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Antibacterial Effectiveness of Mackerel (*Rastrelliger sp*) Fish Oil Emulgel against *Staphylococcus aureus* Bacteria using Well-Diffusion Method

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Abstract

Introduction: The oral cavity is one of the entry points for a wide variety of bacteria. Most of these bacterial species are normal flora. These bacteria can turn into pathogens in poor oral health. One of the common normal flora bacteria that cause infections in the oral cavity is *Staphylococcus aureus*. **Purpose:** To determine the effectiveness of mackerel oil emulgel (*Rastrelliger sp*) in inhibiting *Staphylococcus aureus* bacteria. **Methods:** This research is an experimental study with a Post Test Control Design, with the well-diffusion method. The research sample used mackerel oil emulgel with various concentrations (10%, 5%, 2.5%, 1.25%, 0.62%, and 0.31%), *Clinium*® as positive control, and gel base as negative control. **Results:** The study showed that mackerel fish oil emulgel has effectiveness in inhibiting *Staphylococcus aureus* bacteria with the resulting inhibition diameter of 10% (16.95 mm), 5% (14.73), 2.5% (9.97 mm), 1.25% (8.01 mm), 0.62% (6.71 mm), and 0.31% (6.10 mm). **Conclusion**: Mackerel (*Rastrelliger sp*) oil emulgel has an antibacterial effect against *Staphylococcus aureus* bacteria.

Keywords: Antibacterial; *Rastrelliger sp; Staphylococcus aureus*

Introduction

The oral cavity is one of the entry points for a variety of bacteria. Most of the oral bacterial species are normal flora. These bacteria can turn into pathogens in certain circumstances, such as low oral hygiene. Caries is an infection due to bacteria attached to the teeth, which metabolize sugar to produce acid and will damage the enamel structure if not treated well. It damages the enamel, and tooth protection allows bacteria to enter the pulp, which will cause an abscess. One of the bacteria that commonly causes infections in the oral cavity is *Staphylococcus sp*. Azmi (2020) states that among the types of *Staphylococci*, *Staphylococcus aureus* is a common pathogen that causes infections in the oral cavity.[1] *Staphylococcus aureus* is a gram-positive bacterium that is round forming irregular colonies like grapes. *Staphylococcus aureus* has a characteristic infection, by destroying neutrophils through the release of leucocidin so that an abscess forms.[2]

Bacterial control is done with the use of antibiotics. One of the antibiotics often used in dentistry for oral infections is Clindamycin. However, the use of antibiotics in the long term has a negative impact. The negative impact is the increasing incidence of bacterial resistance to antibiotics.[3] Natural ingredients with low effects are needed due to the high rate of antibiotic

resistance.[4] Natural ingredients that can be used as an antibacterial is mackerel. Mackerel (*Rastrelliger sp*) is a type of marine fish with a nutritional content that is good for health. Marine organisms have a vital source of antimicrobial agents. The content of mackerel that plays an antibacterial role is omega-3. The two main polyunsaturated fatty acids of omega-3 are Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA). DHA and EPA have antibacterial effects against various gram-positive and gram-negative bacteria.[5,6] Inguglia L, et al. (2020) reported that salmon oil extract (*Salmo salar*), which specifically contains DHA and EPA components, has antibacterial activity against gram-positive bacteria.[7] Emulgel is a pharmaceutical formulation that combines two components (gel and emulsion). Emulgels show much stronger antibacterial properties compared to the effect of pure oil. Due to the small particle size, it increases the interaction between the emulgel and bacteria.[8]

Methods

This research is experimental research with a Post Test Control Design. The mackerel oil emulgel was formulated at the Dosage Form Technology Laboratory, Faculty of Pharmacy, Universitas Sumatera Utara, while sample testing was conducted at the Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara. This research was conducted from September to November 2024. The research samples used were *Staphylococcus aureus* bacterial isolates with ATCC. This study used eight treatment groups: emulgel with concentrations of 10%, 5%, 2.5%, 1.25%, 0.62%, 0.31%, *Clinium*® as a positive control, and gel base as a negative control. Positive and negative controls are used for comparison to evaluate the test results. Each group was tested 3 times to prevent bias.

The fish oil extraction process begins with preparing the raw materials. The weight of the fish used in the process was 10 kilograms. The utilized parts of the mackerel (*Rastrelliger sp*) include internal organs such as the liver, stomach, and intestines, while the head is removed. The fish is then thoroughly washed under running water. Next, the fish was steamed using a vacuum oven at 70°C for 30 minutes to remove pathogenic bacteria and rancidity. After steaming, the fish was manually squeezed using a cloth to filter the oil from the solid residue. Next, phase separation was performed on the fish oil using a separatory funnel, and warm water was added to assist the separation. The crude fish oil was then purified by heating it at 60°C for 30 minutes with 1% bentonite while stirring magnetically. The oil was centrifuged to separate the oil from the remaining bentonite. Then, the fish oil was stored in small glass bottles.

The next step involves formulating the fish oil into an emulgel preparation. Emulgel formulation begins with the preparation of the oil and water phases. To make emulgels with various concentrations, fish oil was weighed as much as 5, 2.5, 1.25, 0.625, and 0.156 (g) to obtain concentrations of 10%, 5%, 2.5%, 1.25%, 0.62%, and 0.31%. The emulgel formulation for each concentration and gel base for the negative control is presented in Table 1 below.

Table 1. Emulgel Formulation

	Formula						
Concentration	**10%**	**5%**	**2.5%**	**1.25%**	**0.62%**	**0.31%**	**C-**
Emulsions							
Fish Oil Extract (g)	5	2,5	1,25	0,625	0,312	0,156	-
Span 80 (g)	5	5	5	5	5	5	5
Liquid paraffin (g)	7	7	7	7	7	7	7
Tween 80 (g)	5	5	5	5	5	5	5
Gel							
Propylene glycol (g)	5	5	5	5	5	5	5
Carbopol 940 (g)	0,5	0,5	0,5	0,5	0,5	0,5	0,5
TEA (ml)	1	1	1	1	1	1	1
HPMC (g)	1,5	1,5	1,5	1,5	1,5	1,5	1,5

The initial step involved the preparation of the emulsion. The oil phase was prepared by mixing mackerel fish oil with Span 80 in liquid paraffin, while the water phase was prepared by mixing Tween 80 with distilled water. Next, the gel base was prepared by mixing Carbopol 940 and HPMC until a gel-like consistency. Next, 1 mL of TEA was added to adjust the pH to 6-6.5, followed by continuous mixing until a uniform gel mass was obtained. The gel base and emulsion were mixed until an emulgel was formed, followed by the addition of propylene glycol and continued mixing until a homogeneous formulation was achieved. After emulgels with various concentrations were prepared, viscosity tests were carried out on each emulgel concentration. The viscosity value of a good emulgel preparation is 2000-4000 cPs. [9]

The antibacterial effectiveness test was performed using the well-diffusion method with Mueller-Hinton Agar (MHA) media. The well-diffusion method offers the advantage of facilitating accurate measurement of the inhibition zone, as the active compound diffuses not only across the surface of the nutrient agar but also penetrates its deeper layers. To make wells, a sterile cork borer with a diameter of 6 mm was used aseptically on agar. Petri dishes were divided into four sections, with each zone containing one well filled with various concentrations of mackerel fish oil emulgel, positive control (*Clinium*®), and negative control (Gel base). Petri

dishes were then covered with plastic wrap and incubated at 37°C for 24 hours. The experiment was repeated three times to prevent bias. After incubating for 24 hours, the zone of inhibition was observed and measured using a caliper.

Figure 1. Mackerel Fish Oil Emulgel with Various Concentrations

The data obtained from this research were analyzed using SPSS. A normality test was done using Shapiro-Wilk, and the homogeneity test using Levene's test.

Results

Emulgel formulations at concentrations of 10%, 5%, 2.5%, 1.25%, 0.62%, and 0.31% were tested for viscosity to determine the viscosity of the mackerel oil emulgel. The standard viscosity range for a good emulgel formulation is between 2000-4000 cPs.

Table 2. Viscosity Test of Mackerel (*Rastrelliger sp*) Fish Oil Emulgel

Concentration	Viscosity (cPs)
10%	2954,7 cPs
5%	2902,4 cPs
2.5%	2786,3 cPs
1.25%	2197,5 cPs
0.62%	2083,5 cPs
0.31%	2072,1 cPs

The results of the antibacterial activity test showed that the emulgel of mackerel fish oil (*Rastrelliger sp*) at concentrations of 10%, 5%, 2.5%, 1.25%, 0.62%, and 0.31%, as well as the positive control (*Clinium*®), exhibited antibacterial effectiveness against *Staphylococcus aureus* on Mueller Hinton Agar media, indicated by the formation of clear zones around the wells.

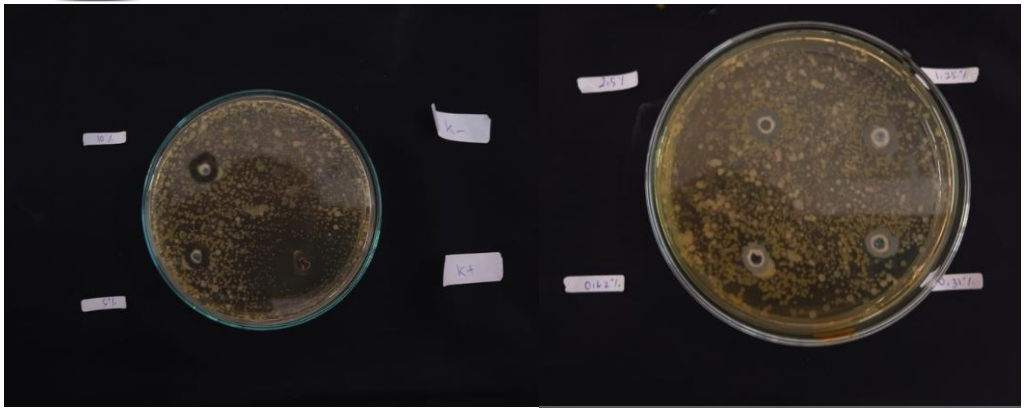

Figure 2. Inhibit Zones on Well-Diffusion Method Agar

Measurement of inhibition is based on the classification by Davis and Stout (1971), which consists of 4 groups of inhibition diameter: weak (<5 mm), moderate (5-10 mm), strong (10-20 mm), and very strong (>20 mm).[10]

Table 3. Inhibitory Diameter Value of Mackerel (*Rastrelliger sp*) Fish Oil Emulgel

Concentration	Repetition			Mean (mm)	Inhibition Strength
	1	2	3		
10%	17,61	16,63	16,63	16,95	Strong
5%	15,23	14,45	14,52	14,73	Strong
2,5%	9,32	10,3	10,31	9,97	Moderate
1,25%	7,32	8,31	8,42	8,01	Moderate
0,62%	7,11	6,72	6,30	6,71	Moderate
0,31%	6,02	6,25	6,04	6,10	Moderate
***Clinium*®**	26,00	27,91	26,11	26,67	Very Strong
Gel Base	0	0	0	0	Weak

The data on inhibition zone diameter were statistically analyzed using the SPSS software. Based on the results of the Shapiro-Wilk normality test, the data from all treatment groups were determined to be not normally distributed ($p < 0.05$), and the results of Levene's homogeneity test indicated that the data across all treatment groups were not homogeneous ($p < 0.05$). Therefore, data analysis was conducted using non-parametric methods, specifically the Kruskal-Wallis test, followed by the Mann-Whitney test for further comparison.

Based on the Kruskal-Wallis test, a significant difference in the mean inhibition zone diameters among all groups was observed ($p = 0.002$; $p < 0.05$). Therefore, the null hypothesis (H_0) was rejected, indicating that the mackerel (*Rastrelliger sp*) fish oil emulgel exhibited

effectiveness against the growth of *Staphylococcus aureus*. Subsequently, a non-parametric Mann-Whitney test was performed to assess the differences in inhibition zone diameters among the treatment groups. The results illustrated in Table 4 showed p-values ≤ 0.05 for each group comparison, indicating that each concentration of mackerel (*Rastrelliger sp*) fish oil emulgel exhibited a significant inhibitory effect on the growth of *Staphylococcus aureus*, except for the negative control group (gel base), which showed no significant effect.

Table 4. Results of Mann-Whitney tests

	Mackerel Fish Oil Emulgel							
	10%	**5%**	**2,5%**	**1,25%**	**0,62%**	**0,31%**	**C+**	**C-**
10%		0,046*	0,046*	0,046*	0,046*	0,046*	0,046*	0,034*
5%			0,050*	0,050*	0,050*	0,050*	0,050*	0,037*
2,5%				0,050*	0,050*	0,050*	0,050*	0,037*
1,25%					0,050*	0,050*	0,050*	0,037*
0,62%						0,050*	0,050*	0,037*
0,31%							0,050*	0,037*
C+								0,037*
C-								

*significant

Discussion

Marine organisms offer numerous benefits and can serve as a significant source of antimicrobial agents. Marine organisms contain omega-3 fatty acids, which are rich in health benefits. Research by Sun et al. (2016) stated that omega-3 exhibits antibacterial activity through several mechanisms.[5] Mackerel (*Rastrelliger sp*) is still primarily utilized as a food source and has not yet been fully optimized for pharmaceutical or medicinal applications. Mackerel (*Rastrelliger sp*) is a marine organism that contains a high level of omega-3 fatty acids. The two main polyunsaturated fatty acids in omega-3 are Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA). Both DHA and EPA exhibit antibacterial effects against a range of gram-positive and gram-negative bacteria. *Staphylococcus aureus* is a gram-positive bacteria that often cause infections in humans and is commonly found in the oral cavity.

This study confirms that mackerel (*Rastrelliger sp*) fish oil emulgel, which is rich in omega-3 fatty acids, exhibits antibacterial activity against *Staphylococcus aureus*. This finding

is supported by research conducted by Kim et al (2018), which reported that omega-3 extracted from herring fish oil inhibited the growth of *Staphylococcus aureus*.[11] Handayani et al. (2023) also reported that the extract from catfish (*Pangasius sp*), which contains omega-3, exhibits antibacterial activity.[12]

Unsaturated fatty acids are known to have an inhibitory effect on bacteria because they are easily absorbed into the outer cell membrane of bacteria.[13] DHA and EPA, exhibits antibacterial activity through several mechanisms. These include targeting both the inner and outer bacterial cell membranes, disrupting the electron transport chain, uncoupling oxidative phosphorylation, inducing cell lysis, inhibiting enzyme activity, reducing nutrient uptake, and promoting peroxidation and auto-oxidation.[5] EPA compounds are effective in rapidly killing gram-positive pathogenic species, which occurs through the process of cell lysis. This compound can also inhibit and kill *Staphylococcus aureus* bacteria.[14] DHA has been shown in various studies to disrupt bacterial cell membrane function. This compound affects the integrity of the bacterial plasma membrane, leading to cellular damage and death. The antibacterial activity of DHA is known to be more potent against gram-positive bacteria.[15]

Emulgel is a pharmaceutical formulation that combines two components, gel and emulsion, with the aim of enhancing the absorption of active ingredients that cannot be effectively delivered by gel systems alone, while also providing a level of comfort not achievable through emulsions. Emulgel also exhibits stronger antibacterial activity compared to pure oil. This is attributed to its small particle size and large surface area, which enhance the interaction between the emulgel and bacteria.[16]

The well-diffusion method has the advantage that it is easier to measure the area of the inhibition zone formed because the active isolate is not only limited to the top surface of the nutrient agar, but also spreads into the layer below. The results of the study can be concluded that mackerel fish oil emulgel with a concentration of 10%, 5% is included in the strong category, in comparison at a concentration of 2.5%, 1.25%, 0.62%, and 0.31% is included in the moderate category. Furthermore, the effectiveness of the positive control (*Clinium*®) belongs to the very strong category, and the negative control (gel base) belongs to the weak category. Each repetition at each concentration exhibited varying inhibition zones. The quality and thickness of the agar medium can influence the diffusion rate of the active compound. Thicker agar tends to hinder the diffusion process, while thinner agar allows for

faster diffusion, and the absorption time of the medium may also affect the diffusion process. Inconsistencies in absorption time could lead to variations in the resulting inhibition zones.[17]

Conclusion

Mackerel (*Rastrelliger sp*) oil emulgel showed antibacterial effectiveness against *Staphylococcus aureus* at concentrations of 10%, 5%, 2.5%, 1.25%, 0.62%, and 0.31%. Despite the antibacterial effect, the mean inhibition zone diameters at all tested emulgel concentrations were still lower compared to the positive control, *Clinium*®.

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