



Utilizing geothermal botanical resources: Evaluating antiplanktonic and biofilm inhibitory effects of Jaboi area plant extracts

[Aprovechamiento de los recursos botánicos geotérmicos: Evaluación de los efectos antiplanctónicos y de inhibición de la biopelícula de los extractos de plantas del área de Jaboi]

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Abstract

Context: Biofilm formation exacerbates infections and stimulates antimicrobial resistance, prompting a search for potent antimicrobial agents from diverse sources. Plants in unique environments, such as geothermal areas, offer prospective medicinal benefits due to their resilience against pathogens.

Aims: To investigate the antiplanktonic and antibiofilm activities of five selected endemic plants from the geothermal area in Sabang comprising *Hydnophytum formicarum* Jack (*Rubiaceae*), *Syzigium myrtifolium* Walp (*Myrtaceae*), *Aporosa octandra* (Buch.-Ham. ex D.Don) Vickery (*Phyllanthaceae*), *Memecylon coeruleum* Jack (*Melastomaceae*) and *Memecylon edule* Roxb (*Melastomaceae*).

Methods: The ethanol extracts were prepared using the maceration method. The identification of phytochemicals was carried out using TLC with specific spraying reagents. The volatile compounds were identified using GC-MS. The evaluation of antiplanktonic and antibiofilm activities was conducted by using the microdilution method against *Staphylococcus aureus* ATCC 29213 and *Pseudomonas aeruginosa* ATCC 27853.

Results: *H. formicarum* and *M. edule* extracts demonstrated the highest antiplanktonic activity against both bacteria. *M. edule* showed superior ability to prevent biofilm formation in *S. aureus*, while *A. octandra* exhibited the highest antibiofilm activity against *P. aeruginosa*. *M. edule* showed relatively potent antiplanktonic and antibiofilm activity. The GC-MS analysis revealed the presence of bioactive compounds from saturated and unsaturated fatty acids, phenols, terpenes, furan compounds, and sugars.

Conclusions: *M. edule* from geothermal areas demonstrated potent antimicrobial activity against *S. aureus* biofilms and *P. aeruginosa*, indicating its potential against biofilm-related infections and antimicrobial resistance.

Keywords: bacterial growth; infection control; plant-derived antimicrobials.

Resumen

Contexto: La formación de biopelículas exacerba las infecciones y estimula la resistencia a los antimicrobianos, lo que impulsa la búsqueda de agentes antimicrobianos potentes de diversas fuentes. Las plantas en entornos únicos, como las áreas geotérmicas, ofrecen posibles beneficios medicinales debido a su resiliencia contra los patógenos.

Objetivos: Investigar las actividades antiplanctónicas y antibiofilm de cinco plantas endémicas seleccionadas del área geotérmica de Sabang que comprenden *Hydnophytum formicarum* Jack (*Rubiaceae*), *Syzigium myrtifolium* Walp (*Myrtaceae*), *Aporosa octandra* (Buch.-Ham. ex D.Don) Vickery (*Phyllanthaceae*), *Memecylon coeruleum* Jack (*Melastomaceae*) y *Memecylon edule* Roxb (*Melastomaceae*).

Métodos: Los extractos de etanol se prepararon utilizando el método de maceración. La identificación de fitoquímicos se llevó a cabo mediante TLC con reactivos de pulverización específicos. Los compuestos volátiles se identificaron mediante GC-MS. La evaluación de las actividades antiplanctónicas y antibiofilm se realizó mediante el método de microdilución contra *Staphylococcus aureus* ATCC 29213 y *Pseudomonas aeruginosa* ATCC 27853.

Resultados: Los extractos de *H. formicarum* y *M. edule* demostraron la mayor actividad antiplanctónica contra ambas bacterias. *M. edule* mostró una capacidad superior para prevenir la formación de biofilm en *S. aureus*, mientras que *A. octandra* exhibió la mayor actividad antibiofilm contra *P. aeruginosa*. *M. edule* mostró una actividad antiplanctónica y antibiofilm relativamente potente. El análisis GC-MS reveló la presencia de compuestos bioactivos de ácidos grasos saturados e insaturados, fenoles, terpenos, compuestos furánicos y azúcares.

Conclusiones: *M. edule* de áreas geotérmicas demostró una potente actividad antimicrobiana contra biofilms de *S. aureus* y *P. aeruginosa*, lo que indica su potencial contra infecciones relacionadas con biofilms y resistencia a los antimicrobianos.

Palabras Clave: antimicrobianos derivados de plantas; crecimiento bacteriano; control de infecciones.

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INTRODUCTION

The formation of biofilms by pathogenic bacteria such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* poses a significant challenge to the conventional antibiotic treatment regimen, resulting in the development of drug resistance and persistent infections (Kemala et al., 2024; Khairan et al., 2023). *S. aureus* secretes various harmful toxins such as α -toxins, toxin 1, and staphylococcal enterotoxins (SEs) and shows resistance to many antibiotics, including vancomycin (Cheung et al., 2021), while *P. aeruginosa* harbors pathogenic factors, including multiple proteases and toxins, as well as attachment tools such as single flagella and type IV pili (Reynolds and Kollef, 2021). These features aid in the bacterium's mobility over surfaces and the development of biofilms, which adds to the infections' extensive distribution.

The problem caused by biofilm formation may be solved by utilizing the plants that grow in geothermal regions, such as those at the Jaboi crater, Sabang, Indonesia. The plants in this area may produce a wide range of secondary metabolites with different quality and quantity as the result of an adaptive process to high temperatures and peculiar soil compositions, i.e., high in carbonate, chloride, and sulfate ions (Idroes et al., 2021). Studies showed that high temperatures affect the quality and quantity of secondary metabolites produced by plants (Imelda et al., 2023; Maulydia et al., 2023). There was an increase of phenolic in *Ribes nigrum*, alkaloids in *Lupinus angustifolius*, and flavonoids in *Quercus rubra* when they were exposed to temperatures 35-40°C (Yang et al., 2018). Studies show that plants growing in geothermal areas exhibit antimicrobial activity, which is suspected to be related to their growth environments (Fakri et al., 2023; Ningsih et al., 2022). This fact highlights the contribution of temperatures in modifying the quality of secondary metabolites used to combat biofilm-forming bacteria.

The antibiofilm and antiplanktonic activities of medicinal plants, including *Curcuma longa*, *Kaempferia rotunda*, *Caesalpinia sappan*, *Cinnamomum burmannii*, *Cinnamomum sintoc*, and *Nymphaea nouchali*, have been shown in several studies (Pratiwi et al., 2015; Suwal et al., 2021). However, the use of plants from geothermal environments for antiplanktonic and antibiofilm agents remains less investigated. The Jaboi geothermal region, with its biodiversity, provides a variety of alternative sources of potential antimicrobials to be scientifically proven, including *Hydnophytum formicarum* Jack, *Syzigium myrtifolium* Walp, *Aporosa octandra* (Buch.-Ham. ex D.Don) Vickery, *Memecylon coeruleum* Jack, and *Memecylon edule* Roxb.

H. formicarum is used as an alternative medicine for a variety of purposes, including wound healing, and has been shown to have antioxidant and anti-inflammatory properties (Ananda et al., 2022). *S. myrtifolium* is traditionally used for digestive problems and scientifically shows antidiarrheal as well as antispasm effects (Uddin et al., 2022). Traditionally, a decoction of the *A. octandra* is used orally to combat inflammation, peptic ulcer, and hypotension (Panda et al., 2018). *M. coeruleum* and *M. edule* species are in the same family as *M. randerianum*, which is used in some countries to treat digestive, skin, and other infections (Hegde and Hungund, 2021). Several studies show the antibacterial activity of *H. formicarum* (Hertiani and Pratiwi, 2015; Srisawat et al., 2021), *S. myrtifolium* (Ahmad et al., 2022), and *M. edule* (Srinivasan et al., 2017).

This study aims to investigate the antiplanktonic and antibiofilm activity of *H. formicarum*, *S. myrtifolium*, *A. octandra*, *M. coeruleum*, and *M. edule*. To the best of our knowledge, this is the first study to investigate the antibiofilm activity of plants specifically from this geothermal region. By filling this research gap, we aim to enhance comprehension of the natural antibacterial strategies found in geothermal environments and facilitate the identification of novel therapeutic medicines.

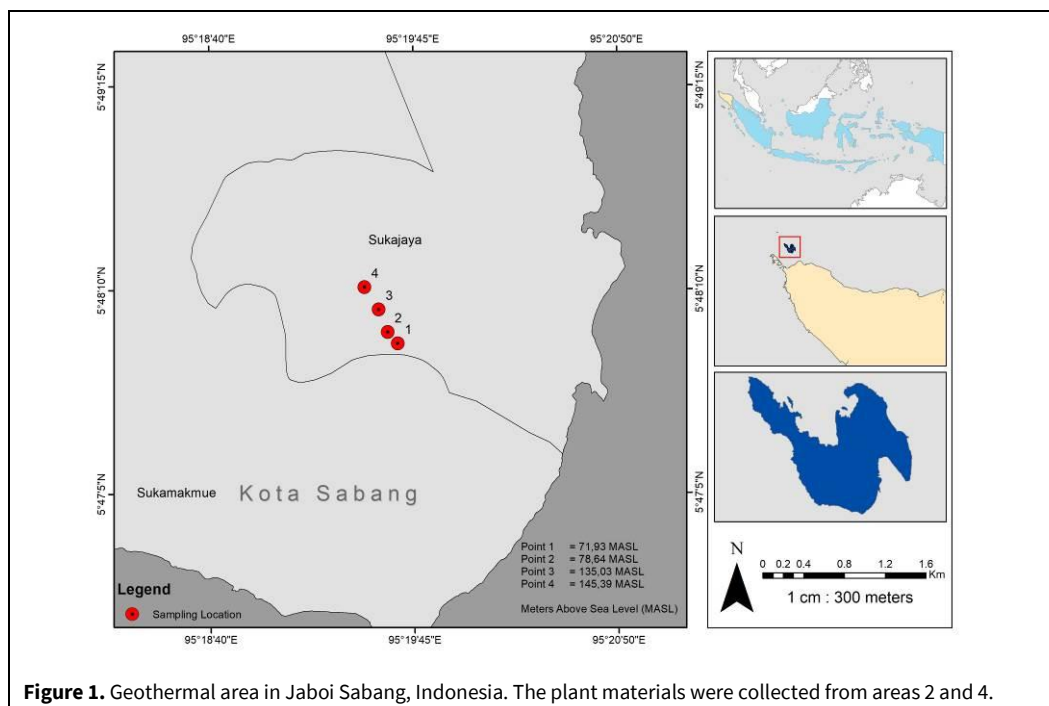
MATERIAL AND METHODS

Plant material

The five plants from the geothermal manifestation area of Jaboi Sabang used in this study were *H. formicarum* (HF), *S. myrtifolium* (SM), *A. octandra* (AO), *M. edule* (ME), and *M. coeruleum* (MC). The *S. myrtifolium* and *A. octandra* were collected from area 2, at coordinates 5°47'55.6"N 95°19'36.4"E (Fig. 1). *H. formicarum* (HF), *M. edule* (ME), and *M. coeruleum* (MC) were collected from area 4 at 5°48'11.0"N 95°19'29.1"E (Fig. 1). The taxonomy was identified at the Herbarium Bogoriense, Directorate of Scientific Collection Management, National Research and Innovation Agency Cibinong, with specimen number B-4237/II.6.2/01.05.07/11/22 by Mr. Wahyudi Santoso.

Test bacteria and culture condition

The bacteria were *S. aureus* ATCC 29213 and *P. aeruginosa* ATCC 27853, obtained from the Microbiology Laboratory, Zainoel Abidin Regional General Hospital, Banda Aceh, Indonesia. *S. aureus* was cultured in Nutrient Agar (NA) (Merck, Darmstadt, Germany), while *P. aeruginosa* in Pseudomonas Cetrimide Agar (PCA) (Oxoid, UK). Incubation temperatures were optimized at 37°C for both bacteria.



Sample preparation

The green leaves (SM, AO, MC, and ME) and tubers (HF) were washed with running water and then cut into small pieces. The leaves of SM, AO, MC, and ME were air-dried at room temperature and were exposed to direct sunlight for 30 days. The tubers of HF were subjected to a drying process within a drying cabinet (Wirastar, Indonesia) maintained at a temperature of 40°C for four days. Subsequently, the dried botanical samples were pulverized into a fine powder using a mill. This fine powder, constituting 300 grams, was homogenously combined with a solution consisting of 70% ethanol. Maceration was performed by mixing the powder with the solvent (1:10, w:v) in a closed container. This mixture was left for 3 × 24 hours with occasional stirring. The solvent was replaced every 24 hours. The mixture was filtered with filter paper (Whatman No. 1). The filtrate underwent desiccation via a vacuum rotary evaporator (Buchi, Indonesia). The five extracts were stored at 4°C for further experiments.

TLC-phytochemical analysis

The extracts were separately eluted on F254 silica gel plates (Merck, Darmstadt, Germany) using ethyl acetate-acetone (9:1)v/v, and spots were observed using a UV observing cabinet (GriyaLab, Indonesia) at 254 and 365 nm. The plates were then sprayed with the following reagents: Dragendorff, AlCl₃, FeCl₃, and vanillin sulfate to identify the phytoconstituents group of compounds in each extract.

GC-MS phytochemical analysis

The volatile compounds of five different extracts were identified using GC-MS. The instrument used was GCMS-QP2010 Ultra (Shimadzu Co., Japan) with a Rtx-5MS capillary column. The analysis conditions were as follows: Using 1 µL of diluted sample, initial injection temperature: 270°C, interface temperature: 270°C. The oven temperature was set at 30°C (held for 2 minutes), then increased at a rate of 10°C per minute to 300°C (held for 5 minutes). The carrier gas flow rate was set at a linear velocity of 39.3 cm/s with a flow rate of 1.2 mL/minute (total flow rate of 47.2 mL/minute). Nitrogen was used as the carrier gas. The mass spectrometer detector (GCMS-QP2010 Ultra) was set in scan mode from m/z 35 to 400 at a scan rate of 1666 for 30 minutes with an interface temperature of 270°C and detector voltage of 0.95 kV. Mass peaks were tentatively interpreted based on the peak mass separation pattern in the mass spectrum and compared with standard mass spectra available in the National Institute of Standards and Technology (NIST) Mass Spectra Database.

Evaluation of antiplanktonic activity

The bacterial suspension was prepared in 0.9% NaCl, and turbidity was assessed using a spectrophotometer (Azure Biosystems, Dublin, USA) at a wavelength of 625 nm. The absorbance ranged from 0.08 to 0.13, corresponding to bacterial concentrations of 10⁸ CFU/mL. The plant extracts were dissolved in dimethyl sulfoxide 5%. Sequential serial dilutions (1:2)

were conducted to prepare five decreasing concentrations (10-0.625 mg/mL). Test wells contained 100 µL of extract and 100 µL of inoculum. The positive control contains 200 µL inoculum in Mueller Hinton Broth (Himedia, India), while the antibiotic control contains 100 µL of gentamicin 1% (Sagestam®, Sanbe, Indonesia) and 100 µL media. The optical density (OD) of the mixture in the microplate was measured at a wavelength of 625 nm using a microplate reader (Azure Biosystems, Dublin, USA). Following the initial measurement, the microplate was incubated for 24 hours at 35 ± 2°C, after which the optical density was measured again. The experiment was conducted in triplicate for each well. The inhibition of bacterial growth was calculated using the formula [1] (Pratiwi et al., 2015).

$$\% \text{ Inhibition} = \left(1 - \left(\frac{\text{ODt24} - \text{ODt0}}{(\text{ODgc24} - \text{ODgc0})} \right) \right) \times 100\%$$

[1]

ODt24 = optical density of the test well after 24 hours incubation; ODt0: optical density of the test well before incubation; ODgc24: optical density of the growth control well after 24 hours incubation; ODgc0: optical density of the growth control well before incubation.

Evaluation of biofilm inhibition activity

Inoculum in Mueller Hinton Broth (Himedia, India) (100 µL) and extract (100 µL) were added to a microplate and then incubated at 35 ± 2°C for 24 hours. The free-floating contents were removed and washed with phosphate-buffered saline (Oxoid, UK) (200 µL) and rinsed three times. Crystal violet 1% (Merck, Darmstadt, Germany) was applied to the microplate and incubated for 15 minutes at room temperature. Subsequently, the unbound crystal violet was rinsed with PBS, washed 15 times, and dried. Ethanol 96% was added to the microplate, and the absorbance was measured using a microplate reader at a wavelength of 625 nm. Optical density readings were obtained with a microplate reader (Azure Biosystems, Dublin, CA, USA) at 625 nm. The experiment was conducted in triplicate for each well. The inhibition of bacterial growth was calculated based on the formula [2] (Pratiwi et al., 2008).

$$\% \text{ Inhibition} = \left(\frac{\text{ODgc} - \text{ODt}}{\text{ODgc}} \right) \times 100\%$$

[2]

ODgc: optical density of the growth control well after 24 hours incubation; ODt: optical density of the test well after 24 hours incubation.

Statistical analysis

The statistical analysis was conducted by comparing the mean values of the treatment and the control antibiotic using two-way analysis of variance (ANOVA) with Dunnett’s post hoc test using SPSS version 26.0 (IBM, NY, USA). Differences were considered statistically significant if the p-value was less than or equal to 0.05.

RESULTS

Yield of extracts

The maceration of plant materials from the five selected plants produced crude extracts with yields ranging from 21% to 34%, as has been presented in Table 1.

TLC-phytochemical analysis

The optimum result of thin-layer chromatography was achieved by using ethyl acetate: acetone (9:1) as the mobile phase. Table 2 shows the results of qualitative analysis for phytochemicals in five extracts. The extracts HF, SM, AO, MC, and ME contained all classes of phytochemicals.

GC-MS analysis

The tentative compounds with a similarity index ≥95, identified in HF, SM, AO, MC, and ME, have been provided in Table 3. The volatile substances in all extracts were saturated or unsaturated fatty acid and their esters, sugar, terpenes, phenols, and furan derivatives. Methyl ricinoleate was abundant in HF, comprising 28.34% of its constitution, while phytol was a major compound within the SM, constituting 16.46% of its composition. In AO and MC, the primary constituents were furan compounds, 5-hydroxymethylfurfural (1.38%) and furfural (28%), respectively. Ethyl oleate was dominant in the ME, constituting 11.45% of its composition. Chromatograms were available as supplementary data.

Table 1. Extraction yields (%).

Species	Dry weight (g)	Extract (g)	Yield percentage (%)
<i>Hydnophytum formicarum</i> Jack	302.2	65.21	21.58
<i>Syzigium myrtifolium</i> Walp.	300.0	102.77	34.26
<i>Aporosa octandra</i> (Buch.-Ham. ex D.Don) Vickery	300.0	70.41	23.47
<i>Memecylon coeruleum</i> Jack	300.0	66.22	22.07
<i>Memecylon edule</i> Roxb	300.0	96.44	32.15

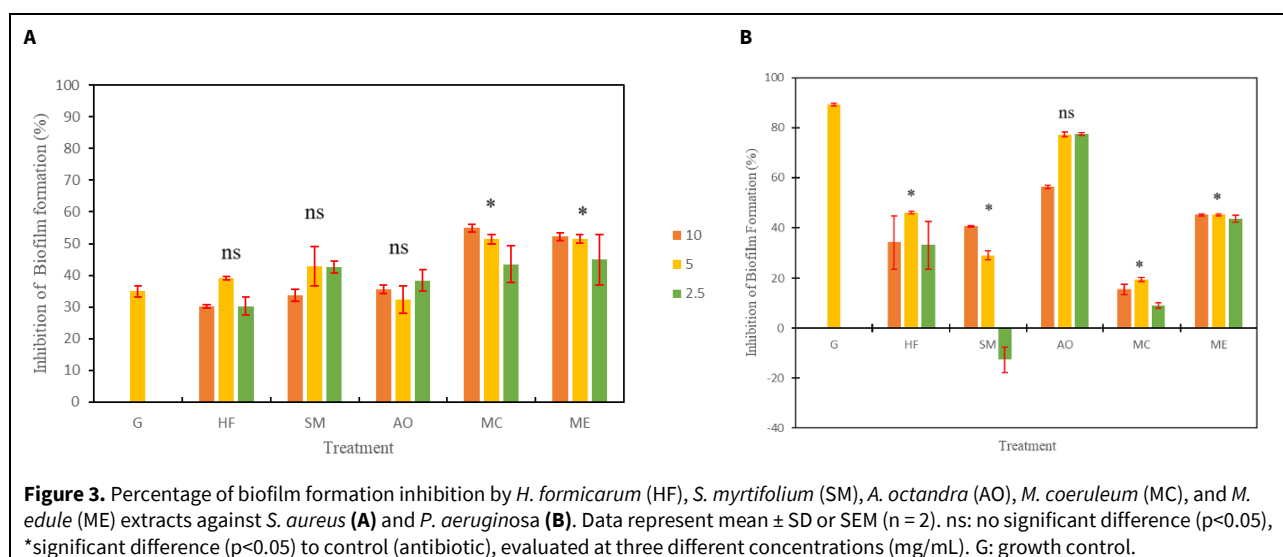
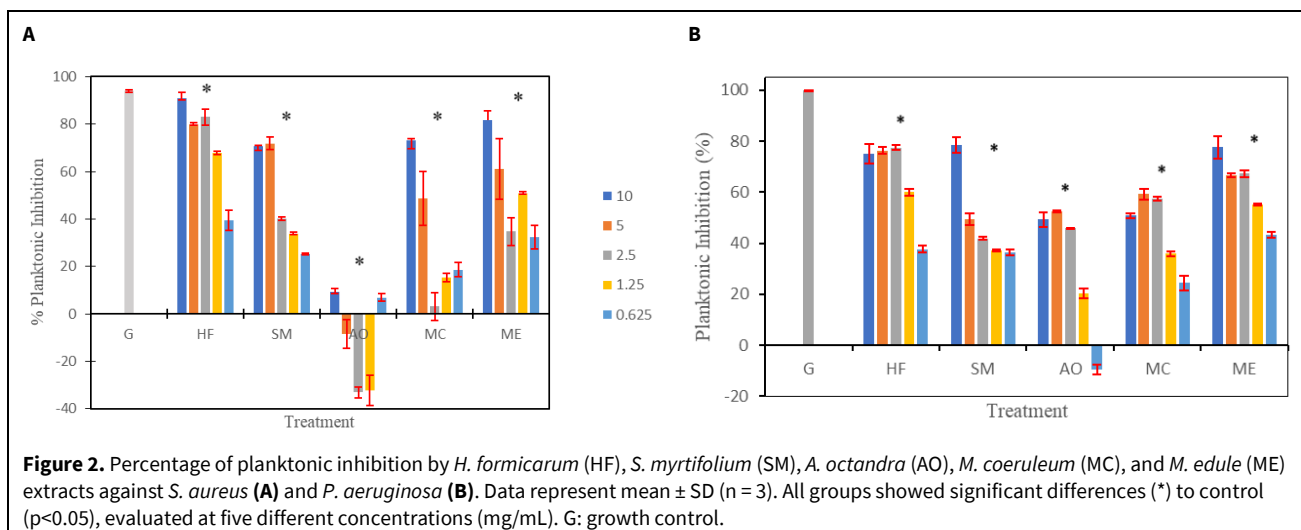
Table 2. Qualitative phytochemical analysis.

Extract	Terpenoid/steroid	Phenol	Alkaloid	Flavonoid
<i>Hydnophytum formicarum</i> Jack	+	+	+	+
<i>Syzygium myrtifolium</i> Walp.	+	+	+	+
<i>Aporosa octandra</i> (Buch.-Ham. ex D.Don) Vickery	+	+	+	+
<i>Memecylon coeruleum</i> Jack	+	+	+	+
<i>Memecylon edule</i> Roxb	+	+	+	+

Table 3. The phytochemicals identified in GC-MS analysis. The data is provided for compounds with a similarity index (SI) ≥ 95.

No	RT	Formula	MW	Name	Area (%)				
					HF	SM	AO	MC	ME
1	4.394	C ₂ H ₄ O ₂	60	Acetic acid	1.35	-	-	0.46	7.43
2	4.730	C ₃ H ₆ O ₂	74	Hydroxyacetone	-	-	-	0.38	5.56
3	6.103	C ₅ H ₄ O ₂	96	Furfural	3.25	4.78	1.21	28.00	-
4	6.992	C ₅ H ₆ O ₂	98	Furfuryl alcohol	-	-	-	-	1.72
5	7.107	C ₅ H ₈ O ₃	116	Acetoxyacetone	-	-	-	-	0.58
6	8.408	C ₆ H ₆ O ₂	110	5 Methyl Furfural	-	0.49	-	3.74	1.10
7	9.752	C ₇ H ₈ O	108	Benzyl alcohol	-	-	-	0.40	-
8	12.775	C ₆ H ₆ O ₃	126	5-Hydroxymethylfurfural	-	2.07	1.38	-	-
9	13.765	C ₆ H ₆ O ₂	110	Hydroquinone	-	-	-	2.15	-
10	14.808	C ₁₅ H ₂₄	204	Copaene	-	0.58	-	-	-
11	14.926	C ₁₅ H ₂₄	204	beta-Patchoulene	-	0.34	-	-	-
12	15.433	C ₁₅ H ₂₄	204	Caryophyllene	-	0.30	-	-	-
13	15.622	C ₁₅ H ₂₄	204	.alpha.-Guaiene	-	0.75	-	-	-
14	15.791	C ₁₅ H ₂₄	204	Seychellene	-	0.45	-	-	-
15	16.466	C ₆ H ₁₂ O ₆	180	D-Allose	-	-	-	0.83	-
16	17.455	C ₁₈ H ₃₈	254	Octadecane	-	0.30	-	-	-
17	19.382	C ₁₄ H ₂₈ O ₂	228	Myristic acid	-	-	0.33	-	-
18	19.900	C ₁₄ H ₂₈ O	212	Myristaldehyde	-	-	0.35	-	-
19	20.595	C ₂₀ H ₃₈	278	Neophytadiene	-	-	-	0.27	-
20	20.793	C ₁₉ H ₃₈ O	282	2-Nonadecanone	-	-	0.22	-	-
21	21.035	C ₁₇ H ₃₄ O ₂	270	Methyl palmitate	3.83	4.64	-	0.31	-
22	21.288	C ₂₀ H ₄₀ O	296	Isophytol	-	-	-	0.25	-
23	21.708	C ₁₈ H ₃₆ O ₂	284	Ethyl palmitate	8.00	1.87	-	1.49	7.54
24	22.700	C ₁₉ H ₃₄ O ₂	294	Methyl linoleate	5.69	-	-	-	-
25	22.748	C ₁₉ H ₃₆ O ₂	296	Methyl cis-11-octadecenoate	6.33	-	-	-	-
26	22.768	C ₁₉ H ₃₆ O ₂	296	Methyl elaidate	-	9.51	-	-	-
27	22.923	C ₂₂ H ₄₂ O ₂	338	Phytol	-	16.46	-	1.52	5.29
28	22.963	C ₁₉ H ₃₈ O ₂	298	Methyl stearate	0.47	3.73	-	-	-
29	23.363	C ₂₀ H ₃₈ O ₂	310	Ethyl oleate	10.13	1.94	-	2.20	11.45
30	23.566	C ₂₀ H ₄₀ O ₂	312	Ethyl stearate	1.45	0.36	-	0.29	1.76
31	24.515	C ₁₉ H ₃₆ O ₃	312	Methyl ricinoleate	28.34	-	-	2.91	-
32	26.313	C ₁₉ H ₃₈ O ₄	330	2-Palmitoylglycerol	-	-	-	0.31	-

HF: *H. formicarum*, SM: *S. myrtifolium*, AO: *A. octandra*, ME: *M. edule*, and MC: *M. coeruleum*.



Antiplanktonic activity

The extracts of HF, SM, AO, MC, and ME show antibacterial activity against planktonic *S. aureus* and *P. aeruginosa* with a minimum inhibitory concentration (MIC) of 0.625 mg/mL and a minimum bactericidal concentration (MBC) of >10 mg/mL. The antiplanktonic activity has been shown in Fig. 2A-B. HF and ME extracts demonstrated the lowest concentration (1.25 mg/mL) in inhibiting more than 50% growth of both *S. aureus* and *P. aeruginosa*. SM, MC, and AO extracts exhibited activity at higher concentrations.

Inhibition of biofilm formation

The results of tests assessing the inhibitory activity of biofilm formation against *S. aureus* and *P. aeruginosa* have been provided in Fig. 3A-B. Biofilm formation inhibitory tests were conducted at concentrations of 10, 5, and 2.5 mg/mL. MC and ME exhibited notable biofilm inhibitory activity against *S. aureus* at a con-

centration of 5 mg/mL, with more than 50% biofilm inhibition, surpassing the antibiotic controls (35%). The inhibitory activity of HF, SM, and AO extracts demonstrated similar levels against biofilm formation, with values ranging from 30% to 40% across all concentrations. For *P. aeruginosa*, plants that provided the best biofilm formation inhibitory activity, starting from the highest to the lowest, were AO, followed by ME, HF, SM, and MC. AO showed the extract with the most significant inhibition with an inhibitory value close to gentamicin (p > 0.05).

DISCUSSION

This study aimed to determine the antiplanktonic and antibiofilm activity of ethanol extracts from *H. formicarum*, *S. myrtifolium*, *A. octandra*, *M. coeruleum*, and *M. edule* against *S. aureus* and *P. aeruginosa*. The plants were obtained from the geothermal region of the Jaboi crater in Sabang, Indonesia. This location

was selected due to its distinct potential for producing phytochemicals possessing antimicrobial properties. The five plants evaluated in this study were abundant and native to the area.

H. formicarum is an epiphytic plant containing fleshy tubers that serve as cavities for ants and other insects, providing nectar and protein for their sustenance. This study found that HF obtained from a geothermal site exhibited antiplanktonic and antibiofilm properties. The previous research demonstrated that HF exhibited inhibitory activity against *Plesiomonas shigelloides* (MIC $\leq$ 60 μ g/mL) (Prachayasittikul et al., 2008). A study showed that the antibiofilm effects of HF against *P. aeruginosa* were concentration-dependent (Hertiani and Pratiwi, 2015). The HF also inhibited *S. aureus* by destroying the internal cell components, which consequently resulted in damage to the membrane and eventual death, assessed by flowcytometry (Srisawat et al., 2021). The result of this study added the potency of HF obtained from geothermal areas related to its antiplanktonic and antibiofilm activity.

There had been very limited data investigating the antibacterial (antiplanktonic) activity of SM, AO, and MC. The result of this study showed that SM, AO, and MC showed antibiofilm activity, which depended on the concentration of extracts for both bacteria. The previous study reported that SM shows antiplanktonic activity against *Acinetobacter baumannii*, *Bacillus cereus*, *Escherichia coli*, *Klebsiella Pneumonia*, *P. aeruginosa*, and *S. aureus* with MIC values of 0.16–1.25 mg/mL (Ahmad et al., 2022). This study demonstrated that SM showed relatively moderate antiplanktonic activity. It was more effective against *S. aureus* than against *P. aeruginosa*. At a concentration of 5 mg/mL, it inhibited 71.78 % of *S. aureus* and 49.46% of *P. aeruginosa*. The antibiofilm activity was relatively low for both bacteria (percentage inhibition was less than 50%, even at 10 mg/mL).

In this study, AO was more potent against *P. aeruginosa* rather than *S. aureus*. The antiplanktonic and antibiofilm against *P. aeruginosa* exceeded 50% inhibition, which was not observed in *S. aureus*. The planktonic inhibition against *S. aureus* was negative, indicating the biofilm grew thicker after incubation. This contrast result was also observed in methanolic extract of *Berginia ciliata*, *Clematis grata*, and *Clematis viticella* that effectively inhibited biofilm formation of *P. aeruginosa* PAO1 but showed no or negative effect on planktonic growth (Alam et al., 2020). This finding indicated that there was a different mechanism between planktonic and biofilm inhibition.

M. coeruleum and *M. edule* are from the same family, which may share the same phytochemicals and

therapeutic activities. To the time this result is written, this is the first study reporting its antiplanktonic and antibiofilm activity against *P. aeruginosa* and *S. aureus*. The MC extract at a concentration of 10 mg/mL inhibited planktonic growth for both bacteria and biofilm of *S. aureus* with inhibition >50%. A notable result was observed in ME, which shows consistent and potent activity for both antiplanktonic and antibiofilm activity against both bacteria. Previous studies showed that ME contains ursolic acid (Srinivasan et al., 2017), which was then separately investigated by the different groups of studies that confirmed its antibiofilm activity (Lyu et al., 2021).

A comprehensive understanding of biofilm stages helps identify the targets of antibacterial agents. The antibiofilm properties of five extracts in the present study could be related to the disruption of the polymer matrix synthesis, cell attachment, and virulence factors production responsible for the quorum sensing network (Guzzo et al., 2020) in bacteria. This concept was affirmed by previous studies that demonstrated the effect of various natural compounds, such as ursolic acid, on biofilm-forming bacteria (Lyu et al., 2021). Ursolic acid was identified in *M. edule*, and its derivative was confirmed to interfere with membrane potential and quorum sensing in colistin-resistant *A. baumannii* (Usmani et al., 2021). A study confirmed the antiplanktonic activity of ursolic acid, which halted colistin efflux in gastrointestinal bacteria (Sundaramoorthy et al., 2019). A gallic acid derivative, 4-O-methyl gallic acid, was isolated from *S. myrsifolium* (Nor et al., 2023). Gallic acid decreased the formation of uropathogenic biofilms and showed superior activity compared to commercially available antibiotics from quinolones and sulphonamides (Abdulshaheed et al., 2024). An alkaloid, coclaurine, was isolated from the bark of *A. octandra*, which improved cognition in animals (Panda et al., 2018). Coclaurine is a precursor for the synthesis of berberine, the widely studied natural antibiofilm agent (Wang et al., 2022), and has been incorporated into medical devices for its benefits (Gao et al., 2021). Potential natural antibiofilm agents identified in *H. formicarum* were isoliquiritigenin, protocatechualdehyde, butin, and butein, which were classified into phenolics (Prachayasittikul et al., 2008). Protocatechualdehyde was reported to show a synergistic effect with ampicillin in inhibiting *S. aureus* biofilm (Wang et al., 2024).

Fatty acids or their ester forms, identified through GC-MS analysis (e.g., methyl ricinoleate and ethyl oleate), may contribute to the extracts evaluated in this study. Fatty acids exert an inhibitory influence on biofilm formation owing to their amphiphilic properties. Fatty acids inhibit surface adhesion, alter cell membrane flexibility, reduce extracellular polymeric

substances (EPS), and impede cell communication through quorum sensing. A study demonstrated that myristoleic acid inhibited *S. aureus* and combined *S. aureus*-*Cutibacterium acnes* biofilm formation at a concentration of 20 µg/mL (Kim et al., 2021).

Sesquiterpenes such as copaene, caryophyllene, sychellene, and guaiane may also contribute to the antimicrobial activity evaluated in this study. The mechanism of action of sesquiterpenoids as antibiofilm agents is attributed to the anti-cell-adhesion properties of terpenoids and the calcium stress induced across the membrane (Lahiri et al., 2019). Another interesting phytochemical identified in extracts was phytol, which was classified as acyclic diterpenoid. Phytol was the active antimicrobial compound isolated from *Adhatoda vasica*. It increased the rate of fish viability infected by *Bacillus licheniformis*, a pathogenic bacteria causing infection in ornamental fish farms in India (Saha and Bandyopadhyay, 2020).

Considering the antiplanktonic and antibiofilm activity presented in this study, further research is needed to examine the *in vivo* activity using animals to confirm the therapeutic activity of the plants. A study aiming to design the formulation and delivery methods of these plant extracts is also important for potential clinical applications in the future.

CONCLUSION

Plant extract obtained from the geothermal area showed antiplanktonic and antibiofilm activity against *S. aureus* and *P. aeruginosa*. *H. formicarum* and *M. edule* exhibited the highest antiplanktonic activity, followed by *S. myrtifolium*, *M. coeruleum*, and *A. octandra* for both *S. aureus* and *P. aeruginosa*. In terms of biofilm formation inhibition, MC and ME were the most effective, followed by ME, SM, HF, and AO against *S. aureus*. Concerning *P. aeruginosa*, AO exhibited the highest inhibition, followed by ME, HF, SM, and MC. *M. edule* showed the highest potencies as antibiofilm and antiplanktonic due to its consistency, highlighting the potential for further research to identify the bioactive constituents. The phytochemicals in the extract may interfere with the synthesis of the polymer matrix, cell adhesion, and the production of virulence factors that regulate the quorum sensing network in bacteria to inhibit biofilm. Advanced studies focusing on the isolation of active components in *M. edule* may provide deeper insights and potential applications in the development of new drugs or alternative treatments against bacterial infections.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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AUTHOR CONTRIBUTION:

Contribution	Suryawati S	Salsabila A	Balqisa SR	Suardi HN	Triana Hertiani	Khairan K	Idroes R
Concepts or ideas	x				x	x	x
Design	x				x	x	x
Definition of intellectual content					x		x
Literature search	x	x	x	x			
Experimental studies	x	x	x				
Data acquisition	x	x	x				
Data analysis	x				x	x	x
Statistical analysis				x			
Manuscript preparation	x				x	x	x
Manuscript editing	x			x	x	x	x
Manuscript review	x	x	x	x	x	x	x

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Supplementary data

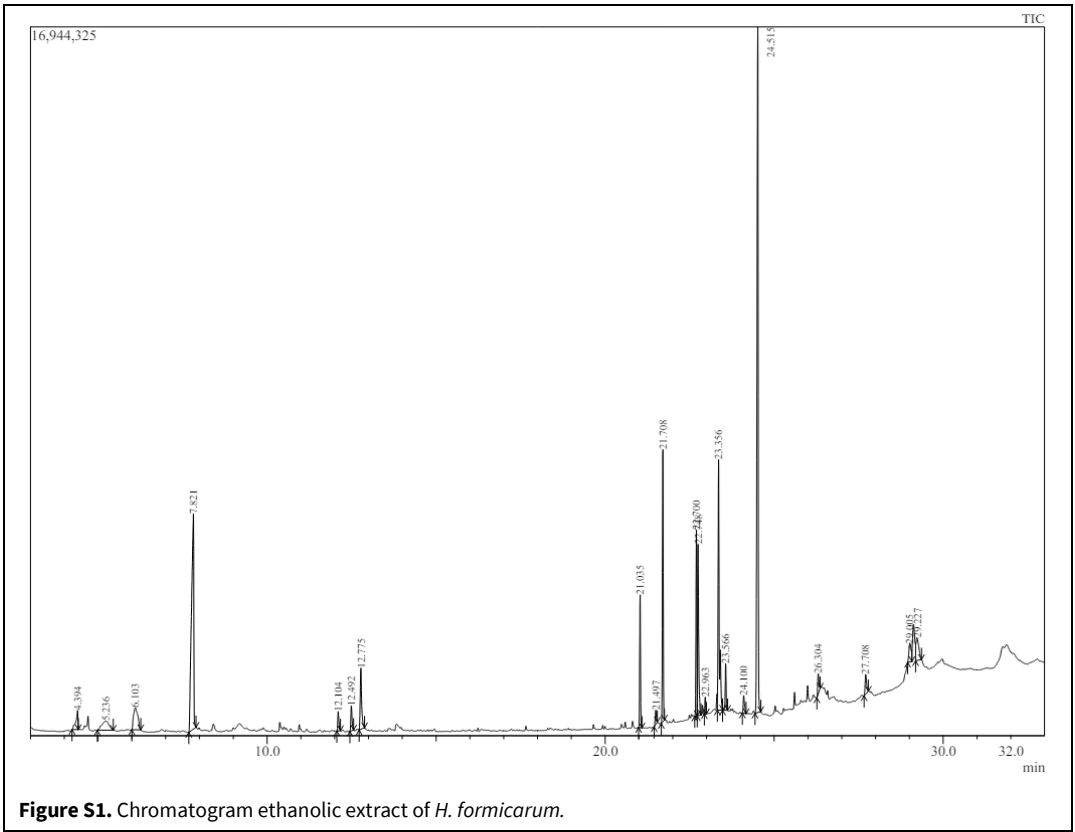


Figure S1. Chromatogram ethanolic extract of *H. formicarum*.



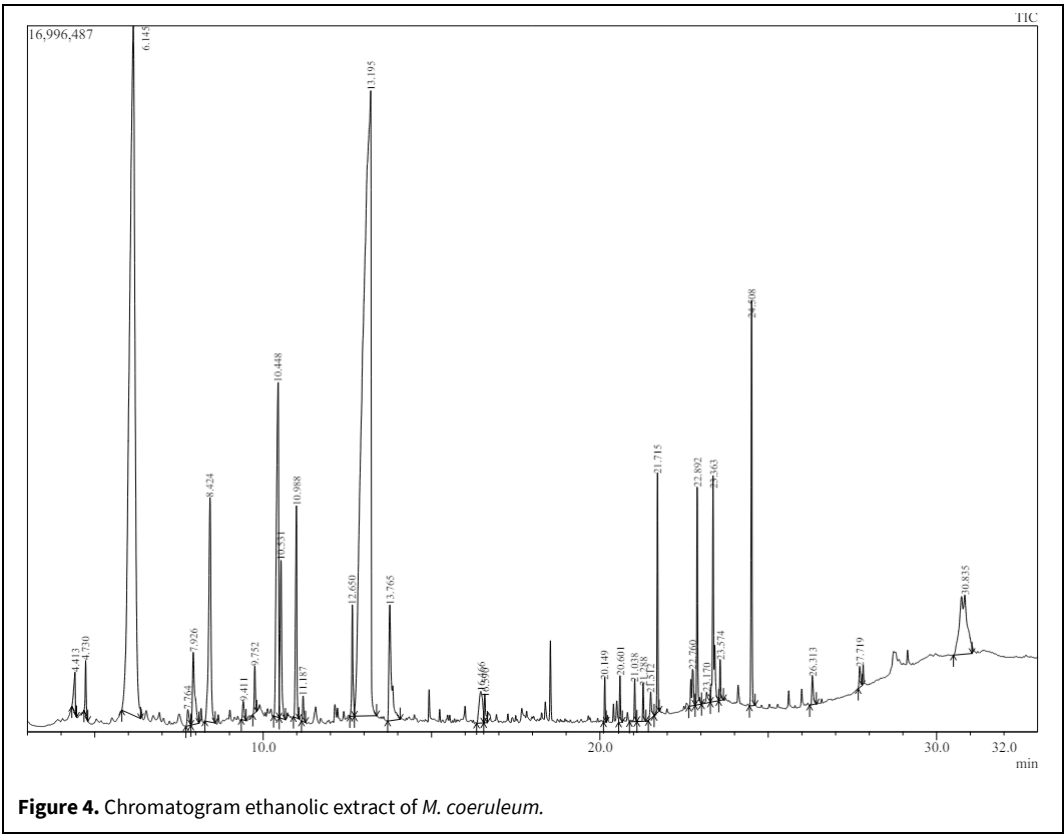


Figure 4. Chromatogram ethanolic extract of *M. coeruleum*.

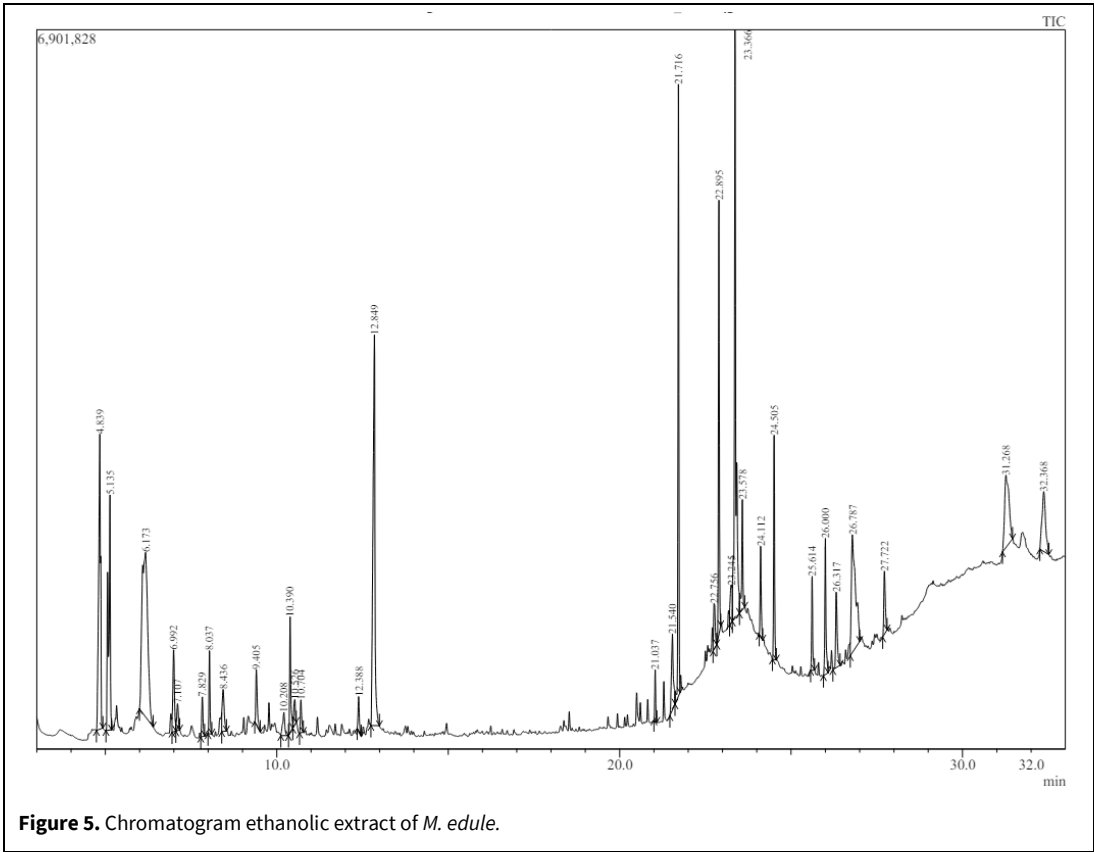


Figure 5. Chromatogram ethanolic extract of *M. edule*.