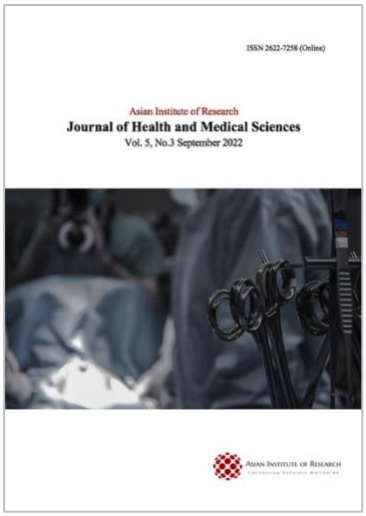

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Antibacterial Effects of Various Concentrations of Natural Ingredients Snail Mucus (*Achatina fulica*) Against Inhibition Zones of *Fusobacterium nucleatum* Causes Periodontitis In Vitro

Swastini IGAAP[1], Ni Nengah Sumerti[2]

[1,2] Politeknik Kesehatan Kemenkes Denpasar, Denpasar, Bali, Indonesia

Correspondence: Swastini IGAAP, Politeknik Kesehatan Kemenkes Denpasar, Jl. Sanitasi No.1, Sidakarya, Denpasar, Bali, Indonesia. E-mail: agungswastini18@gmail.com

Abstract
Periodontitis is a microorganism that invades the subgingival area, thereby triggering an inflammatory response of the periodontal tissue. *Fusobacterium nucleatum* is one of the most abundant gram-negative bacilli colonizing the subgingival plaque and closely associated with periodontal disease. The aim of study was to calculate the inhibition of snail mucus against *Fusobacterium nucleatum* bacteria with concentrations of 12.5%, 25%, 50%, and 100%. This study used a laboratory experimental test method and was conducted at the Oral Biology Laboratory, Faculty of Dentistry, Airlangga University. The steps were performed are: plant germ stock with sterile osse on BHI broth media then incubate for 48 hours, observe the turbidity of the germs then standardize with mc farland standard 0.5, plant germs on Hilton Muller agar media with the spreading technique, treat the test sample on sterile paper disk as much as 0, 01 ml with a sterile micropipette, then paste it on the surface of the media so that it is then incubated for 48 hours. Then, observe and measure the diameter of the clear zone. The results show that the average inhibition in the control group is 25.65 mm, and the snail mucus group is 12.5%, 0 mm, 25%, 12.40 mm, 50%, 16.70 mm and 100% 19.60 mm. There is a significant difference in the diameter of the inhibitory power between the treatment groups. The conclusion of the antibacterial effect of natural ingredients of snail mucus from the 25% treatment showed strong criteria, and the greatest inhibitory power was the concentration of 100%.

Keywords: Antibacterial, Snail Slime, Fusobacterium Nucleatum

1. Introduction

Periodontitis is a disease that attacks the supporting tissues of the teeth characterized by chronic inflammation, caused by bacteria present in dental plaque. This disorder begins with the accumulation of plaque containing pathogenic bacteria and toxins. The interaction of plaque and bacteria causes ulcers in the four periodontal tissues (gingiva, periodontal membrane, cementum, and alveolar bone) (Evan Wijaksana, 2016). Figure 1 shows the condition of teeth diagnosed having periodontitis.

Figure 1: Teeth with periodontitis diagnosis

The use of natural ingredients as medicinal ingredients by the people of Indonesia has been used for tens or even hundreds of years ago, especially in rural communities. Natural ingredients are often used because people believe they can cure several diseases, such as snail slime which is used for wound healing by farmers. A research says that the use of snail mucus can reduce pain in dental caries, where the material can eliminate tooth pain (Swastini, 2011). The healing of gingivitis as well as periodontitis has been carried out, with the result that in gingivitis it can reduce the grade of gingivitis, and in healing periodontitis with parameters of levels MDA, VEGF, TGF beta and the number of fibroblasts, proved to be curative, and this is still being done in mice (Swastini, 2019). Snail mucus is known to contain several substances that are very useful for healing diseases, including the protein achasin. This protein has antibacterial activity that works by inhibiting the formation of common parts of bacterial strains such as the peptidoglycan layer and cytoplasmic membrane. The work activity of achasin against Gram-positive bacteria is by attacking the cytoplasmic membrane and causing the cell wall to peel and sink into the cytoplasm (Berniyanti & Suwarno, 2010). The absolute requirement for the growth of this bacterium is iron. It is a Gram-negative bacterium in the form of an obligatory anaerobic, non-motile, asaccharolytic stem, which forms pigmented black colonies on a blood agar plate (How et al., 2016). Figure 2 shows a step how to collect the snail slime.

Figure 2: The snail and how to collect snail slime

Fusobacterium nucleatum is an obligate, gram-negative, rod-shaped anaerobic bacterium and included in the bacterioidaceae family. *Fusobacterium nucleatum* bacteria are frequently found in dental disease, especially with periodontitis disorders, and which produce tissue irritants such as butyric acid, proteases and cytokines (Scannapieco & Dongari-Bagtzoglou, 2021). Supragingival plaque on the surface of a tooth has about 109 bacterial cells attached to it. In a healthy periodontal pocket, there are 103 bacterial cells while in a deep periodontal pocket there are about 108 bacterial cells. Biomolecular identification found about 500 types of microbes found in dental plaque (Newman et al., 2012). Dental plaque, which initiates calculus, is a complex bacterial ecosystem. Some of the bacteria that play a role in periodontal disease are gram-negative bacteria such as *Phorpyromonas gingivalis*, *Fusobacterium nucleatum*, *Aggregatobacter actinomycetemcomitans*, and gram-positive bacteria such as *Lactobacillus*, *Streptococcus, Actinomyces israelli* (Perry & Beemsterboer, 2007). *Fusobacterium* in supragingival and subgingival plaque although in small numbers. *Fusobacterium nucleatum* is one of the most abundant gram-negative bacilli colonizing the subgingival plaque and closely associated with periodontal disease. To culture these bacteria can be done on blood agar media under anaerobic conditions with colonies that are not bright, granular in shape, rhizoid edges, irregular in shape. *Fusobacterium* can remove sulfur from cysteine and methionine to produce hydrogen sulfide and methylmercaptan odors associated with halitosis (Samaranayake, 2012).

The purpose of this study was to determine the effectiveness of the antibacterial snail slime with concentrations of 12.5%, 25%, 50% and 100%, against the bacteria *Fusobacterium nucleatum*. Figure 3 shows the bacteria of *Fusobacterium nucleatum.*

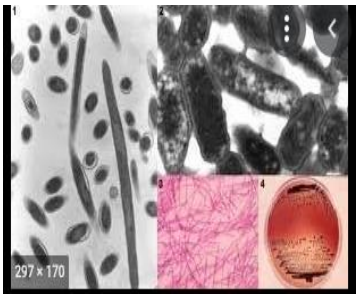


Figure 3: Fusobacterium nucleatum

2. Method

This research is purely a laboratory research with a post test control group design, and was conducted in the oral biology laboratory of the Faculty of Dentistry, Airlangga University, Surabaya, in June 2021.

2.1 Snail slime collection

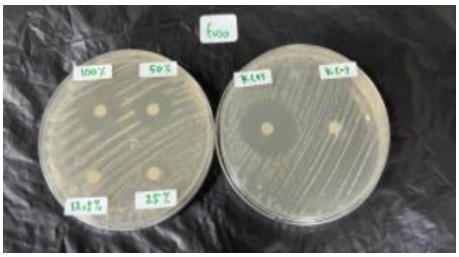
Snail slime was collected from a community plantation in Nyalian village, Banjarangkan, Klungkung, Bali. The weight of the snails used is in the range of 200-250 grams, then the mucus is taken using the tip of a needle to be scratched on the flesh of the snail, then the mucus is collected into a sterile bottle, followed by centrifugation for 30 minutes.

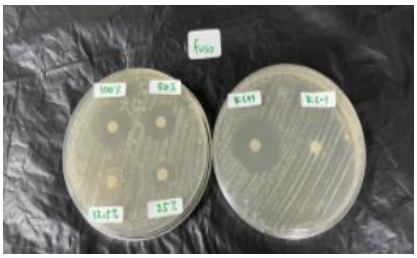
2.2 Planting the suspension of Fusobacterium nucleatum

Planting the stock of germs using sterile osse on 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 paperdisk 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. Bacteria stock taken from ATCC 25586 PK/5.

3. Results

In the present study, the results of the research on the inhibitory capability of mucous slime on the growth of Fusobacterium nucleatum bacteria growth can be seen in Figure 4, below:





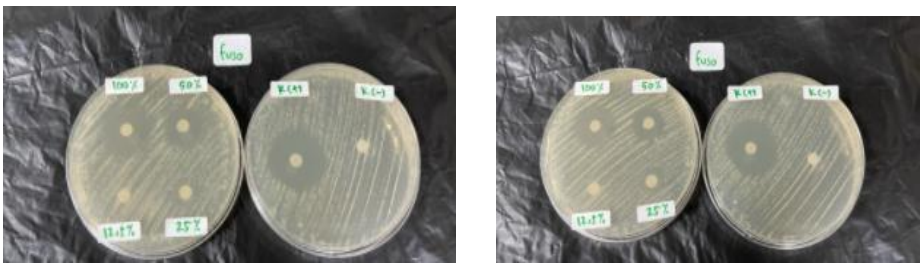


Figure 4: Inhibitory zone of snail slime against Fusobacterium nucleatum

Table 1: The width of Fusobacterium nucleatum inhibitory zone in the treatment group

Subject Group	N	Mean ± Fusobacterium nucleatum Inhibition Zone (millimeters)	Total bacteria (CFU/m l) (No)	P
Control	4	25.65±1,04	0.5 Mc Farland	0.001*
Snail slime 12.5%	4	0 ± 0.00		
Snail slime 25%	4	12.40 ± 0.2		
Snail slime 50%	4	16.70 ± 0.73		
Snail slime 100%	4	19.60 ± 0.78		

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

Table 1 illustrates that the inhibitory power of the control group is higher than the treatment group with snail slime, where in the control group the inhibitory power is 25.65±1,04 while the 100% concentration of snail slime is 19.60± 0.78. Meanwhile table 2 shows the number of difference of inhibitory zone of *Fusobacterium nucleatum* between the treatment groups.

Table 2: The difference of inhibitory zone Fusobacterium nucleatum between the treatment groups

Variable	Group I	Group J	Mean difference (I-J)	P
Snail Slime	Control	12.5%	25.20	1,00
		25%	12.40	<0.001*
		50%	9.00	<0.001*
		100%	6.00	<0.001*
	12.5%	25%	-12.40	<0.001*
		50%	-16.20	<0.001*
		100%	-19.20	<0.001*
		control	-25.20	<0.001*
	25%	50%	-3.80	<0.001*
		100%	-6.80	<0.001*
		control	-12.80	<0.001*
		12,5%	12.40	<0.001*
	50%	100%	-3.00	<0.001*
		control	-9.00	<0.001*
		12,5%	16.20	<0.001*
		25%	3.80	<0.001*
	100%	control	6.00	<0.001*
		12,5%	19.20	<0.001*
		25%	6.80	<0.001*
		50%	3.80	<0.001*

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

4. Discussion

The results shown that the research on the inhibition of snail mucus against the growth of *Fusobacterium nucleatum* bacteria can be seen in Figure 4. While the average inhibition of snail mucus against *Fusobacterium nucleatum* bacteria in this study was tested using the One Way Anova test. The results are shown in table 1. Zone diameter inhibition of snail mucus against *Fusobacterium nucleatum* bacteria has the highest concentration at 100% with an average diameter of 19.60 mm.

The results of the research that has been carried out showed that the diameter of the inhibition zone was the highest in snail slime with a concentration of 100% with an average of 19.60 mm in the strong category. According to Davis and Stout (1971) the diameter of the clear zone between 0±10mm has moderate inhibition, 11-20 mm is in the strong category, above 20 mm is very strong (Davis & Stout, 1971).

One of the soft animal species (mollusca) is snail (*Achatina fulica*). This animal was very much used by ancient farmers because the healing properties of wounds are very good. Snails produce mucus which has many health benefits. Snail mucus is produced in the body wall of the snail and is a lymph substance. Snail mucus that flows in the snail's body has the activity of eradicating bacteria and foreign objects. The components of snail mucus include analgesic, anti-septic, and antimicrobial peptides (Achasin). Achasin works by attacking or inhibiting the formation of common parts of bacterial strains such as: the peptidoglycan layer and the cytoplasmic membrane (Carranza et al., 2012).

Achasin is a protein contained in snail mucus, is a protein that has important biological functions other than those intended to prevent evaporation, assisting smooth movement is also needed to protect the body from mechanical injuries (Merglova et al., 2014). Bacteria that cause periodontitis are generally gram-negative bacterial species that colonize sub-gingival plaque, including *Porphyromynas gingivalis, Prevotella intermedia, Actinobacillus (agregibacter) actinomycetemcomitans* and *Fusobacterium nucleatum* (Merglova et al., 2014). One of the most dominant bacteria in periodontal disease is *Fusobacterium nucleatum* compared to other gram-negative bacteria. Fusobacterium nucleatum has a percentage of 55% while in Peptostreptococcus micros is 3%, Tannerella forsythia 4% and in Porphyromynas gingivalis 1% (Park et al., 2016).

Fusobacterium nucleatum is a gram-negative anaerobic bacteria that has a role in bridging the early and late colonies during plaque formation (Manson, 2013). These bacteria appear in high numbers 2 after 24 hours and can multiply for 48 hours in dental plaque. An increase in the number of *Fusobacterium nucleatum* can cause gingival inflammation, pocket deepening and periodontal tissue damage (Liu et al., 2014). *Fusobacterium nucleatum* is one of the most abundant gram-negative bacilli colonizing the subgingival plaque and closely associated with periodontal disease. This bacterium is often found in chronic gingivitis and chronic periodontitis because it plays a role in killing the normal proliferation of fibroblasts in the periodontal tissue. F. nucleatum may be an important contributor to periodontal disease either directly or by serving as a mediator of plaque colonization for other virulent anaerobes (Han et al., 2000).

The results of the data obtained were tested for normality and homogeneity as a condition for conducting the One Way ANOVA test, the Shapiro-Wilk test ($p>0.05$) which showed that all groups are normally distributed, and the Lavene test of all variances are homogeneous. The results of the One Way ANOVA test showed that the p-value <0.05 means that there are significant differences in the antibacterial power of various concentrations of snail mucus against *Fusobacterium nucleatum* bacteria in vitro. The difference in the results of the One Way ANOVA test was then carried out with the Post Hoc Least Significant Different (LSD) test to find out the significant differences between the treatment groups. The results showed that there were differences in the clear zone around the well which was dripped with snail mucus with various concentrations. All concentrations of snail mucus used showed significant differences in inhibition.

Antibacterial Effects of Various Concentrations of Natural Ingredients Snail Mucus (Achatina fulica) Against the Increase in the Number of Bacterial Inhibitions of Fusobacterium nucleatum that Cause Periodontitis In Vitro starting from a concentration of 25% indicating a strong diameter of inhibition zone and the highest inhibition is with a concentration of 100% snail mucus. The average inhibition in the control group was 25.65 mm, and the snail mucus group was 12.5%, 0 mm, 25%, 12.40 mm, 50%, 16.70 mm and 100% 19.60 mm. There is a significant difference in the diameter of the inhibitory power between the treatment groups. The conclusion of the antibacterial effect of natural ingredients of snail mucus from the 25% treatment showed strong criteria, and the greatest inhibitory power is the concentration of 100%.

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