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Efficacy of Aloe Vera Hydrogel in Inhibition the Growth of *Campylobacter rectus* and *Provetella intermedia* Bacteria

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ABSTRACT

Background: Campylobacter rectus and Provetella intermedia bacteria as the main etiology causes of periodontal disease. One of the locally synthesized antimicrobials that is widely used for the treatment of periodontitis is Chlorhexidine, however this drug has side effects when used continuously. To overcome this, an alternative periodontitis therapy with medicinal plants is needed. This study aims to determine the effectiveness of hydrogel Aloe vera antibacterial concentrations of 2.5%, 5%, 10% and 20% in the bacteria Campylobacter rectus and Provetella intermedia. **Methods:** This study was a laboratory experimental study with posttest only control group design in vitro. The samples were Campylobacter rectus and Provetella intermedia. The number of groups in this study was six. The sample size was determined by Federer's formula and obtained four replications for each group. Data collection by measuring the inhibition diameter using a slide caliper. Data were analyzed using oneway ANOVA and posthoc LSD. **Results:** The results showed that there was a difference in the diameter of the hydrogel Aloe vera inhibition with a concentration of 2.5%, 5%, 10%, 20% and a positive control for Campylobacter rectus and Provetella intermedia bacteria. **Conclusion:** This study concluded that there is an antibacterial effectiveness of hydrogel Aloe vera in concentrations of 2.5%, 5%, 10% and 20% on Campylobacter rectus and Provetella intermedia bacteria.

1. Introduction

Periodontal disease is a common oral health problem after caries [1,2]. According to the 2017 Global Burden of Disease Study, this disease is the 11th most common disease, affecting nearly 20% - 50% of the global population and will increase as humans grow older [3,4]. Chronic periodontitis most commonly occurs in adults over 30 years of age, characterized by complex and progressive chronic inflammation (5-9). Campylobacter rectus and Provetella intermedia are two bacterial pathogens which are the main etiologies of periodontal disease [10-14].

The basis of periodontal treatment is eliminating bacterial growth with antibacterials [7]. Antibacterial

drugs are administered in various dosage forms, including hydrogel [15-17]. This preparation is suitable for administration of antibacterial drugs because of its high hydrophilicity, unique three-dimensional tissue and cell adhesion [18]. Currently, hydrogels with antibacterial function are the main focus of biomedical research [19].

A locally synthesized antimicrobial that is widely used in treating periodontitis is Chlorhexidine [20]. From the research results of Binshabaib et al (2020), chlorhexidine 0.2% effectively decreased the growth of P. gingivalis bacteria [21-22]. However, daily use of chlorhexidine has side effects [23]. To overcome this,

alternative therapy with medicinal plants is needed [24].

Aloe vera is a medicinal plant that comes from the Liliaceae family. Since ancient times, Aloe vera has been used as anti-inflammatory, antimicrobial, and immune boosting [25]. Based on the results of research by Gharibi et al (2015), aloe vera extract has antibacterial activity against *S.aureus*, MRSA, *P. aeruginosa* and *K. pneumoniae* bacteria [26]. Aloe vera contains several bioactive components that have antibacterial properties, namely anthraquinones [25]. The most anthraquinone content is found in the latex or exudate layer which is brownish yellow between the skin and the meat of aloe vera [27]).

Many sophisticated antibacterial hydrogels have been developed, but researchers still have not found studies on the effectiveness of the 20% concentration of aloe vera hydrogel antibacterial against *Campylobacter rectus* and *Provetella intermedia* bacteria. From the above thought, the researcher was interested in conducting research on "The Antibacterial Effectiveness of Hydrogel Aloe Vera on *Campylobacter rectus* and *Provetella intermedia* Bacteria".

2. Method

This type of research is an experimental laboratory with posttest only control group design. The research sample is the bacteria *Campylobacter rectus* and *Provetella intermedia*. This study consisted of 5 groups, namely hydrogel aloe vera 2.5%, 5%, 10%, 20%, and negative control (hydrogel). The study sample size was determined according to Federer's formula and obtained replications of 5 times for each group. Aloe vera used in this study was taken purposively and has been identified in USU's Herbarium Medenesse. The process of making aloe vera extract by maceration method uses 70% ethanol. In this study, the main basic ingredient for the manufacture of hydrogel preparations is na-alginate mixed with other supporting ingredients to obtain 10 ml of aloe vera hydrogel for each concentration. Then, the hydrogel aloe vera antibacterial activity test was performed against *Campylobacter rectus* and *Provetella intermedia* by diffusion. The clear zone formed around

the media in order to measure its diameter using a sliding caliper. The collected data were then analyzed by using the oneway ANOVA and posthoc LSD statistical tests.

3. Results

Based on the results of the study, the mean diameter of hydrogel aloe vera inhibition was 2.5%, 5%, 10% and 20% in *Campylobacter rectus* bacteria were 8.21 ± 0.35 mm, 10.97 ± 0.26 mm, 13.00 ± 0.25 mm, and 16.35 ± 0.42 mm, whereas there were no obstacles in the negative control.

Based on the results of the study, the mean diameter of hydrogel aloe vera inhibition concentration of 2.5%, 5%, 10% and 20% in *Provetella intermedia* bacteria was 8.54 ± 0.18 mm, 10.48 ± 0.43 mm, 12.17 ± 0.65 mm, and 16.87 ± 0.42 mm, whereas there were no obstacles in the negative control.

Before testing the effectiveness of hydrogel aloe vera antibacterial on *Campylobacter rectus* and *Provetella intermedia* bacteria, first all data were carried out for normality with the Shapiro Wilik statistical test.

Based on the results of the normality test, the value of $p > 0.05$ was obtained, which means that all data were normally distributed. Furthermore, data analysis with oneway Anova and posthoc LSD. to determine the antibacterial effectiveness of hydrogel aloe vera 2,5%, 5%, 10% and 20% in the bacteria *Campylobacter rectus* and *Provetella intermedia*.

Based on the results of the oneway Anova statistical test on *Campylobacter rectus* and *Provetella intermedia* bacteria, each obtained p value = 0.00, which means that there is a significant difference in inhibition diameter between hydrogel Aloe vera 2.5%, 5%, 10%, and 20%. From these results it can be stated that there is an antibacterial effectiveness of hydrogel Aloe vera 2.5%, 5%, 10% and 20% on *Campylobacter rectus* and *Provetella intermedia* bacteria.

The next statistical test is LSD which aims to see the difference in effectiveness between the two treatment groups. Based on the results of the LSD posthoc statistical test, it can be seen that all tests obtained p value = 0.00, which means that there is a

significant difference in the diameter of antibacterial inhibition between the two treatment groups in the

bacteria *Campylobacter rectus* and *Provetella intermedia*.

Table 1 Average diameter of hydrogel aloe vera antibacterial inhibition 2.5%, 5%, 10% and 20% in *Campylobacter rectus* bacteria

Group	Inhibitor Diameter (mm)					Mean	SD
	1	2	3	4	5		
I	16.40	15.95	16.20	17.05	16.15	16.35	0.42
II	12.80	12.95	13.40	13.05	12.80	13.00	0.25
III	11.40	10.95	10.80	10.95	10.75	10.97	0.26
IV	8.00	8.15	8.40	7.80	8.20	8.21	0.35
V	-	-	-	-	-	-	-

Remarks: (-) there are no obstacles

Group I : Hydrogel Aloe vera 20%

Group II : Hydrogel Aloe vera 10%

Group III : Hydrogel Aloe vera 5%

Group IV : Hydrogel Aloe vera 2.5%

Group V : Negative control (hydrogel)

Table 2 Average diameter of hydrogel aloe vera antibacterial inhibition 2.5%, 5%, 10% and 20% in *Provetella intermedia* bacteria

Group	Inhibitor Diameter (mm)					Mean	SD
	1	2	3	4	5		
I	17.20	16.80	16.40	17.40	16.55	16.87	0.42
II	12.40	12.80	11.40	11.55	12.60	12.17	0.65
III	10.60	10.95	10.05	10.00	10.80	10.48	0.43
IV	8.60	8.40	8.80	8.55	8.35	8.54	0.18
V	-	-	-	-	-	-	-

Remarks: (-) there are no obstacles

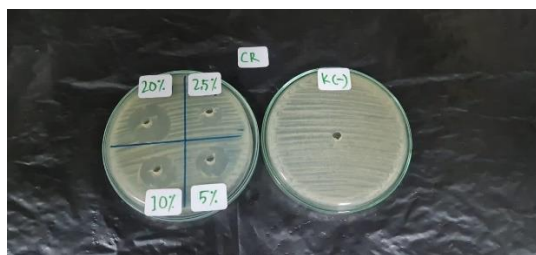
Group I : Hydrogel Aloe vera 20%

Group II : Hydrogel Aloe vera 10%

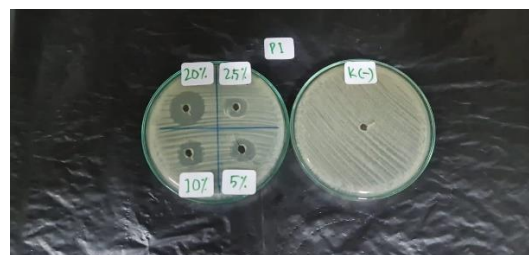
Group III : Hydrogel Aloe vera 5%

Group IV : Hydrogel Aloe vera 2.5%

Group V : Negative control (hydrogel)



(a)



(b)

Figure 1 Inhibition Diameter of the Treatment Group on Disc Discs
(a) *Campylobacter rectus*, (b) *Provetella intermedia*

Table 3 Oneway Anova Test Results

Group	<i>Campylobacter rectus</i>			<i>Provetella intermedia</i>		
	Average	SD	p value	Average	SD	p value
Hydrogel Aloe vera 20%	16.35	0.42	0.00	16.87	0.42	0.00
Hydrogel Aloe vera 10%	13.00	0.25		12.17	0.65	
Hydrogel Aloe vera 5%	10.97	0.26		10.48	0.43	
Hydrogel Aloe vera 2.5%	8.21	0.35		8.54	0.18	

Table 4 Posthoc LSD Test Results

Group	<i>Campylobacter rectus</i>	<i>Provetella intermedia</i>
	p value	p value
I – II	0.00	0.00
I – III	0.00	0.00
I – IV	0.00	0.00
II – III	0.00	0.00
II – IV	0.00	0.00
III – IV	0.00	0.00

4. Discussion

This study aims to determine the effectiveness of Aloe vera hydrogel in inhibiting the growth of *Campylobacter rectus* and *Provetella intermedia* bacteria. The concentrations of Aloe vera hydrogel tested were 2.5%, 5%, 10%, and 20%. The hydrogel in this study served as a control group. Each of these materials is dropped into agar media that has been cultured by bacteria. This action was incubated for 1 day and the treatment was replicated 5 times. The antibacterial effectiveness of Aloe vera hydrogel was evaluated by the inhibition zone diameter on agar media and measured by shear calipers.

Based on the research results, it was found that the largest inhibition diameter in inhibiting the growth of *Campylobacter rectus* bacteria was seen in the 20% Aloe vera hydrogel group with a mean of 16.35 ± 0.42 mm, while the smallest inhibition diameter was 2.5% of the Aloe vera hydrogel, namely 8.21 ± 0.35 mm. In *Provetella intermedia* bacteria, the results also showed that the largest diameter of inhibition was found in 20% Aloe vera hydrogel and 2.5% Aloe vera hydrogel. From these results it can be stated that the increasing concentration of Aloe vera hydrogel, the diameter size of the inhibition zone also increases [28].

In this study, it has been proven that the Aloe vera hydrogel has a significant effectiveness in inhibiting the growth of *Campylobacter rectus* and *Provetella intermedia* bacteria. The antibacterial effectiveness of Aloe vera hydrogel against *Campylobacter rectus* and *Provetella intermedia* was also proven from the oneway ANOVA results ($p < 0.05$). Susanto & Girsang's research (2020) shows results that are in line with this study that 5% and 10% Aloe vera hydrogel has significant inhibitory power against *Fusobacterium nucleatum* bacteria with an average inhibitory power in the 5% Aloe vera hydrogel group 13.00 ± 0.224 mm and hydrogel. Aloe vera 10% was 18.00 ± 0.261 mm [28].

The results of the research by Jain et al (2016) also showed that Aloe vera extract gel had antibacterial activity against *A. actinomycetemcomitans*, *C. bacili*, *S. mutans*, and *S. aureus*. The antibacterial effect of Aloe vera gel is 100% with an average inhibitory power of 6.9 mm against *A. actinomycetemcomitans*, 6.3 mm against *C. bacili*, 6.8 mm against *S. mutans* and 6.6 mm against *S. aureus* [25]. Supported by the research results of Adzitey et al (2019) that there is antibacterial effectiveness of Aloe vera extract gel against *Escherichia coli* and *Salmonella enterica* bacteria. Aloe vera extract gel can be used prophylactically to reduce

bacterial infections, especially *Escherichia coli* and *Salmonella enteric* bacteria [29].

Campylobacter rectus and *Provetella intermedia* bacteria are anaerobic gram-negative bacteria that cause periodontal disease. Jain et al (2016) stated that Aloe vera gel is effective in fighting infections, whether it is caused by gram-positive or gram-negative bacteria. The results of this study indicated that Aloe vera hydrogel was more effective against *Provetella intermedia* bacteria compared to *Campylobacter rectus* as evidenced by a larger inhibitory diameter [25].

The results of the hydrogel inhibition test of Aloe vera i 2.5%, 5%, 10% and 20% against *Campylobacter rectus* and *Provetella intermedia* bacteria showed a clear zone on NA media in the shape of a circle around the disc paper. The clear zone indicates the antibacterial effectiveness of each treatment material. Based on the research of Davis and Stout, the diameter of inhibition formed in the diffusion test measuring <5 mm has weak resistance, 5-10 mm is moderate, 10-19 mm is strong and 20 mm or more is very strong [28]. From this statement, hydrogel Aloe vera concentrations of 5%, 10%, and 20% have a strong antibacterial effect. This is in line with the research of Susanto & Girsang (2020) that the 5% and 10% Aloe vera hydrogels have a strong antibacterial effect.

The antibacterial effectiveness of the Aloe vera hydrogel against *Campylobacter rectus* and *Provetella intermedia* bacteria is probably due to the active compounds in it, especially Aloin and Aloemodin (antroquinone) which inhibit protein synthesis by bacterial cells [25 These results are also in accordance with the research of Yang et al (2018) that hydrogels with the addition of several antimicrobial agents are more effective against periodontal pathogenic bacteria which are anaerobic and the release of these antimicrobial agents is more controlled [18].

This study has proven that Aloe vera hydrogel has antibacterial effectiveness on *Campylobacter rectus* and *Provetella intermedia*. So, this Aloe vera hydrogel can be used as an alternative antibacterial agent to prevent and treat several oral infectious diseases.

5. Conclusion

This study resulted in several conclusions, including the mean diameter of hydrogel aloe vera inhibition concentration of 2.5%, 5%, 10% and 20% in *Campylobacter rectus* bacteria was 16.06 ± 0.19 mm, 8.21 ± 0.35 mm, 10.97 ± 0.26 mm, 13.00 ± 0.25 mm, and 16.35 ± 0.42 mm. The mean diameter of hydrogel aloe vera inhibition concentration of 2.5%, 5%, 10% and 20% in *Provetella intermedia* bacteria was 8.54 ± 0.18 mm, 10.48 ± 0.43 mm, 12.17 ± 0.65 mm, and 16.87 ± 0.42 mm. There is an antibacterial effectiveness of hydrogel Aloe vera concentrations of 2.5%, 5%, 10% and 20% on *Campylobacter rectus* and *Provetella intermedia* bacteria.

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