Dentika Dental Journal
Journal homepage: https://talenta.usu.ac.id/dentika

Antibacterial Effectiveness of Mackerel (Rastrelliger sp) Fish Oil Emulgel against Staphylococcus aureus Bacteria Using Disk-Diffusion Method.

Isnandar[1], Olivia Avriyanti Hanafiah [1], Indra Basar Siregar[1], Salsabila[2]
[1]*Department of of Dental Surgery Dentistry and Maxillofacial , Faculty of Dentistry, Universitas Sumatera Utara, Medan, 20155 Indonesia*
[2]*Faculty of Dentistry, Universitas Sumatera Utara, Medan, 20155 Indonesia*
Corresponding Author: isnandar@usu.ac.id

ARTICLE INFO

Article history:
Received 24 July 2025
Revised 13 November 2025
Accepted 11 December 2025
Available online December 2025

E-ISSN: 2615-854X
P-ISSN: 1693-671X

How to cite:
Isnandar, Hanafiah OA, Siregar IB, Salsabila. Antibacterial Effectiveness of Mackerel (Rastrelliger sp) Fish Oil Emulgel against Staphylococcus aureus Bacteria Using Disk-Diffusion Method.. Dentika Dental Journal 2025; 28(2): 113-117

http://doi.org/10.32734/dentika.v28i2.22238

ABSTRACT

Oral health is a major concern in Indonesia, which is caused by bacteria infections in oral cavity.[1] Bacteria such as *Staphylococcus* play a crucial role in the development of dental diseases, showing the need for improving oral hygiene to prevent infections.[2] Therefore, this research aimed to evaluate the effectiveness of mackerel fish oil (*Rastrelliger sp*.) emulgel against *Staphylococcus aureus* using the disk diffusion method in an *in vitro.* The investigation was carried out using an experimental laboratory design with a post-test only control group through the Kirby-Bauer disk diffusion method. The samples consisted of various concentrations of mackerel fish oil emulgel (10, 5, 2.5, 1.25, 0.62, and 0.31%). *Clinium® gel 1%* was used as a positive control, while gel base was served as a negative control. The formation of inhibition zone diameter after 24 hours of incubation was measured in millimeters (mm) using a vernier caliper. The results showed that mackerel fish oil (*Rastrelliger sp*.) emulgel had antibacterial activity against *Staphylococcus aureus*. The mean inhibition zone diameters observed at concentrations 10, 5, 2.5, 1.25, 0.62, and 0.31% were 16.99 mm, 13.23 mm, 10.32 mm, 7.18 mm, and 6.23 mm. Mackerel fish oil (*Rastrelliger sp*.) emulgel showed effective antibacterial activity against *Staphylococcus aureus* in an in vitro setting.

Keywords: *Rastrelliger Sp*, Mackerel Fish, *Staphylococcus Aureus,* Antibacterial

ABSTRAK

Kesehatan rongga mulut masyarakat Indonesia masih dipermasalahkan hingga saat ini. Permasalahan kesehatan rongga mulut paling umum diakibatkan oleh infeksi bakteri pada rongga mulut.[1] Bakteri seperti *Staphylococus aureus* ikut berperan dalam menyebabkan masalah gigi dan mulut. Oleh sebab itu, diperlukan peningkatan perilaku pemeliharaan kesehatan gigi dan mulut untuk menjadi lebih baik.[2] Oleh karena itu, tujuan penelitian ini adalah untuk mengetahui efektivitas pemberian emulgel minyak ikan kembung *(Rastrelliger sp*) terhadap bakteri *Staphylococcus aureus* menggunakan metode difusi cakram secara in vitro. Jenis penelitian ini merupakan eksperimental laboratorium rancangan *post-test only control design*, menggunakan metode difusi cakram *Kirby-Bauer*. Sampel penelitian ini terdiri dari berbagai konsentrasi emulgel minyak ikan kembung (10, 5, 2.5, 1.25, 0.62, dan 0.31%). *Clinium®* gel 1 % sebagai kontrol positif dan basis gel sebagai kelompok kontrol negatif. Zona bening yang yang terbentuk setelah 24 jam inkubasi diukur dalam satuan milimeter(mm), dengan menggunakan jangka sorong. Hasil penelitian bahwa Emulgel minyak ikan kembung (*Rastrelliger sp*.) menunjukkan adanya aktivitas antibakteri terhadap *Staphylococcus aureus* dengan diameter zona hambat pada konsentrasi 10, 5, 2.5, 1.25, 0.62, and 0.31% sebesar 16.99 mm, 13.23 mm, 10.32 mm, 7.18 mm, and 6.23 mm. Emulgel minyak ikan kembung (*Rastrelliger sp*.) efektif sebagai antibakteri terhadap *Staphylococcus aureus* secara in vitro.

Kata kunci: *Rastrelliger Sp*, Ikan Makerel, *Staphylococcus Aureus,* Antibakteri

1. Introduction

Oral health is a major concern in Indonesia, which is caused by bacteria infections in oral cavity [1]. Based on data from Indonesian basic health research (Riskesdas) in 2018, dental problems, including caries, toothache, and tooth damage, contribute 45.3% of dental issues. Therefore, poor oral hygiene practices must be improved to prevent and manage these conditions [2].

Staphylococcus aureus is recognized as one of the primary bacteria in oral cavity, which is frequently found in oral environment, particularly in periodontal abscesses, showing the need for antibiotic therapy for treatment.[3] However, the inappropriate and prolonged use of antibiotics can lead to bacteria resistance through various mechanisms, including drug inactivation, enzymatic modifications, low drug accumulation in resistant cells, and metabolic pathway alterations [3,4]. Therefore, to limit *Staphylococcus aureus* resistance to antibiotics, natural antibacterial agents like mackerel fish oil emulgel can be considered.

Mackerel fish (*Rastrelliger sp*.) contains various bioactive compounds such as proteins, lipids, phosphorus, calcium, iron, vitamin A, and vitamin B, alongside omega-3 and omega-6 fatty acids with antimicrobial properties [5]. Le et al. (2017) further showed that eicosapentaenoic acid, as an omega-3 fatty acid, effectively eliminated gram-positive bacteria through rapid cell lysis and inhibited *Staphylococcus aureus* [6]. Therefore, this research aims to examine antibacterial properties of mackerel fish oil emulgel as a natural therapeutic agent inhibiting the growth of *Staphylococcus aureus*.

2. Materials and Methods

This research used laboratory experimental design with a post-test only control group and Kirby-Bauer disk diffusion method, which was fast, inexpensive, and easy to use [7]. The experiment was performed from September to November 2024 at the Microbiology Laboratory in the Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara. A total of 24 samples were tested across eight treatment groups, each replicated three times. The test samples consisted of mackerel fish oil emulgel at various concentrations (10, 5, 2.5, 1.25, 0.62, and 0.31), with *Clinium® gel 1%* and gel base serving as positive and negative control, respectively.

Mackerel fish oil was prepared by washing fish thoroughly, followed by cutting into several pieces, and heating in a vacuum oven at 70°C for 30 minutes. After heating, fish was manually wrapped in a cloth and pressed to extract oil. Purification was performed using a magnetic stirrer by heating the extracted oil at 60°C for 30 minutes and adding 1% bentonite. After changing color, oil was separated from coarse bentonite particles using a centrifuge. The purified mackerel fish oil was stored in small glass bottles for further formulation.

For emulgel formulation process, oil phase was made by mixing 12 mL of mackerel fish oil with Span 80 in liquid paraffin. The aqueous phase, prepared separately by mixing Tween 80 with distilled water, was heated to 70–75°C. Subsequently, the aqueous phase was added gradually to the oil phase under continuous stirring to form a stable emulsion. The gel base was prepared by mixing carbomer and *hydroxypropyl methylcellulose* (HPMC), and adjusted to pH 6-6.5 with 1 mL Triethanolamine (TEA), followed by continuous stirring. The emulsion was incorporated into the gel base to form the final emulgel formulation, with propylene glycol added to enhance homogeneity [8].

Antibacterial activity of mackerel fish oil emulgel was tested against *Staphylococcus aureus* ATCC 25923 using *Mueller-Hinton Agar* (MHA) as the culture medium. Sterile filter paper discs were impregnated with various concentrations at 10, 5, 2.5, 1.25, 0.62, and 0.31, alongside *Clinium®* gel 1% as the positive control and gel base as the negative control. The discs were carefully placed onto the inoculated agar using sterile forceps, ensuring full contact, followed by labeling and incubating for 24 hours at 37°C. Measurement of inhibition zone diameter was performed after incubation using a vernier caliper and analyzed with SPSS version 25. The data obtained were analyzed using One-Way Analysis of Variance (ANOVA) to evaluate, with a $p < 0.05$ showing statistically significant difference among treatments. This suggested that the treatments had a significant and effective influence on the tested variable.

3. Results

Inhibition zone test was performed by measuring the diameter of clear zone formed around the bacteria samples using a vernier caliper. The data obtained were analyzed statistically, and antibacterial activity was assessed using mackerel fish oil emulgel (0.31-10%) compared to *Clinium*® 1% and gel base. The viscosity of mackerel fish oil emulgel formulations was measured with Brookfield Viscometer to determine preparation consistency, with acceptable values ranging from 2,000–4,000 cPs [9].

Table 1. Viscosity test of mackerel fish emulgel (*Rastrelliger sp.*)

Concentrations of mackerel fish oil emulgel (*Rastrelliger sp.*)	Result
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

Table 2. Diameter of Inhibition Zone

No	Group	Antimicrobial Activity (mm)				
		U1	U2	U3	Mean ± SD	Sig.
1	Control+	26.2	22.91	22.11	23.74 ± 2.17	0.002*
2	Control-	0	0	0	0 ± 0	
3	10%	16.63	19.72	14.63	17 ± 2.56	
4	5%	13.25	13.35	13.11	13.24 ± 0.12	
5	2.50%	10.13	10.61	10.23	10.32 ± 0.25	
6	1.25%	8.12	9.02	8.31	8.48 ± 0.47	
7	0.62%	7.31	7.21	7.02	7.19 ± 0.15	
8	0.31%	6.21	6.45	6.04	6.23 ± 0.21	

* = statistically significant

Table 3. Comparison of Inhibition Zone

Group	Control +	Control -	10%	5%	2.50%	1.25%	0.62%	0,31%
Control+	-	0,012*	0,176	0,047*	0,035*	0,023*	0,024*	0,021*
Control-	-	-	0,033*	0,005*	0,001*	0,005*	0,001*	0,002*
10%	-	-	-	0,563	0,186	0,114	0,093	0,077
5%	-	-	-	-	0,053	0,009*	0,016*	0,010*
2.50%	-	-	-	-	-	0,056	0,002*	0,001*
1.25%	-	-	-	-	-	-	0,153	0,038*
0.62%	-	-	-	-	-	-	-	0,030*
0.31%	-	-	-	-	-	-	-	-

* = statistically significant

Inhibition zone diameter can indicate antibacterial response. Based on the classification by Davis and Stout (1971), the inhibition power criteria were categorized into four groups, namely weak (<5 mm), moderate (5-10 mm), strong (11-20 mm), and very strong (>20 mm) [10]. The results showed a p-value of 0.002 ($p < 0.05$), indicating a statistically significant difference in the mean number of *Staphylococcus aureus* colonies among all treatment groups. Therefore, the null hypothesis (H_0) was rejected, showing that mackerel fish oil had effectiveness against the growth of *Staphylococcus aureus*.

Mackerel fish oil emulgel showed the potential to inhibit the growth of *Staphylococcus aureus* at multiple levels. Moderate inhibition was observed at concentrations of 1.25, 0.62, and 0.31% with inhibition

zone diameter of 10, 5, and 2.5% with 16.99, 13.23, and 10.32 mm, respectively. Meanwhile, very strong inhibition was observed in the positive control group (*Clinium® Gel 1%*), which showed inhibition zone diameter of 23.74 mm.

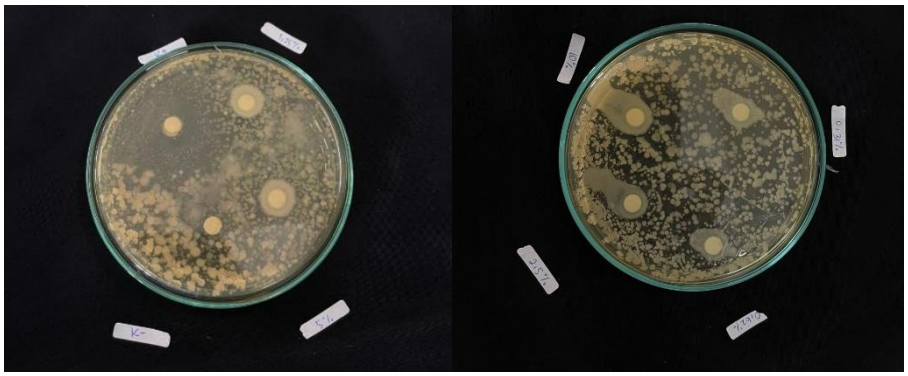

Figure 1. Observation of Inhibition Zones

4. Discussion

The mean inhibition zone diameter for mackerel fish oil emulgel at concentrations of 10, 5, 2.5, 1.25, 0.62, and 0.31% had values of 17±2.56, 13.24±0.12, 10.32±0.25, 8.48±0.47, 7.19±0.15, and 6.23±0.21 mm, respectively. The positive control (*Clinium® gel 1%*) produced an average inhibition zone diameter of 23.74±2.17 mm, while the negative control (gel base) did not produce any inhibition zone. The inhibitory activity test result of mackerel fish oil emulgel showed moderate to strong inhibition of *Staphylococcus aureus* growth, significant antibacterial activity compared to 0.31-10% (*p* value: 0.002).

Previous research has shown antibacterial activity of marine oil-based formulations against *Staphylococcus aureus*, supporting the present results. Kim et al. (2018) reported that herring oil and the omega-3 fatty acid components, particularly DHA and EPA, inhibited *Staphylococcus aureus* biofilm formation and exceeded the expression of virulence genes such as hla encoding α-hemolysin [11]. Similarly, Coraça-Huber et al. (2021) found that omega-3 polyunsaturated fatty acids showed significant antibacterial and anti-biofilm activity against multidrug-resistant *Staphylococcus aureus* strains isolated from prosthetic joint infections (PubMed). These results were similar to the concentration-dependent inhibition observed in this research. Therefore, antibacterial effect of Rastrelliger sp. oil emulgel was largely attributable to the omega-3 fatty acid content acting through membrane disruption and inhibition of bacterial adhesion [12].

Viscosity test of mackerel fish oil emulgel at concentrations of 10, 5, 2.5, 1.25, 0.62, and 0.31% was conducted using a digital viscometer to determine formulation consistency. Viscosity is a measure of a fluid's resistance to flow, indicating the degree of internal friction in the fluid. Increase in viscosity affects the difficulty for the fluid to flow and the movement of objects. Previous report observed that the optimal viscosity range for gel formulations suitable for application was 2000–4000 cPs (*centipoise*) [9]. In this research, viscosity of emulgel met the optimal viscosity range, with each concentration being more than 2000 cPs. The viscosity measurement for each emulgel concentration was performed at a rotational speed of 6 RPM, and the obtained results were recorded. Viscosity represents the resistance of a liquid to flow, and a higher value correlates with greater antibacterial activity. The highest viscosity was observed in the 10% mackerel fish oil emulgel formulation, which was 2954,7 cPs. In formulations with the same base composition, an increase in emulgel concentration produced higher viscosity [13].

Antibacterial effectiveness of mackerel fish oil is attributed to omega-3 fatty acid. Similarly, Dewi et al. (2023) reported that Pangasius sp. extract, a fish rich in omega-3 fatty acids, showed significant antibacterial activity [14]. Emulgel formulation effectively reduced the colony count of *Staphylococcus aureus* in a concentration-dependent manner. This was due to higher concentrations of emulgel, which contained greater amounts of active compounds, enhancing antibacterial efficacy [15].

Components of omega-3 fatty acids, such as *Icosapentaenoic acid* (EPA) and *Docosahexaenoic acid* (DHA), show antibacterial properties through several mechanisms. These include disrupting bacteria membrane by interrupting the electron transport chain, uncoupling oxidative phosphorylation, causing death

of cell by lysis, inhibiting enzymatic activity, reducing nutrient absorption, and inducing peroxidation and auto-oxidation [12]. Le et al. (2017) stated that EPA compounds were highly effective in rapidly eliminating Gram-positive bacteria through cell lysis. Additionally, EPA can inhibit and eliminate *Staphylococcus aureus* [6]. Homens et al. reported that DHA disrupted bacteria cell membrane function, leading to structural damage and cell death. Antibacterial activity of DHA is more potent against Gram-positive bacteria [16].

5. Conclusion

In conclusion, this research shows that mackerel fish oil emulgel has moderate to strong antibacterial effectiveness against *Staphylococcus aureus* in vitro. The 5% and 10% concentrations produce the largest inhibition zone diameter, indicating a concentration-dependent antibacterial effect. Compared to previous reports on herring or salmon oil, this research shows that mackerel fish oil possesses significant antibacterial potential when formulated as an emulgel. These results contribute to the growing understanding of marine-derived omega-3 fatty acids as natural antibacterial agents, showing the potential of mackerel fish oil emulgel as locally available alternative for future.

6. Acknowledgement

The authors gratefully acknowledge the staff of the Faculty of Pharmacy Technology Laboratory and the Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, for providing research facilities under the Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Universitas Sumatera Utara, Medan, Indonesia, 2025

References

Kristanti Y, Dessy D. Pengaruh ozonated water sebagai antiseptik dalam menghambat pertumbuhan staphilococcus aureus (in vitro). Maj Kedokt Gig Indones 2012; 19(1): 25–8.

Azzahirah M, Berliyanti D. Efektivitas ekstrak cengkeh terhadap bakteri permasalahan gigi. J Kesehat Tambusai 2024; 5(1): 1875–86.

Hartanto R, Khang V, Trinh T, Novelya N, Antibiotik WCEPEDMAP. Klindamisin dalam menghambat pertumbuhan bakteri staphylococcus aureus. J Prima Med Sains 2020; 2(1): 14–7.

Erikawati D, Santosaningsih D, Santoso S. Tingginya prevalensi MRSA pada isolat klinik periode 2010-2014 di RSUD Dr. Saiful Anwar Malang, Indonesia. J Kedokt Brawijaya 2010; 29(2): 149–56.

Sallamah E, Hendrawan. Y. Studi tentang asam lemak omega-3 dari bagian-bagian tubuh ikan kembung ikan laki-laki (rastrelliger kanagurta). J Pengolah Has Perikan Indones 2010; 7(2): 30–6.

Le PNT, Desbois AP. Antibacterial effect of eicosapentaenoic acid against bacillus cereus and staphylococcus aureus: Killing kinetics, selection for resistance, and potential cellular target. Mar Drugs 2017; 15(11).

Handayani R, Qamariah N, Mardova SA. The inhibitory test of ethanol extract saluang belum stem to escherichia coli. Borneo J Pharm 2018; 1(1): 16–8.

Rakhma DN, Najih YA, Pratiwi FE. Pengaruh rasio karbomer dan HPMC terhadap karakteristik dan stabilitas fisik emulgel minyak ikan salmon. J Pharmasci 2020; 5(2): 43–7.

Djuwarno EN, Hiola F, Isa I. Formulasi sediaan emulgel ekstrak daun kelor (moringa oleifera lam. dan uji aktivitas antioksidan menggunakan metode DPPh. Indones J Pharm Educ 2021; 1(1): 10–9.

Surjowardojo P, Susilorini TE, Sirait GRB. Daya hambat dekok kulit apel manalagi (malus sylvestrs mill.) terhadap pertumbuhan staphylococcus aureus dan pseudomonas sp. penyebab mastitis pada sapi perah. J Ternak Trop 2015; 16(2): 40–8.

Kim YG, Lee JH, Raorane CJ. Herring oil and omega fatty acids inhibit staphylococcus aureus biofilm formation and virulence. Front Microbiol 2018; 9.

Coraça-Huber DC, Steixner S, Wurm A, Nogler M. Antibacterial and anti-biofilm activity of omega-3 polyunsaturated fatty acids against periprosthetic joint infections-isolated multi-drug resistant strains. Biomedicines 2021; 9(4).

Zats L, Gregory KG, Liebermen H, Rieger M, Banker G. Pharmaceutical dosage forms: Disperse systems vol. 2. London: Marcel Dekker Inc, 1996: 400–403 p.

Suroso E, Utomo TP, Hidayati S, Nuraini A. Pengasapan ikan kembung menggunakan asap cair dari kayu karet hasil redestilasi. J Pengolah Has Perikan Indones 2018; 21(1): 42–53.

Safiti M, Anhar A, Putri D. Uji aktivitas antibakteri ekstrak etanol varietas kulit jeruk siam. Serambi Biol 2023; 8(1): 61–6.

Mil-Homens D, Bernardes N, Fialho AM. The antibacterial properties of docosahexaenoic omega-3 fatty acid against the cystic fibrosis multiresistant pathogen Burkholderia cenocepacia. FEMS Microbiol Lett. 2012; 328(1): 61–9.