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Antibacterial Potential of Geothermal Plant Extracts from Jaboi Crater, Indonesia: A Thin Layer Chromatography-Bioautography Approach

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Abstract

Antimicrobial resistance (AMR) poses an urgent global health concern, prompting the need for alternative therapeutic agents. This study evaluated the antimicrobial potential of ethyl acetate extracts from five medicinal plant species (*Memecylon edule*, *Garcinia dioica*, *Syzygium* sp., *Memecylon caeruleum*, and *Aporosa octandra*) collected from the geothermal Jaboi Crater in Aceh, Indonesia. Phytochemical profiling was performed using thin layer chromatography (TLC), and antimicrobial activity was assessed via TLC-bioautography against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. The results revealed the presence of phenolic and terpenoid compounds, with antibacterial activity observed only against *E. coli*. No inhibition was detected against *S. aureus* or *C. albicans*. The study highlights the selective antimicrobial potential of geothermal plant extracts and underscores the relevance of bioautography as a rapid screening tool. While preliminary, these findings support further investigation into geothermal flora as a source of antibacterial compounds and call for advanced studies to isolate active constituents and explore their mechanisms of action.

1. Introduction

The escalating prevalence of antimicrobial resistance (AMR) poses a significant threat to global public health, rendering many conventional antibiotics increasingly ineffective [1]. In response, there is a growing interest in exploring alternative antimicrobial agents, particularly those derived from medicinal plants, which have been utilized in traditional medicine for centuries [2]. Medicinal plants are rich sources of bioactive secondary metabolites, notably phenolic compounds and terpenoids, which exhibit diverse pharmacological activities [3–5]. Phenolic compounds, including flavonoids and tannins, have demonstrated the ability to disrupt microbial cell walls, inhibit enzyme activity, and induce oxidative stress, leading to microbial cell death [6]. Terpenoids, another prominent class of plant metabolites, have been reported to possess antibacterial, antifungal, antiviral, and antimalarial activities [7].

Recent studies have highlighted the potential of these plant-derived compounds to act synergistically with conventional antibiotics, enhancing their efficacy against resistant bacterial strains [8]. For instance, combinations of plant extracts with antibiotics have shown significant antibacterial activity against various pathogens [9]. This

synergistic interaction not only enhances the antibacterial efficacy but also may reduce the required dosage of antibiotics, thereby minimizing potential side effects and slowing the development of resistance [10].

The present study investigates the antimicrobial properties of ethyl acetate extracts from five selected plant species, namely *Memecylon edule, Garcinia dioica, Syzygium* sp., *Memecylon caeruleum,* and *Aporosa octandra,* collected from the Jaboi Sabang Crater, a geothermal ecosystem located on Weh Island, Aceh, Indonesia. The rationale for selecting these five species is grounded in both ethnopharmacological and phytochemical evidence. *Memecylon edule* Roxb. (Melastamataceae family) is a small evergreen tree reported as having ethnobotanical and pharmacological properties. *M. edule* has been traditionally used for skin infections and has shown antibacterial activity [11]. *Syzygium* species, including *S. cumini*, are well-documented for their health-promoting applications including antimicrobial activity [12]. *A. octandra* is used in Southeast Asian traditional medicine and shows promise in anti-inflammatory and antimicrobial screening [13].

Geothermal ecosystems represent unique ecological niches characterized by extreme physicochemical conditions, including elevated temperatures, mineral-rich substrates, and distinctive microbial communities [14–16]. These environmental stressors are known to influence the biosynthesis of secondary metabolites in plants, leading to the production of compounds with enhanced biological activity [17]. Comparative studies have shown that geothermal plants often exhibit stronger antimicrobial properties than non-geothermal counterparts, likely due to adaptive metabolic responses [18, 19]. Jaboi, one of the active geothermal fields in Indonesia, is located on Weh Island, Aceh, within the volcanic arc of Sumatra. The geothermal manifestations in this region are typified by fumaroles, hot springs, and solfatara fields, which contribute to a chemically complex environment with high concentrations of sulphur and heavy metals. These environmental pressures drive the evolution of plant species that exhibit unique adaptations at the metabolic level, leading to the enhanced biosynthesis of secondary metabolites with potential antimicrobial properties [20]. Comparative phytochemical analyses have revealed that plants from geothermal regions frequently exhibit superior antimicrobial properties relative to their non-geothermal counterparts due to the adaptive evolution of their metabolic pathways in response to extreme conditions [21].

Despite this potential, the biodiversity of the Jaboi geothermal area remains underexplored. Very few systematic studies have investigated its plant-derived secondary metabolites or their antimicrobial properties. Thus, this study aims to address this specific knowledge gap by evaluating the antimicrobial potential of selected Jaboi geothermal plants and identifying their active components. While many studies have explored plant-based antimicrobials, there is a lack of data focusing on plants from extreme environments such as geothermal fields, especially in Southeast Asia. To this end, we employ thin-layer chromatography (TLC) for phytochemical profiling and TLC-bioautography for antimicrobial activity assessment. TLC is a rapid and cost-effective technique widely used for preliminary phytochemical screening, especially when working with crude extracts in early-phase studies [22]. The integration of TLC with bioautography provides a powerful tool for directly associating specific phytochemical bands with biological activity, making it ideal for detecting antibacterial compounds in complex plant matrices [23]. This study contributes to the understanding of geothermal flora and their bioactive potential and serves as a preliminary step in the discovery of novel antimicrobial compounds that may be further developed for pharmaceutical applications.

2. Materials and Methods

2.1. Sampling Point and Plant Identification

Plant sampling was conducted in the geothermal manifestation zone of the Jaboi crater. The Jaboi crater geothermal region is located in Jaboi village, Suka Jaya district, Sabang city, Aceh province. The geothermal features of Jaboi include hot springs, fumaroles, sulphur springs, and changed rocks, with temperatures ranging from 30 to 39 °C. Preliminary findings indicate that plant samples were collected from the *Jaboi Kawah 2* (JK2) and *Jaboi Kawah 4* (JK4) regions. Both locations possess coordinate points established with a global positioning system (GPS) device: for JK2, E = 095°39′49.4″; N = 05°28′23.4″, and for JK4, E = 095°39′49.4″; N = 05°28'23.7". Both regions exhibit temperature and pH measurements; for JK2, the temperature averages around 79.17 ± 0.02°C and the pH value approximately 2.29 ± 0.01, whereas for JK4, the temperature averages approximately 96.07 ± 0.02°C and the pH value approximately 2.66 ± 0.01 (Figure 1).

2.2. Extraction of Plant Samples

Leaf and bark samples were thoroughly washed with distilled water to eliminate surface contaminants and subsequently air-dried at ambient temperature under shade for one week. Dried leaves were pulverized using a mechanical grinder, and ethyl acetate extraction was performed via maceration. A total of 100 g of powdered

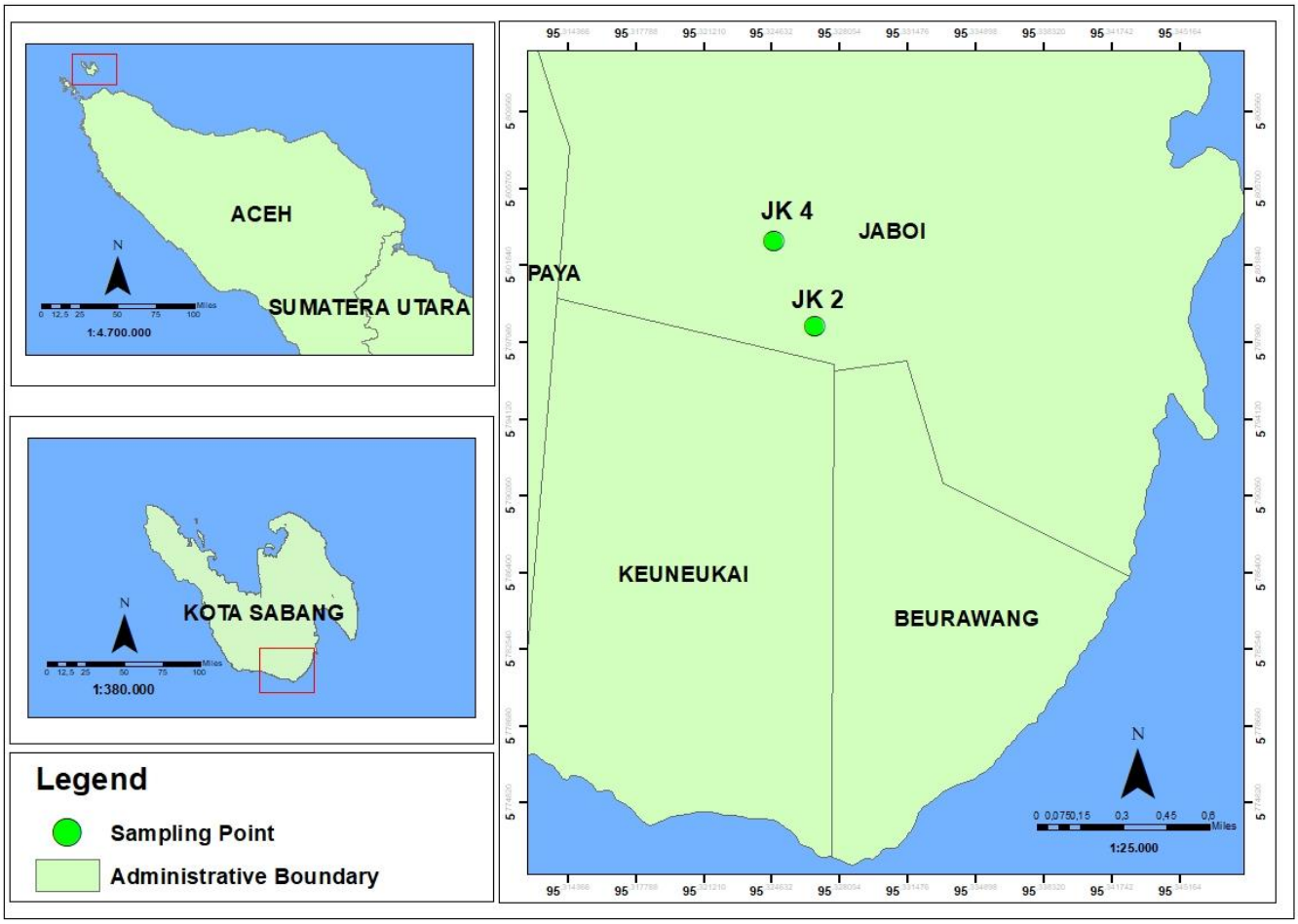

Figure 1. Sampling point in Jaboi Sabang located in JK2 and JK4.

plant material was immersed in 1000 mL of ethyl acetate for 84 hours with periodic agitation. The extract was filtered through Whatman No. 1 filter paper and concentrated using rotary evaporation at 40°C under reduced pressure. The crude extracts were stored at 4°C until use. For antimicrobial testing, extracts were resuspended in dimethyl sulfoxide (DMSO) to a working concentration of 100 mg/mL.

2.3. Microbial Strains and Culture Preparation

Test organisms included *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Candida albicans* ATCC 10231. These were maintained on Mueller-Hinton Agar (MHA) for bacteria and Sabouraud Dextrose Agar (SDA) for fungi. Before testing, microbial suspensions were prepared in sterile saline and adjusted to a 0.5 McFarland standard (~1.5 × 10^8 CFU/mL) to ensure standardized inoculum density.

2.4. Thin Layer Chromatography (TLC) and Bioautography Assay

Bioautography was used to detect bioactive compounds with antimicrobial properties. TLC plates (Silica gel 60 F_{254}, 1 × 10 cm) were used for all assays. Each plate was spotted with 20 µL of plant extract (100 mg/mL in DMSO) along a baseline 1 cm from the bottom edge using a micropipette. Plates were developed to 8 cm in a saturated glass chamber using *n*-hexane:ethyl acetate (7:3, v/v) as the mobile phase. This solvent system was selected based on preliminary optimization and literature precedent for effectively separating moderately polar compounds, including phenolics and terpenoids. After development, TLC plates were air-dried and placed face-down onto freshly seeded MHA (for bacteria) or SDA (for fungi) in Petri dishes. Each TLC plate remained in contact with the agar surface for 1 hour at room temperature to allow compound diffusion. Afterward, the plates were removed and the Petri dishes were incubated for 24 hours at 37°C for bacteria and 48 hours at 30°C for fungi. Zones of inhibition were observed as clear areas around spots on the media surface. Each test was conducted in triplicate. Although no commercial antimicrobial standards or control samples were used in this exploratory study, only clearly visible inhibition zones that were consistent across at least two replicates were recorded as positive.

Table 1. The list of the tested plant species from Jaboi Sabang.

Plant name	Family name	Part Testes	Area	Traditional uses*
Garcinia dioica Blume	Clusiaceae	Leaves and Bark	JK2	Headache
Aporosa octandra (Buch.-Ham. Ex D.Don) Vikery	Phyllanthaceae	Leaves and Bark	JK2	Inflammation
Syzygium sp.	Myrtaceae	Leaves and Bark	JK2	Stomachache
Memecylon edule Roxb.	Melastomaceae	Leaves	JK4	Astringent
Memecylon caeruleum Jack	Melastomaceae	Leaves	JK4	Malaise

Description: (*) data collected from Dr. Duke's Phytochemical and Ethnobotanical databases [24]

2.5. Detection of Compound Classes on TLC Plates

Subsequently, an additional chromatogram underwent TLC analysis and was examined under UV light at 254 nm and 365 nm. Multiple reagents were applied to the Silica gel F_{254} plate, specifically Dragendorf reagent for alkaloid identification, Liebermann-Burchard reagent for terpenoid identification, and $FeCl_3$ reagent for phenolic identification [25]. For reagent-based detection, TLC plates were sprayed evenly and dried in a 60°C oven for 5 minutes. The position of the clear zone on the petri dish corresponds with the location of the colourful spots observed in the TLC test findings. The distinct zone created by the colored spot from the TLC test is recognized and classified as a bioactive molecule with antibacterial properties. $FeCl_3$ yielded green-black spots for phenolics, Liebermann-Burchard reagent produced red-purple coloration for terpenoids, and Dragendorff's reagent developed orange-brown zones for alkaloids. The colored spots that developed were quantified for their Rf (retention factor) values, and their compound classifications were identified [23, 26].

2.6. Data Analysis

TLC bioautography and secondary metabolite results were documented photographically. All experimental assays, including TLC-bioautography and inhibition zone measurements, were performed in triplicate. Inhibition zone diameters were recorded in millimeters and expressed as mean ± standard deviation.

3. Results and Discussion

Antimicrobial resistance (AMR) is becoming more and more threatening; thus, new therapeutic agents must be explored. Medicinal plants show promise due to their abundance of bioactive secondary metabolites. This study investigated the antibacterial activity of ethyl acetate extracts from five geothermal plant species: *Memecylon edule*, *Garcinia dioica*, *Syzygium* sp., *Memecylon caeruleum*, and *Aporosa octandra,* traditionally used in ethnomedicine (Table 1).

Bioautography is a method that involves the utilization of an appropriate chromatographic procedure, which is then followed by a biological detection system, in order to identify a single hit or lead compound [26]. The bioautography assay provided direct evidence of antimicrobial activity, with distinct inhibition zones observed in extracts rich in phenolics and terpenoids. The ability of these bioactive metabolites to interact with bacterial membranes likely contributes to their antimicrobial efficacy [27]. Thin-layer chromatography (TLC) followed by bioautography revealed visible inhibition zones against *Escherichia coli* ATCC 25922 in extracts from all five plant species. No inhibition zones were observed against *Staphylococcus aureus* or C*andida albicans*. Extracts rich in phenolic or terpenoid compounds showed the strongest inhibition, particularly in *G. dioica*, *A. octandra*, *Syzygium* sp., *M. edule*, and *M. caeruleum* (Table 2).

The phytochemical analysis of the ethyl acetate extracts from five plant species using TLC revealed a diverse array of secondary metabolites, including phenolic and terpenoid compounds. The selected mobile phase system (*n*-hexane: ethyl acetate, 7:3) facilitated the effective separation of these bioactive constituents, which were subsequently identified using chemical derivatization and UV visualization. Notably, phenolic compounds were found to predominate in *M. edule*, G*. dioica*, and *Syzygium* sp., whereas terpenoids were particularly abundant in *M. caeruleum* and *A. octandra*. These findings align with prior research emphasizing the antimicrobial potential of phenolics and terpenoids due to their ability to disrupt microbial membranes and interfere with enzymatic activity.

The bioautography assay on *S. aureus* and *C. albicans* showed no inhibitory zone. This is considered to result from the disparity in cell wall architecture between Gram-positive and Gram-negative bacteria, which is thought to influence the test bacteria's reaction to the ethyl acetate extract. The bacterium *S. aureus* is a class of Gram-positive bacteria characterized by a cell wall consisting of 90% or more peptidoglycan. The presence of *E. coli* in water indicates prior contamination with human feces and suggests the possible presence of intestinal pathogens [28]. *E. coli* is a Gram-negative bacterium characterized by a thin peptidoglycan layer and an outer membrane, which can influence its susceptibility to antimicrobial agents. The chemicals in the ethyl acetate

Table 2. Activity of plant extract in three target bacteria.

Plant name	Part Testes	*Staphylococcus aureus*		*Escherichia coli*		*Candida albicans*	
		Rf	Metabolites	Rf	Metabolites	Rf	Metabolites
G. dioica	Leaves	Nd	Nd	0.68	Phenolics	Nd	Nd
	Bark	Nd	Nd	Nd	Nd	Nd	Nd
A. octandra	Leaves	Nd	Nd	0.75	Terpenoids	Nd	Nd
	Bark	Nd	Nd	Nd	Nd	Nd	Nd
Syzygium sp.	Leaves	Nd	Nd	0.68	Phenolics	Nd	Nd
	Bark	Nd	Nd	Nd	Nd	Nd	Nd
M. edule	Leaves	Nd	Nd	0.68	Phenolics	Nd	Nd
M. caeruleum	Leaves	Nd	Nd	0.71	Terpenoids	Nd	Nd

Descriptions= Rf = retention factor; Nd = Not detected

extract are unable to traverse the peptidoglycan layer of *S. aureus*, leading to the lack of an inhibitory zone in that bacteria. Simultaneously, the cell wall composition of the fungus *C. albicans* comprises chitin and ergosterol polymers, which exhibit greater strength than the cell walls of the majority of bacteria. Consequently, it is hypothesized that the *C. albicans* fungus exhibited no inhibitory zone in this investigation. Although all experiments were conducted in triplicate, the semi-quantitative nature of TLC-bioautography limited statistical analysis. In future work, inhibition zone diameters and minimum inhibitory concentrations (MICs) will be determined using disc diffusion and broth microdilution assays [29].

Compound identification was conducted using TLC to ascertain the class of compounds responsible for the antibacterial action against *E. coli* bacteria. The results of the compound identification are illustrated in Figure 2. According to Figure 2, the TLC test results were obtained at wavelengths of 254 and 366 nm. Both wavelengths have been widely employed in TLC tests, with great results for spotting spots on TLC plates. UV detection at 254 nm reveals the presence of quenching, which appears as dark dots on a green fluorescent backdrop. This means it detects the presence of an organic component. Observation utilizing UV detection at 366 nm reveals red fluorescent patches, suggesting the existence of molecules with conjugated double bonds that glow under UV 366 nm irradiation.

The bioautography results against *E. coli* align with TLC-based phytochemical identification, where phenolic-rich extracts (e.g., *G. dioica*, *M. edule*, *Syzygium* sp.) exhibited antimicrobial activity. Phenolics such as flavonoids are known to form complexes with bacterial cell walls, disrupt membranes, and inhibit nucleic acid synthesis [30]. Likewise, terpenoid-rich extracts (e.g., *M. caeruleum*, *A. octandra*) showed activity consistent with their lipophilic properties that disrupt microbial membranes. The utilization of Dragendorf's reagent to ascertain the existence of alkaloid compounds; a good outcome is indicated by the appearance of brownish spots. The spraying performed in this investigation yielded favorable outcomes for the *Garcinia dioica* bark sample, with an Rf value of 0.1. The results indicate that the sample suspected of harboring *E. coli* bacteria from the bioautography activity test remains unidentified. The use of the Lieberman-Burchard reagent to ascertain the presence of terpenoid compounds; a favourable outcome is shown by blue to purple dots or red to purple under visible light [25]. The spraying performed in this study yielded favourable outcomes for the ethyl acetate extract leaf samples from plants in the Jaboi Sabang geothermal region; however, the bark samples exhibited unfavourable results. The samples identified as potentially responsible for the antibacterial activity against *E. coli* in the bioautography test include the leaves of *M. caeruleum*, exhibiting an Rf value of 0.78 and appearing blue, and the leaves of *A. octandra*, with an Rf value of 0.76, which are likewise blue (Figure 2). The application of the $FeCl_3$ reagent for the identification of phenolic compounds; a good outcome is indicated by the appearance of green, red-purple, blue, or intense black stains [25]. The spraying performed in this investigation yielded favourable outcomes for the leaf and stem bark samples of the ethyl acetate extract from flora in the Jaboi Sabang geothermal region. The samples identified as potentially responsible for antibacterial activity against *E. coli* in the bioautography test include the leaves of *M. edule* with an Rf value of 0.7 in black, the leaves of *Garcinia dioica* with an Rf value of 0.73 in green, and the leaves of *Syzygium* sp with an Rf value of 0.71 in black. These secondary metabolites are well documented for their antimicrobial properties. Phenolic compounds, such as flavonoids and tannins, can disrupt microbial cell walls, inhibit enzymatic activity, and induce oxidative stress, leading to microbial cell death [6]. Terpenoids are known to interfere with microbial membrane integrity and respiratory functions, contributing to their antimicrobial efficacy [7]. Previous studies reported antibacterial activity of *M. edule* against *E. coli* and *S. aureus*, supporting our findings [11, 31]. Similarly, *A. octandra* has

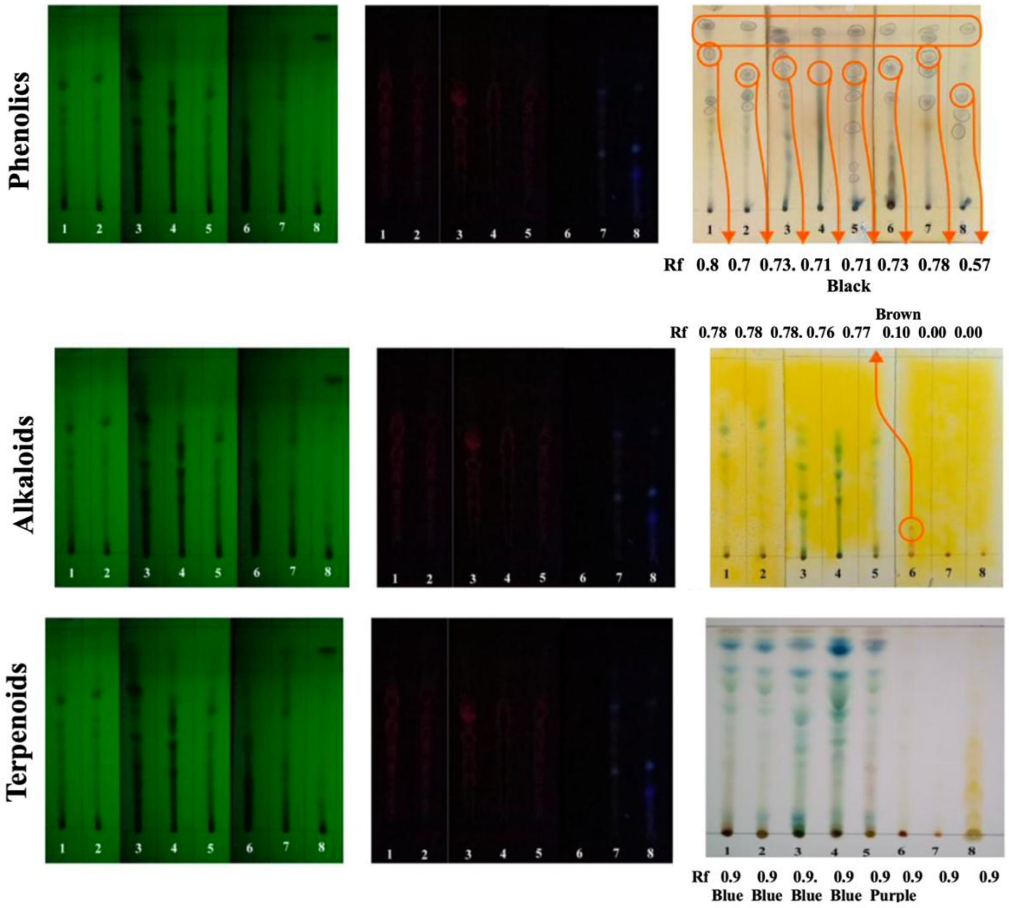

Figure 2. The results of the identification of phenolics, alkaloids, and terpenoids compound by TLC. (1) *M. caeruleum* leaf sample, (2) *M. edule* leaf sample, (3) *G. dioica* leaf sample, (4) *A. octandra* leaf sample, (5) *Syzygium* sp. leaf.

been reported to show activity against Gram-negative bacteria in preliminary screening [13]. The results are also in line with [21], who reported stronger antimicrobial activity in geothermal flora from Ie Seuum geothermal outflow, Indonesia.

Interestingly, although all five plant extracts exhibited visible inhibition against *E. coli*, none were active against *S. aureus* or *C. albicans*. This selective activity can be attributed to several interrelated factors. While the structural differences in microbial cell walls are often cited—*E. coli* being Gram-negative with a thinner peptidoglycan layer, and *S. aureus* being Gram-positive with a thicker peptidoglycan barrier. Other possibilities include compound-specific permeability, efflux mechanisms, target site availability, or concentration-dependent thresholds not reached in *S. aureus* or *C. albicans* [32]. Furthermore, the fungal cell wall in *C. albicans* contains chitin and glucan polymers, and its membrane is rich in ergosterol, which could contribute to its inherent resistance to plant-derived compounds used in this study.

Despite these promising findings, certain limitations must be acknowledged. A limitation of this study is the absence of positive control antibiotics such as ciprofloxacin or ampicillin. Including standard references in future bioautography assays will allow benchmarking of plant extract potency and validate the observed inhibition zones. The use of crude plant extracts, rather than isolated bioactive compounds, may introduce variability in antimicrobial potency. Additionally, the in vitro nature of this study necessitates further in vivo validation to ascertain clinical relevance. Future research should focus on isolating and characterizing the specific compounds responsible for antimicrobial activity and elucidating their mechanisms of action. Investigating the potential synergistic effects of these compounds with conventional antibiotics could further contribute to developing effective strategies against AMR.

4. Conclusions

This study investigated the antimicrobial properties of ethyl acetate extracts from five medicinal plant species traditionally used in ethnomedicine and collected from the Jaboi geothermal area. Phytochemical profiling confirmed the presence of phenolic and terpenoid compounds, and qualitative bioautography assays revealed antibacterial activity specifically

against *Escherichia coli* ATCC 25922. However, no inhibitory effects were observed against *Staphylococcus aureus* or *Candida albicans*. While these findings suggest selective antimicrobial potential, they remain preliminary due to the absence of quantitative data such as inhibition zone diameters, minimum inhibitory concentrations (MIC), and comparisons with standard antibiotics. To build upon these initial findings, future research should prioritize the isolation and structural characterization of the specific bioactive compounds responsible for the antibacterial effects. Quantitative antimicrobial assays, including MIC and MBC testing, should be employed to confirm potency. Additionally, comparative studies using known antibiotics as benchmarks will help contextualize the activity of these plant-derived compounds. Mechanistic investigations are also needed to elucidate how these metabolites exert their effects, and in vivo studies will be essential to assess their therapeutic potential and safety. Exploring the synergistic interactions between these natural compounds and conventional antibiotics may also offer promising avenues to address antimicrobial resistance.

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