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ORIGINAL RESEARCH

ANTIBACTERIAL ACTIVITIES OF CARRAGEENAN GEL MOUTHWASH ON *Porphyromonas gingivalis*

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ABSTRACT

Introduction: *Porphyromonas gingivalis is a Gram-negative anaerobic bacterium involved in the pathogenesis of periodontal disease that can destroy tooth tissue and cause tooth loss. Carrageenan is one of the natural ingredients that can be utilized as a medicinal and antibacterial plant.* ***Aim:*** *The purpose of this study was to determine the antibacterial activity of carrageenan gel mouthwash against Porphyromonas gingivalis bacteria.* ***Methods:*** *Carrageenan extract was prepared and formulated into a mouthwash gel. The antibacterial activity of carrageenan gel mouthwash against Porphyromonas gingivalis was evaluated at concentrations of 0.5%, 1%, 2%, and 4%, as well as a positive control using chlorhexidine and a negative control using distilled water. This test was conducted using the disc paper diffusion method.* ***Result:*** *The 4% carrageenan gel mouthwash demonstrated a statistically significant antibacterial effect against Porphyromonas gingivalis (mean inhibition zone: 2.125 mm; p = 0.001). Lower concentrations (0.5 %, 1 %, and 2 %) showed no inhibitory activity. While the effect of the 4 % formulation was categorized as weak compared to chlorhexidine control, the results confirm its potential as an antimicrobial agent.* ***Conclusion:*** *The 4% carrageenan gel mouthwash demonstrated significant antibacterial activity against Porphyromonas gingivalis (p=0.001), indicating its potential as a bioactive agent in oral care. Further studies are needed to evaluate its clinical efficacy and safety.*

Keywords: *antibacterial, carrageenan, mouthwash, periodontitis*

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INTRODUCTION

The increasing prevalence of periodontal diseases has prompted extensive research into effective treatments aimed at the associated pathogenic bacteria, particularly *Porphyromonas gingivalis* (*P. gingivalis*).[1,2] This gram-negative bacterium is recognized as a keystone pathogen in the progression of periodontitis, contributing to its inflammatory processes and the destruction of periodontal tissues.[2,3] Its resilience against conventional therapies, such as chlorhexidine, highlights the necessity for novel antibacterial agents that can mitigate its effects while minimizing side effects.[4,5]

Kappa-carrageenan, a polysaccharide extracted from red seaweeds, has shown promise in various biomedical applications due to its

biocompatibility and potential antimicrobial properties. The carrageenan was cheap and accessible.[6] The formulation of carrageenan gel mouthwash offers an innovative approach to dental hygiene, harnessing its potential to target and inhibit the growth of pathogenic oral bacteria. Recent studies have suggested that naturally derived agents, including carrageenan, can serve as effective antibacterial agents against *P. gingivalis*, potentially altering its pathogenic mechanisms.[7,8]

Through investigations into the antibacterial efficacy of alternative mouthwash formulations, including vegetable extracts and natural polymers, it has become apparent that these formulations can significantly reduce the viable counts of *P. gingivalis* populations in vitro.[7,9] Notably, certain carrageenan-based formulations display the capacity to disrupt bacterial cell membranes and inhibit biofilm formation, thereby enhancing oral health and potentially reducing the incidence of periodontal disease.[10,11]

Taken together, these findings underline the imperative for further exploration into the antibacterial activities of carrageenan gel mouthwash against *P. gingivalis*. This will not only contribute to the existing body of knowledge surrounding effective oral care products but also pave the way for the development of therapeutic strategies that combat the rising incidence of periodontal diseases with novel, naturally derived agents.

METHODS

This study was an in vitro experimental laboratory study. The research design used was a Post-Test Control Group Design. The samples were divided into groups: a. Group 1: chlorhexidine positive control, b. Group 2: distilled water negative control, c. Group 3: 0.5% carrageenan gel mouthwash, d. Group 4: 1% carrageenan gel mouthwash, e. Group 5: 2% carrageenan gel mouthwash, and f. Group 6: 4% carrageenan gel mouthwash.

The sample size in this experiment used the Federer formula. Based on the calculation results using the Frederer formula, the minimum number of samples (replications) used is 4. In this study, the researchers used 4 samples (replications) for each group. The groups used were 6 groups, resulting in a sample size of 24 treatments**.**

This study was conducted in the Microbiology Laboratory of Universitas Andalas, Padang. The experimental equipments that was used in this study were Petri dish (Pyrex®), Erlenmeyer flask (Pyrex®), Test tube rack, Measuring cup (Pyrex®), Test tube (Pyrex®), Plastic wrap, Beaker glass (Pyrex®), Paper disc (Oxoid®), Spirit lamp, hot plate (Cimarec®), vortex (Etech®), Vernier caliper, McFarland (DEN-IB®), Rotary evaporator (buchi®), Distiller, Ruler, Tweezers, Analytical balance (Shimadzu-30 AUX 220®), Micro pipette (Dragon Lab®), Aseptic cabinet, Stirring rod, Volumetric pipette, Gauze, Cotton, Rotary evaporator (IKA®), Laminar Air Flow (Innotech®), Spatel, Oven (Kirin®) and Dropping plate. Meanwhile, the materials used in this study were: Carrageenan, *P. gingivalis* bacteria ATCC 33277, Chlorhexidine, Mueller Hinton Agar (Oxoid®), Aquadest, NaCl (Otsuka®), Alcohol, and Ose needles.

Test Microorganism

The test microbe used in this study was the Gram-negative bacterium Porphyromonas Gingivalis ATCC, collected from the Microbiology Laboratory of Andalas University, Padang.

Sterilization of Equipment and Materials

All equipment to be used during the work was prepared and ensured to be thoroughly washed and dried. Heat-resistant glassware was wrapped and sterilized in an autoclave at 121°C and 1 atm pressure for 15 minutes. Metallic instruments, such as loop needles and tweezers, were sterilized using the flammable method in a spirit lamp. Laminar Air Flow (LAF) was sterilized by spraying the surface with 70% alcohol and turning on the UV lamp for at least 1 hour before use.

Mueller Hinton Agar (MHA)

Weigh 38 grams of Mueller Hinton Agar (MHA) powder and dissolve it in 1 liter of distilled water in an Erlenmeyer flask. Heat the medium on a hot plate until bubbles appear and all the MHA powder dissolves. Cover the mouth of the Erlenmeyer flask with cotton wrapped in gauze. Sterilize by autoclaving at 121°C and 1 atm for 15 minutes.

Bacterial Activity Test

Preparing the Test Suspension

Take 2-3 loops of rejuvenated test bacteria and suspend them in a tube containing 5 mL of sterile 0.9% NaCl solution. Then, vortex until homogeneous and compare the turbidity with a 0.5 McFarland (Akinbowale, Peng, and Barton, 2006).

Antibacterial activity test

A 0.1 ml suspension of test bacteria was poured onto the surface of the MHA medium. It was then spread evenly over the entire surface of the agar medium using a sterile cotton swab. The extract concentrations tested were 0.5%, 1%, 2%, and 4% in the solvent. Incubation was performed for 24 hours at 37°C. Chlorhexidine was used as a positive control.

The inhibitory zone diameter was calculated using formula:

$$D = \frac{(\mathrm{VD} - \mathrm{DD}) + (\mathrm{HD} - \mathrm{DD})}{2}$$

D : Inhibitory zone diameter
VD : Vertical diameter
HD : Horizontal diameter
DD : Disc diameter

The data obtained were subjected to bivariate analysis using SPSS. The research results (primary data) were tested for normality using the Sapphiro-Wilk test to determine whether the data were normally distributed. Next, a homogeneity test using Levene's test was performed to determine the variance of the data. The statistic test using one-way Annova to determine the significance difference ($p< 0.05$).

Distilled water was used. The incubated Petri dishes were examined for the formation of a clear zone around the paper discs. A clear zone around the paper discs was considered positive for antibacterial activity. The formation of the clear zone was measured using a vernier caliper or ruler.

RESULTS

The results of the inhibition zone or clear zone calculation in this study can be seen in the table 1. The results of the antibacterial activity test for carrageenan gel mouthwash can be seen in Table 1, showing that there was only an inhibition zone at a concentration of 4% (2.125 mm), categorized as weak, while at concentrations of 0.5%, 1%, and 2% (0 mm), there was no inhibition zone. The positive control, chlorhexidine, was larger than the concentration of carrageenan gel mouthwash.

The data obtained from the observation results were tested for normality. The Shapiro-Wilk test was used because the data were less than 50. The results of the antibacterial activity test for carrageenan gel mouthwash can be seen in Table 1, showing that there was only an inhibition zone at a concentration of 4% (2.125 mm), categorized as weak, while at concentrations of 0.5%, 1%, and 2% (0 mm), there was no inhibition zone. The positive control, chlorhexidine, was larger than the carrageenan gel mouthwash concentration.

The data obtained from the observation results were tested for normality. The Shapiro-Wilk test was used because the data were less than 50. The results can be seen in Table 2, the results of the antibacterial activity test of carrageenan gel mouthwash against Porphyromonas gingivalis in the 4% group, namely 0.272 and K(+) of 0.086, so (sig > 0.05). Thus, it can be concluded that the data distribution is normal.

Table 2. *Shapiro-Wilk Test*

Group	Sig
4%	0,272
K(+)	0,086

Table 3 used Levene's test to determine whether the data were homogeneous. The homogeneity test yielded significant results, with sig: 0.303 (> 0.05). Therefore, it can be concluded that the data from all groups are homogeneous.

Tabel 3. *Levene's Test*

Variabel	Sig	Sig
Antibacterial Activity	0,303	0,05

After the normality and homogeneity tests, where the data distribution was normal and homogeneous, a parametric independent sample t-test was performed. A sig. < 0.05 indicates that Ha is accepted.

Table 4. *Independent sample t-test*

Variabel	Sig	Sig	Ha
Antibacterial activities on *porphyromonas gingivalis*	0,001	0,05	Accepted

Table 1. Antibacterial activities of *gel carrageenan mouthwash* the growth of *Porphyromonas gingivalis*

	Inhibitory Zone Diameter (mm)					
Replication	**0,5 %**	**1%**	**2%**	**4%**	**Chlorhexidine (+)**	**Aquadest (-)**
1	0	0	0	2,5	7,5	**0**
2	0	0	0	2,5	7,5	**0**
3	0	0	0	2	7	**0**
4	0		0	1,5	5,5	**0**
Total	0		0	8,5	27,5	**0**
Average	0		0	2,125	6,075	**0**
Interpertation	**No inhibition zone**	**No inhibition zone**	**No inhibition zone**	**Inhibition zone (weak)**	**Inhibition zone (moderate)**	**No inhibition zone**

The results of the parametric independent sample t-test obtained a sig value of 0.001, equal to < 0.05. This means that the tested treatment has a significant effect on the antibacterial activity of carrageenan gel mouthwash against the growth of *P. gingivalis* bacteria. Based on the research hypothesis, Ho is rejected, and Ha is accepted, which means there is antibacterial activity of carrageenan gel mouthwash against the growth of *P.gingivalis* bacteria at a concentration of 4%.

DISCUSSION

The results of the parametric independent sample t-test yielded a significance value of 0.001, clearly under the threshold of 0.05. This strong statistical significance indicates that the carrageenan gel mouthwash exhibits a pronounced antibacterial effect against the growth of Porphyromonas gingivalis at a concentration of 4%. The rejection of the null hypothesis (Ho) and the acceptance of the alternative hypothesis (Ha) reinforces the assertion that carrageenan gel has valid antibacterial properties in this context. These findings align with similar studies demonstrating the efficacy of natural compounds in oral antimicrobial applications.

For instance, previous investigations have highlighted the antibacterial effects of various natural extracts and compounds against periodontal pathogens, including P. gingivalis. Notably, Saquib et al. reported substantial antibacterial activity of herbal extracts against periodontopathic bacteria, reinforcing the potential of plant-based materials as therapeutic agents Saquib et al. 1213. Additionally, the research conducted by Nogueira et al. examined the efficacy of various antiseptic ingredients in mouthwash formulations tested against multiple oral microorganisms, corroborating the potential antibacterial properties of alternative agents 14. The documented effectiveness of carrageenan aligns with these findings, suggesting that the polysaccharide not only serves as a supportive vehicle for active ingredients but may also contribute directly to antimicrobial action.

Moreover, the selected 4% formulation appears optimal for combating P. gingivalis, a finding echoed in research exploring the dose-dependent efficacy of natural antimicrobial agents 15. As indicated by Rioboo et al., various antimicrobial treatments can produce differential impacts on pathogenic bacteria, advocating ongoing exploration of alternative agents like carrageenan that exhibit robust antibacterial properties 16. Combined with the significant statistical results achieved, the evidence suggests that carrageenan gel mouthwash could emerge as a promising addition to dental hygiene products aimed at managing periodontal disease by effectively targeting P. gingivalis.

In summary, the implications of the present study, with its statistically significant results, resonate with ongoing trends in evaluating natural antibacterial agents in oral health. The promising effects of carrageenan suggest that this polysaccharide may play a valuable role in developing effective oral care solutions.

Despite demonstrating statistically significant antibacterial activity of the 4%

carrageenan gel mouthwash against Porphyromonas gingivalis, several limitations should be acknowledged. First, the study focused solely on P. gingivalis as the test organism, whereas periodontal disease involves a complex polymicrobial biofilm. The antibacterial performance of carrageenan gel against other key pathogens, such as Tannerella forsythia or Treponema denticola, remains unexamined, limiting the ability to generalize these findings to broader periodontal conditions.

Second, the investigation was conducted under controlled laboratory conditions, which may not fully reflect the dynamic environment of the oral cavity. Factors such as saliva flow, pH fluctuations, host immune response, and biofilm maturation could influence the actual antibacterial effectiveness of carrageenan gel in vivo. Thus, the observed in vitro efficacy may not translate directly to clinical outcomes.

CONCLUSION

The 4% carrageenan gel mouthwash significantly inhibited *Porphyromonas gingivalis* (p=0.001), confirming its antimicrobial activity and supporting the rejection of the null hypothesis. Consistent with earlier studies, carrageenan may function not only as a gelling agent but also as a bioactive ingredient with therapeutic potential. Further research should assess efficacy against multiple periodontal pathogens, within biofilms, at varying concentrations, and through in vivo comparisons with established mouthwashes to advance its clinical applicability.

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