinomycetemcomitans*.

Material and Methods

This research is a pure laboratory experiment with the design of Post Test Control Group Design. This research was conducted in the Oral Biology Laboratory of the Faculty of Dentistry, Airlangga University, Surabaya, in July 2017. The technical procedures are as follows:

Preparation of Snail Slime

This study used slime snail extracted from the garden in Banjar Umaanyar Nyalian Village Banjarangkan District of Klungkung. 50 snails were picked up, slime was collected by touching or stimulating snail meat with the tip of the straw, before the snail shell was sterilized first to prevent bacterial contamination to the slime. The slime stored in a bottle and then added ethanol was centrifuged for 30 minutes at the analytical laboratory of the Faculty of Chemistry, Udayana University, Denpasar Bali.

Media Culture of the Bacterium *A. actinomycetemcomitans*.

A. actinomycetemcomitans in AaGM media at 37°C for 24 hours with an anaerobic atmosphere. Then test bacterial confirmation with gram staining. Making suspension of A. *actinomycetemcomitans* in 5 ml TSB liquid media and homogenized with Vortex.

Cell strain was calculated using spectrophotometry with a wavelength of 625 nm with an absorbance value of 0.08-0.10. The media was divided into eight petri dishes and then be await until it's become solid. One bacterial stock was from ATCC 702358 [8].

Planting the Suspension of Bacteria *A. actinomycetemcomitans*

The population in this study was the bacteria *A. actinomycetemcomitans* in agar brain heart infusion (BHI) media. Variables of influence in this study snail slime were made with concentrations of 12.5%, 25%, 50% and 100%. Germ stock was taken with osse stick, then plant on BHI broth media, then incubated in 37°C for 24 hours.

Plant germs on BHI media so that with the swab technique to evenly spread on the surface, then contact the paper disc that has been given each concentration of 12.5%, 25%, 50%, 100%, with tweezers on the surface as much as 10 micro millimeters, then incubate 37°C for 48 hours, eight repetitions are carried out. Observation of clear zones on the surface so that there is a paper disc with the calculation using a caliper (mm). The area without visually apparent bacterial growth (clear zone) around each disc was observed [9].

Results

Table 1: The width of Actinobacilus actinomycetemcomitans inhibitory Zone in The Treatment Group

Subject Group	n	Mean ± SD *A. actinomycetemcomitans* Inhibition Zone (millimeters)	p
Control	8	0 ± 0.00	0.001*
LB 12.5%	8	0 ± 0.00	
LB 25%	8	11.00 ± 0.55	
LB 50%	8	14.00 ± 0.34	
LB 100%	8	17.90 ± 0.53	

*Analysis with One-Way Anova Test; Significant at p<0.05

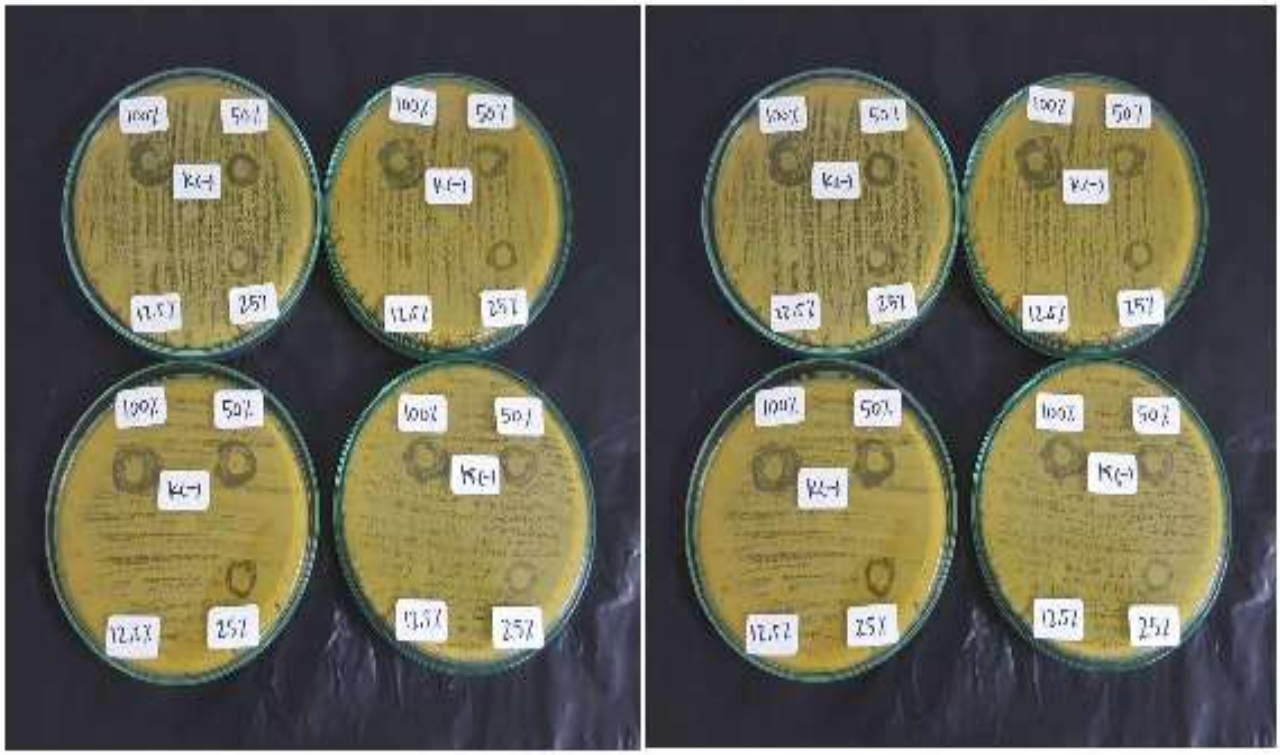

Figure 1: The inhibitory zone of Snail slime against *A. actinomycetemcomitans* bacteria (in millimeters)

Table 2: The Difference of *A. actinomycetemcomitans* Inhibitory Zone between the Treatment Groups

Variable	Group (I)	Group (J)	Mean Difference (I-J)	p
Snail Slime	Control	12.5%	0.00	1.00
		25%	-11.01	<0.001*
		50%	-13.96	<0.001*
		100	-17.92	<0.001*
	12.5%	Control	0.00	1.00
		25%	-11.01	<0.001*
		50%	-13.96	<0.001*
		100%	-17.92	<0.001*
	25%	Control	11.01	<0.001*
		12.5%	11.01	<0.001*
		50%	-2.95	<0.001*
		100%	-6.91	<0.001*
	50%	Control	13.96	<0.001*
		12.5%	13.96	<0.001*
		25%	2.95	<0.001*
		100%	-3.96	<0.001*
	100%	Control	-17.92	<0.001*
		12.5%	-17.92	<0.001*
		25%	6.91	<0.001*
		50%	3.96	<0.001*

*Analysis with Post Hoc Test; Significant at p<0.05

The results of the research on the inhibitory capability of Snail slime on growth of *Actinobacillus actinomycetemcomitans* can be seen in Figure 1. The mean Snail slime inhibition zone diameter of the *Actinobacilus actinomycetemcomitans* bacteria in the treatment group was testing using One-Way ANOVA. The results from Table 1 shows that the mean diameter of the inhibitory zone of the LB had significant differences in between groups of intervention (p<0.05).

Table 2 shows the mean difference of the inhibitory zone between controls and the LB treatment groups. The mean differences between LB 25%, 50%.And 100% groups in control group were statistically significant (p<0.05*)*, but there was no significant

difference between LB 12.5% and control group (p>0.05).

Discussion

Mucin glycoprotein is the main macromolecular constituent of snail mucus. Glycoproteins, such as achacin, are components involved in antimicrobial activity10. Achacin, besides inhibiting bacterial growth, also appears to attack the bacterial plasma membrane.

However, achacin has the ability to catalyze oxidative deamination that produces ketoacid, hydrogen peroxide, and ammonia [10, 11]. The results of various concentrations of snail mucus extract against the bacteria *A. actinomycetemcomitans* show that the formation of clear zones around the wells has been treated with a concentration of 12.5% slime. 25 %, 50 %, and 100 %, and negative controls with eight repetitions.

The results of the data obtained were tested for normality and homogeneity as a condition for conducting the One Way ANOVA test, Shapiro-Wilk test (p>0.05) indicating that all groups were distributed normal, and Lavene tests all homogeneous variances. The One Way ANOVA test results showed that the p-value <0.05 means that there is a significant difference in the antibacterial power of various concentrations of snail mucus to in vitro bacteria *A. actinomycetemcomitans*.

The difference in the results of the One Way ANOVA test was then carried out by the Post Hoc Least Significant Different (LSD) test to determine the significant differences between the treatment groups. The results showed that there were differences in the clear zones around the well which were dripped with various concentrations of snail mucus. At all concentrations of snail mucus used showed a significant difference in inhibitory power.

Increasing the concentration of snail mucus from 12.5% 25%, 50% and 100% there are differences in inhibition. The inhibition of snail mucus is the highest concentration of 100%, this is in accordance with Palcter et al. the higher the concentration of an antibacterial ingredient, the antibacterial ratification showed by the inhibition strength is stronger, but the snail mucus the higher the concentration it produces more inhibitory power high, but still in strong criteria, associated with the provisions of the criteria for inhibitory activity adopted by David et al.

Inhibition zones formed ≥ 20 mm are considered to have very strong inhibitory activity, 10-20 mm stated to have strong inhibitory activity, 5-10 mm was stated to have moderate inhibitory activity and ≤ 5 mm was stated to have a weak inhibitory activity [12, 13]. Snail slime with a concentration of 25% has been able to inhibit the growth of bacteria with antibacterial power with strong criteria. This shows that snail slime contains achasin isolate as an antibacterial active protein molecule [14].

Achasin protein is a protein that has important biological functions. Achasin can work by attacking or inhibiting the formation of strains of bacteria such as peptidoglikan, which is a cell-forming component wherein strong cell walls are needed to resist external pressure [15].

There are two ways to synthesize peptidoglycan in elongation and septation, this requires penicillin-binding protein (PBPs) which is an enzyme, the transpeptidase enzyme that has a function as a catalyst in biosynthesis in the final stage. Achasin refers to the bacterial cytoplasmic membrane which causes the cell wall to peel and enter the cytoplasm. Hyaluronic acid content in snail mucus has viscoelastic properties; this can inhibit the penetration of germs and viruses in the wound. Hyaluronic acid is very effective at inhibiting the growth of *A. actinomycetemcomitans* in periodontitis [16, 17].

Conclusion

Based on the research that has been done in the oral biology laboratory of the Faculty of Dentistry, Airlangga University, Surabaya, Indonesia it can be concluded that snail slime can inhibit the growth of *A. actinomycetemcomitans* bacteria with strong criteria at concentrations of 25 %, 50 % and 100 %.

The concentration of 100% snail slime has the highest inhibitory power, against the growth of the *A. actinomycetemcomitans* bacteria, The 12.5 % concentration snail slime showed no antibacterial activity against the growth *A. actinomycetemcomitans.*

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