



Bioactive Phytochemicals from *Memecylon edule*: Targeting Planktonic and Biofilm States of *Pseudomonas aeruginosa*

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Abstract

The rise in antimicrobial resistance has prompted the search for potent antimicrobial agents, with plants from unique environments, such as geothermal areas, offering potential due to their diverse phytochemical profiles. This study evaluated the antiplanktonic and antibiofilm activity of *M. edule* fractions from the geothermal area, Jaboi, Sabang. Crude extracts of *M. edule* were sequentially fractionated using hexane, ethyl acetate, and ethanol. The resulting fractions were further analyzed for their phytochemical content and antiplanktonic and antibiofilm activities. The ethyl acetate fraction demonstrated superior antiplanktonic and antibiofilm activities compared to other fractions, inhibiting 50% of *Pseudomonas aeruginosa* planktonic and biofilm formation at a concentration of 10 mg/mL. The most potent fraction exhibited the phenolic content, 672.84 mg GAE/g extract, surpassing the other fractions. The GC-MS analysis shows the presence of pyrogallol, hexadecanoic acid, cedran-diol, and sitosterol. These findings highlight the potential of the ethyl acetate fraction of *M. edule* as a source of bioactive compounds with promising antiplanktonic and antibiofilm properties, laying the groundwork for future research into its therapeutic applications against biofilm-associated infections.



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1. Introduction

Microbial resistance continues to pose challenges to the efficacy of infectious disease treatment in many developing countries [1, 2]. According to a study investigating antimicrobial-related deaths in 204 countries in 2019, mortality related to antimicrobial resistance reached 4.95 million [3]. This high mortality rate occurred due to the uncontrolled use of antimicrobials, accelerating mutations in pathogens [4], including *Pseudomonas aeruginosa*.

In 2024, the Centers for Disease Control and Prevention (CDC) reported that multidrug-resistant *P. aeruginosa* infections were one of the U.S. burden seven antimicrobial-resistant pathogens in healthcare settings. These resistant pathogens rose by 20% during the COVID-19 pandemic, with cases peaking in 2021 and remaining above pre-pandemic levels in 2022 [5]. A study in China covering the data from 2017 to 2021 shows that *P. aeruginosa* demonstrates significant resistance to potent antibiotics, including carbapenems, aztreonam, and



Figure 1. *M. edule* leaves.

cefepime, posing a serious challenge in treating infections. This resistance increases healthcare costs due to the need for expensive alternative therapies, prolonged hospital stays, and enhanced infection control measures [6].

P. aeruginosa is a primary pathogen responsible for nosocomial infections, including urinary and respiratory tract infections. Its surface adhesion is primarily mediated by adhesins, such as a single polar flagellum and type IV pili, which enhance surface motility and promote biofilm formation [7]. The pathogenic factors of *P. aeruginosa* include various proteases, toxins, lipopolysaccharides, pili, flagella, leukocidins, siderophores, urease, and membrane proteins [8]. The serious pathogenicity of *P. aeruginosa* indicates the need for effective therapies for infection control.

Herbal offers a promising solution to the issue of antimicrobial resistance, potentially providing effective phytochemicals for infectious diseases [9]. One species that has gained interest is *Memecylon edule* (Figure 1), a member of the *Melastomataceae*. *M. edule* has been confirmed to show antibacterial activity against bacteria causing digestive, skin, and other infections [10], antidiabetes [11], and anti-inflammatory [12]. The antibacterial activity of other species of *Memecylon* has been investigated by employing diffusion and dilution techniques, including *M. Malabaricum* [13–15], *M. embellatum* [16], *M. talbotanum* [17], and *M. heyneanum* [18]. Although the mechanisms of action of these plants remain not fully understood, most of the extracts exhibit activity against both Gram-positive and Gram-negative bacteria.

In Aceh, *M. edule* grows in the geothermal area of Jaboi, which is characterized by variations in water types, including chloride, sulfate, and bicarbonate-sulfate mixtures [19]. This area is also a source of plant biodiversity with the potential as an antimicrobial candidate. Studies suggest that the antimicrobial activity of plants in geothermal areas is potentially influenced by

their unique growth environments [20–22]. A geothermal area produces heat from the Earth's interior and is accessible at or near the surface. Plants in geothermal areas adapt to unique environmental conditions, potentially producing distinct phytochemicals. Studies reveal that these areas yield greater secondary metabolite diversity and higher concentrations than non-geothermal regions [23].

Previous studies have demonstrated that crude extracts of *Hydnophytum formicarum*, *Syzygium myrtifolium*, *Aporosa octandra*, *Memecylon coeruleum*, and *M. edule* from this region exhibit antiplanktonic activity against *Staphylococcus aureus* and *P. aeruginosa* with MIC₅₀ values ranging from 1.25 to 5 mg/mL. In the context of the antibiofilm activity, *M. edule* exhibited consistent antimicrobial activity, with planktonic minimum inhibition concentration 50% (PMIC₅₀) of 1.25 mg/mL and biofilm minimum inhibition concentration 50% (BMIC₅₀) of 5 mg/mL. This activity is relatively potent compared to the other four plants. The phytochemistry profile of *M. edule* shows the presence of alkaloid, phenolic, flavonoid, and terpenoid/steroid [24]. The findings highlight the promising potential of *M. edule* as a candidate therapeutic agent, particularly for antiplanktonic and antibiofilm applications.

This study evaluates the antiplanktonic and antibiofilm activities of fractionated extracts of *M. edule* and their phytochemical contents. Fractionation with a stepwise solvent extraction method helps to group compounds with similar solubility, with the ultimate goal of identifying active metabolites [25]. This method can potentially optimize yields, enhance efficiency, and reduce solvent consumption while enabling the targeted isolation of specific metabolites.

This study promotes a novel investigation into the antibiofilm and antiplanktonic activities of fractionated *M. edule*. To date, this is the first study that evaluates the bioactivity of *M. edule* sourced from a geothermal environment. This setting may produce different bioactive secondary metabolites due to unique environmental stressors. Furthermore, earlier studies identified compounds such as EGCG and myricetin in *M. edule*, but their specific active agents related to antibiofilm activity have not been investigated. This study result would provide a valuable foundation for identifying novel antibiofilm agents to solve antimicrobial resistance.

2. Materials and Methods

2.1. Bacteria

The bacterial strain, *Pseudomonas aeruginosa* ATCC 27853, was obtained from the Microbiology Laboratory of

Zainoel Abidin Regional General Hospital, Banda Aceh, Indonesia. The strain was cultured on Pseudomonas Cetrimide Agar (Oxoid, UK) with the incubation temperature of 37°C.

2.2. Plants

The leaves of *M. edule* were collected from the geothermal manifestation area of Jaboi Sabang JK4, located at coordinates 05°28'23.7"N, 095°39'49.4"E. The taxonomic identification was conducted at the Herbarium Bogoriense, Directorate of Scientific Collection Management, National Research and Innovation Agency (BRIN), Cibinong, and recorded under specimen number B-4237/II.6.2/01.05.07/11/22 by Mr. Wahyudi Santoso.

2.3. Samples Preparation

The dried leaves were powdered (2430 grams) and macerated with ethanol 96% (1:10, w:v) in a closed container for 72 hours with occasional stirring. The fresh solvent was used every 24 hours. The resulting mixture was filtered using filter paper (Whatman No 1). The filtrate was collected, and the solvent was removed by a rotary evaporator (Buchi, Indonesia), resulting in crude extract (528.625 grams). The crude extract was successively partitioned with hexane, ethyl acetate, and ethanol in a separatory funnel. The three filtrates were concentrated using a rotary evaporator, resulting in a hexane fraction (2.36 grams), ethyl acetate fraction (12.12 grams), and ethanol fraction (313.66 grams). The three fractions, hexane (Me1), ethyl acetate (Me2), and ethanol (Me3), were stored in a refrigerator at 4°C for further experiments.

2.4. Gas Chromatography-Mass Spectroscopy Analysis

Gas chromatography-mass spectrometry (GC-MS) analysis of Me1, Me2, and Me3 was carried out in TRACE 1300 GC with an ISQ 7000 MS detector (Thermo Fisher Scientific, USA) equipped with a TG-5MS capillary column as previously described [26]. The GC operated with a separate flow rate of 50 mL/min and a cleaning flow rate of 5 mL/min. The gas reduced the flow to 1 mL/min during idle periods. The inlet temperature was set to 250°C. The column used was a TG-5MS column (30 m length, 0.250 mm inner diameter, 0.25 µm film thickness). The carrier gas flowed at a constant rate of 1.0 mL/min. The temperature program began with equilibrium at 40°C. A single injection was performed using Injector A with a 10 µL syringe and a sample volume of 1 µL.

2.5. Determination of Total Phenolic Content

The total phenolic content was determined following the procedure outlined in the previous study [27]. The

experiment used the Folin-Ciocalteu reagent, and absorbance was measured at 765 nm. The experiments were conducted in triplicate, and absorbance was measured using a UV-Vis spectrophotometer (Shimadzu UVmini-1240, Kyoto, Japan).

2.6. Antiplanktonic Activity

Antiplanktonic activity was evaluated following the procedure described in the previous study [24]. *P. aeruginosa* was cultured on Pseudomonas Cetrimide Agar (PCA) (Oxoid, UK) at 37°C. A 0.9% NaCl suspension was prepared, and turbidity was measured at 625 nm (absorbance 0.08–0.13), corresponding to 10⁸ CFU/mL. The test samples were dissolved in 5% DMSO and diluted with Mueller Hinton Broth (Himedia, India) using a serial dilution method (1:2) to prepare three test concentrations (10, 5, and 2.5 mg/mL). To each test sample, 100 µL of bacterial inoculum was added. Growth control contained the inoculum in the media, while an antibiotic control contained gentamicin 1% (Sagestam®, Sanbe, Indonesia) and inoculum in media. The mixtures' optical density (OD) was measured before and after incubation using a microplate reader (Azure Biosystems, Dublin, USA) at 625 nm. Bacterial growth inhibition was calculated using a formula from the study mentioned before.

2.7. Antibiofilm Activity

Test samples were prepared following the same procedure as described for antiplanktonic measurements. After incubation, planktonic cells were removed, and the remaining wells's content was washed with phosphate-buffered saline (PBS, Oxoid, UK). To visualize the biofilm formed, 1% crystal violet (Merck, Darmstadt, Germany) was added and incubated for 15 minutes at room temperature. The unbound crystal violet was rinsed off with PBS. Biofilm was fixed using 100 µL of 95% ethanol, followed by a spectrophotometer at 625 nm absorbance measurement. Biofilm inhibition was assessed by comparing the absorbance of the growth control with that of the test samples using a formula from the study mentioned before [24].

2.8. Statistical Analysis

The mean values of the treatment groups were compared using a two-way analysis of variance (ANOVA) followed by an LSD test. The analysis used SPSS software version 20.0 (SPSS Inc., Chicago, USA). A *p*-value of ≤ 0.05 was considered to indicate statistically significant differences.

Table 1. Tentative compounds identified in the hexane, ethyl acetate, and ethanol fractions of *M. edule* with a similarity index >90%.

No.	Retention Time (min)	Compounds	Class of Secondary Metabolite	Relative Area (%)		
				Me1	Me2	Me3
1	4,181	Formamide, N-methoxy-	Amide	-	-	1.09
2	20,122	1,2,3-Benzenetriol	Phenol	-	8.34	31.1
3	24,679	1-Hexadecanol	Fatty alcohols	3.08	-	-
4	28,454	Melezitose	Trisaccharide	-	-	2.17
5	29,087	E-15-Heptadecenal	Fatty aldehyde	3.84	-	-
6	31,771	Pentadecanoic acid, 14-methyl-, methyl ester	Fatty ester	-	2.65	4.93
7	31,804	Hexadecanoic acid, methyl ester	Fatty ester	7,86	5,95	-
8	32,509	n-Hexadecanoic acid	Fatty acid	-	-	19.69
9	32,6	Tetrahydropyran 12-tetradecyn-1-ol ether	Ether derivates	1.43	-	-
10	33,053	Cyclopropanetetradecanoic acid, 2-octyl-, methyl ester	Fatty ester	-	-	1.02
11	33,08	1-Heneicosanol	Fatty alcohols	3.41	-	-
12	35,046	11-Octadecenoic acid, methyl ester	Fatty ester	-	-	3.53
13	35,09	Trans-13-Octadecenoic acid, methyl ester	Fatty ester	3.94	-	-
14	35,097	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	Fatty ester	-	6.28	1.36
15	35,324	Phytol	Diterpenoid acyclic	3.35	-	-
16	35,529	2-Hexadecenoic acid, methyl ester, (E)-	Fatty ester	-	-	1.03
17	35,549	Methyl stearate	Fatty ester	1.2	-	-
18	35,784	Z-8-Methyl-9-tetradecenoic acid	Fatty acid	-	-	4.55
19	36,73	Heptacos-1-ene	Alkane	2.81	-	-
20	40,07	n-Tetracosanol-1	Alcohol	1.89	-	-
21	42,222	5-Ethyl-5-methyl-2-phenyl-2-oxazoline	Oksazoline	-	1.06	-
22	43,171	Nonacos-1-ene	Alkane	1.28	-	-
23	46,599	Squalene	Triterpenoid	21.56	-	-
24	50,147	Stigmasta-3,5-diene	phytosterol	-	-	9.5
26	52,953	β-Sitosterol	phytosterol	4.51	2.4	5.01
27	54,103	Cedran-diol, (8S,14)-	Terpenoid	-	1.06	-
28	54.126	Cucurbitacin b, 25-desacetoxy-	Terpen tetracyclic	-	-	1.26
29	54,224	Lupeol	Triterpenoid pentacyclic	14.74	-	-

3. Results and Discussion

The phytochemistry analysis was conducted to identify the compounds contributing to the activities. Fractions were obtained through sequential extraction with hexane, ethyl acetate, and ethanol, which were selected for their polarities to extract secondary metabolites effectively. Lipophilic compounds, like terpenes, would be concentrated in the hexane fraction, while polar compounds, such as phenols, could be found in the ethyl acetate and ethanol fractions.

The total phenolic compounds in Me1, Me2, and Me3 are 43.21±21.38, 672.84±77.10, and 500±37.04 mg GAE/g extract. The ethyl acetate fraction contains the highest levels of phenols, followed by ethanol and hexane. The high phenol content in ethyl acetate is linked to the polarity of the compounds, as these semi-polar compounds are more easily extracted with ethyl acetate. Given their known antimicrobial properties [28], the high phenol content suggests that this fraction is a promising candidate for further biological activity testing.

Table 1 presents phytochemical compounds identified by GC-MS with a similarity index (SI) >90% from the three fractions, including their retention times and relative areas. The percentage of SI is crucial in GC-MS analysis

because it indicates the confidence level of the reference spectra in a database, the NIST library. This suggests a strong match between the experimental spectrum and the reference, increasing the reliability of compound identification. The relative area (%) provides a proportional estimate of each compound's abundance. It helps to assess the composition and sample comparison by identifying the most and least abundant components. It also shows the possible transformation during the assessment.

The compounds are classified into several classes: amide, fatty acids and esters, alkanes, alcohols, aldehydes, carbohydrates, phenols, sterols, and terpenes. While all of these compounds may work synergistically for antiplanktonic and antibiofilm activity, only a subset of them would benefit drug discovery due to their drug-like properties, such as the low molecular weight.

The antibacterial activity of fatty acids may stem from their ability to disrupt bacterial cell membranes, resulting in enhanced permeability and subsequent cell lysis. For example, saturated fatty acids such as lauric and myristic acids have been found to decrease the production of virulence factors, which play a key role in the

pathogenicity of *P. aeruginosa* [29]. Organic compounds such as alkanes, alcohols, aldehydes, and carbohydrates contribute significantly to the antibacterial activity of extracts through various mechanisms, including membrane disruption.

Phenols pose hydroxyl groups that form ionic bonds or hydrogen bonds with the target protein, resulting in target inactivation [30]. Terpenes form covalent bonds and change the target protein through electrophilic or nucleophilic substitution reactions. They also reduce the integrity of cell membranes and cause changes in ion concentration, leading to cell leakage and cell death [31].

Due to their lipophilic nature, squalene and lupeol were identified as prominent compounds in the hexane fraction. Lipophilic substances are highly soluble in non-polar solvents like hexane. Their hydrophobic characteristics cause them to be extracted well into the non-polar solvent, leading to their high concentration in the hexane fraction. Their role in inhibiting bacterial growth has been well-documented in various studies. Notably, squalene is reported as a major constituent in the ethyl acetate extract of *Excoecaria agallocha*, demonstrating antibacterial activity against *Staphylococcus aureus* [32]. Lupeol is identified in significant amounts in the hexane fraction of *Pulicaria crispa* leaves, which has been reported as the most active fraction for antibiofilm activity [33].

The phenolic compound, 1,2,3-Benzenetriol or pyrogallol, is the dominant compound in the ethyl acetate and ethanol fractions. Pyrogallol has been shown to inhibit biofilm formation in *P. aeruginosa*, possibly by disrupting quorum sensing [34]. It also inhibits biofilm in *Staphylococcus epidermidis* and *S. aureus*, which is supposed to be related to oxidative stress induction. This hypothesis is supported by its stronger effect in catalase-deficient strains and the restoration of biofilm formation by exogenous catalase, paralleling the dose-dependent action of hydrogen peroxide [35]. The three extracts also contain phytosterol, β -Sitosterol. Ethanol extract contains stigmasta-3,5-diene, phytosterol derivative. β -Sitosterol exhibited weak antibacterial activity against *P. aeruginosa* [36]. The study focusing solely on stigmasta-3,5-diene activity against *P. aeruginosa* is limited.

Antiplanktonic activity of *M. edule* fractions was assessed at concentrations of 2.5, 5, and 10 mg/mL following preliminary tests that determined PMIC₅₀ occurred at doses exceeding 2.5 mg/mL. The results of the antiplanktonic activity of *M. edule* fractions presented in Figure 2. The ethanol (Me3) and ethyl acetate (Me2) fractions exhibited antibacterial activity against *P. aeruginosa* planktonic cells, with PMIC a 2.5 mg/mL. In

contrast, the hexane fraction demonstrated the lowest inhibitory effect, with a PMIC of 10 mg/mL.

Within each fraction, varying concentrations resulted in statistically significant differences in planktonic inhibition ($p < 0.05$). Similarly, at identical concentrations, significant differences in inhibition were observed among the different fractions ($p < 0.05$). At a concentration of 10 mg/mL, the ethyl acetate fraction demonstrated 50% planktonic inhibition, a level of activity not achieved by the other two fractions. This finding highlights the contribution of phytochemicals as antibacterial agents. These findings are consistent with those of Srinivasan et al., who demonstrated that using ethyl acetate in the extraction process results in potent antibacterial activity against pathogenic bacteria [37].

The high phenolic content in the ethyl acetate fraction potentially contributes significantly to its superior antimicrobial activity against *P. aeruginosa*. Phenolic compounds are known for their antimicrobial properties, primarily due to their ability to interfere with microbial cell membranes and protein synthesis enzymes. In this case, the correlation between phenolic content and antiplanktonic efficacy suggests that higher concentrations of phenolic compounds enhance the fraction's ability to inhibit planktonic growth and biofilm formation.

The previous study identified several phenolic compounds in the ethyl acetate fraction of *M. edule*, including epigallocatechin gallate (EGCG) [12]. EGCG exhibits antiplanktonic (antibacterial) activity by inhibiting the function of membrane proteins, including penicillin-binding proteins [38] and disrupting protein synthesis [39].

Terpenoid compounds, including ursolic acid, contribute to the antiplanktonic activity, as shown in previous studies [37]. Ursolic acid can also be detected through GC-MS analysis [40]. However, GC-MS analysis in this study did not identify ursolic acid, which may be attributed to various factors.

An antibacterial candidate is categorized as bactericidal if its MBC value is no more than four times the MIC value [41]. In this study, a 10 mg/mL concentration, four times the PMIC, does not exhibit 100% inhibition. Nevertheless, the results provide a preliminary step toward identifying active compounds with inhibitory activity, which can serve as a foundation for further development.

The inhibition of biofilm formation by the hexane (Me1), ethyl acetate (Me2), and ethanol (Me3) fractions against *P. aeruginosa* is shown in Figure 3. The tested concentrations were 2.5, 5, and 10 mg/mL. In the Me2

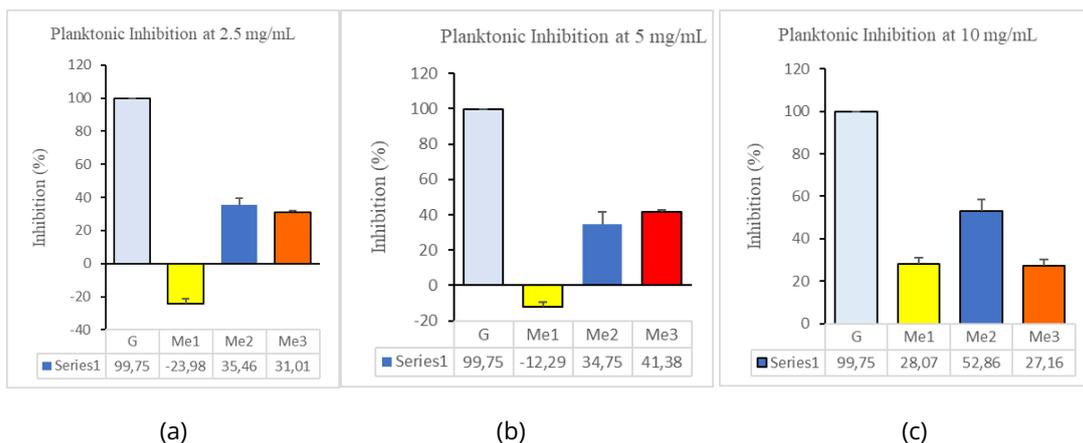


Figure 2. The percentage of planktonic inhibition by the hexane (Me1), ethyl acetate (Me2), and ethanol (Me3) fractions against *Pseudomonas aeruginosa* at concentrations of 2.5 mg/mL (a), 5 mg/mL (b), and 10 mg/mL (c).

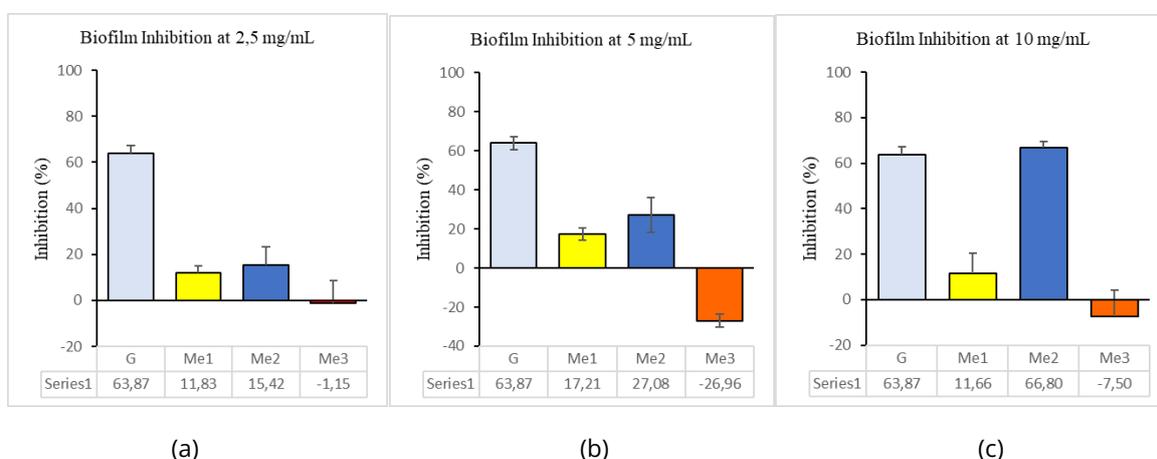


Figure 3. The percentage of biofilm inhibition by the hexane (Me1), ethyl acetate (Me2), and ethanol (Me3) fractions against *P. aeruginosa* at concentrations of 2.5 mg/mL (a), 5 mg/mL (b), and 10 mg/mL (c).

group, differences in concentration significantly affected antibiofilm activity ($p < 0.05$). The Me2 group exhibited a $BMIC_{50}$ of 10 mg/mL, making it the only treatment group to show inhibition exceeding 50% in the test bacteria. In the Me1 group, variations in dose did not result in significant differences in activity ($p > 0.05$). The Me3 group demonstrates no biofilm growth inhibition at the tested concentrations (2.5-10 mg/mL). Therefore, the ethyl acetate fraction displays the most potent antibiofilm activity.

In practical terms, Me2 could be a promising candidate for further development as an anti-biofilm agent, particularly against *P. aeruginosa*. It could be explored in medical devices, wound dressings, or adjunctive therapy in infections where biofilm formation complicates treatment. While it may not be as potent as Me2, Me3 could still be considered for applications where moderate antibiofilm activity is sufficient, or its active components could be further isolated and optimized. The absence of antibiofilm activity across tested concentrations suggests that Me3 is not a viable option for antibiofilm purposes at

these doses. However, this fraction might still possess other pharmacological properties worth exploring, such as antimicrobial or anti-inflammatory effects.

The more potent antibiofilm activity of the ethyl acetate fraction compared to the hexane and ethanol fractions is possibly associated with its phenolic content. Phenolic compounds reduce biofilm formation in *P. aeruginosa* [42]. These compounds can inhibit the attachment of planktonic cells to surfaces or disrupt the communication process within the biofilm complex, known as quorum sensing. In *P. aeruginosa*, quorum sensing is regulated by three systems: Las, Rhl, and Pqs. These systems control the production of autoinducers, which act as signaling molecules to trigger genes expressing bacterial virulence factors. Phytochemical compounds can interfere with these stages of quorum sensing [43].

The superior activity of Me2 fractions containing pyrogallol or cedran-diol may be attributed to their ability to form hydrogen bonds and their balanced hydrophobic-hydrophilic nature. Me1 fraction with phytol or lupeol might show weaker activity due to their limited

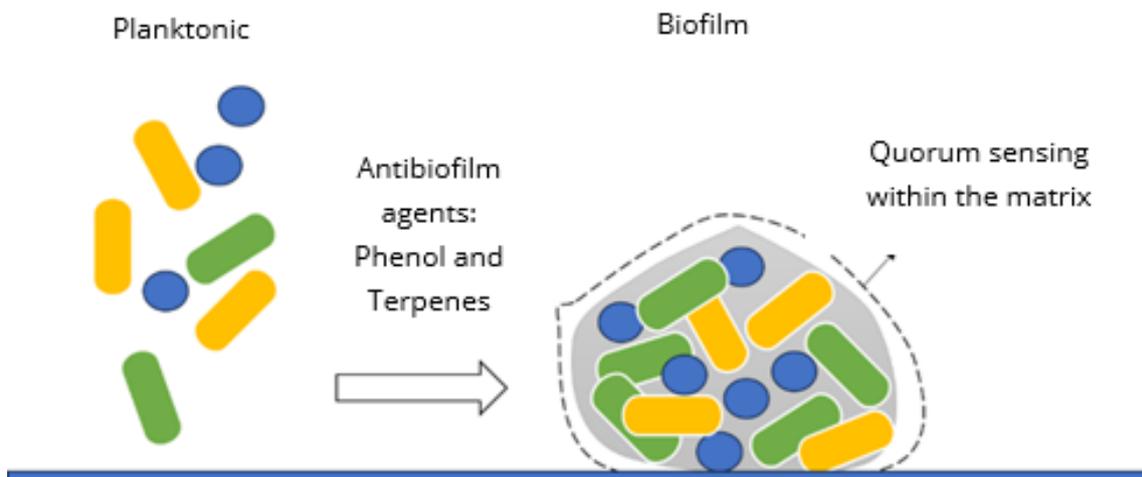


Figure 4. The proposed mechanism and active compounds in active fractions.

polar interactions and bulkier structures. The weaker antibiofilm activity of the ethanol fraction likely results from its reliance on pyrogallol and sterols, which are less effective in disrupting bacterial membranes and biofilm structures than the more balanced and diverse active compounds present in the ethyl acetate fraction. Both the weaker activity fractions contain more sterols than Me2.

To date, research evaluating the activity of *Memecylon* species extracts remains limited. The results of a study investigating the antibiofilm activity of *M. talbotianum* indicated that a concentration equal to the minimum inhibitory concentration (1xMIC) is required to prevent biofilm formation [17]. In this study, a concentration of 1x MIC also inhibits biofilm formation.

EGCG identified in *M. edule* in one study [12] exhibits antibiofilm effects by inhibiting quorum sensing in *P. aeruginosa* [44]. Ursolic acid, which has been reported as the active compound in ethyl acetate fraction [37], exerts its effects, including competing with substrates for enzymatic reactions involved in extracellular polysaccharide synthesis and directly affecting bacterial cell viability within the biofilm [45]. The active fraction in this study may contain phenols like EGCG and terpenoids like ursolic acid, which may work by the mechanism described above and summarized in Figure 4. However, these compounds were not detected in GC-MS in this study.

M. edule used in this study was obtained from a geothermal area, which is expected to be a potential source for identifying effective antibiofilm candidates. Further investigation into the mechanisms underlying the observed antibiofilm activity will provide valuable insights into the therapeutic potential of this plant. Comparative

analyses with the same species from non-geothermal regions could show the strength of plants from geothermal areas compared to non-geothermal areas. This will also identify the differences or common bioactive compounds, thereby advancing the understanding of the antimicrobial properties inherent to geothermal flora. These findings are a foundation for future pharmacological research and the development of natural antimicrobial agents specifically targeting biofilm-associated infections.

4. Conclusions

The *M. edule* fractions obtained from the geothermal area of Jaboi Sabang exhibited significant antiplanktonic and antibiofilm activities against *P. aeruginosa*, with the ethyl acetate fraction (Me2) showing the most potent antimicrobial effects compared to hexane and ethanol fractions. GC-MS analysis of Me2 revealed phenols, sitosterols, and terpenoids, which likely contribute to its bioactivity by inhibiting bacterial adhesion and interfering with quorum sensing. Me2 holds promise as an antibiofilm agent for medical applications, such as coating catheters, implants, and wound dressings, to prevent biofilm-related infections and enhance healing. Additionally, it could serve as an adjunctive therapy to improve the efficacy of antibiotics against biofilm-resistant *P. aeruginosa* in chronic infections. These findings highlight the potential of plant-derived compounds in addressing antimicrobial resistance (AMR) and developing biofilm-targeting therapies. Future research should focus on isolating and characterizing active compounds, investigating their mechanisms of action, including quorum sensing disruption and biofilm matrix interference, and conducting in vivo studies to validate their therapeutic potential and safety for clinical use.

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