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RESEARCH ARTICLE

In Vitro Antibacterial Bioassay of Methanol Extract Fraction ($R_f > 0.5$) from *Rhizophora mucronata* L. (Rhizophoraceae) Leaves Against Multidrug-Resistant *Escherichia coli*

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Abstract

A bioactivity evaluation was carried out in January 2018 on the methanolic leaf extract fraction ($R_f > 0.5$) of *Rhizophora mucronata* L. (Rhizophoraceae) against antibiotic-resistant Escherichia coli. Isolation of secondary metabolites was conducted at the Marine Chemistry Laboratory, Faculty of Marine and Fisheries, while antibacterial assays were performed at the Microbiology Laboratory, Faculty of Medicine, Universitas Syiah Kuala. Thin-layer chromatography (TLC) was employed to identify bioactive fractions, and phytochemical screening indicated the presence of alkaloid constituents. The tested extract exhibited inhibition zones ranging from 7.50 to 8.50 mm, reflecting moderate antibacterial activity against resistant *Escherichia coli* strains.

Keywords: Rhizophora mucronata leaves, alkaloids, antibacteria-resistant *Escherichia coli*.

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1 Introduction

Escherichia coli (*E.coli*) is a gram-negative bacterium that naturally inhabits the gastrointestinal tract of humans and animals. Although most strains are commensal, pathogenic variants such as Enteropathogenic *E. coli* (EPEC) can cause severe gastrointestinal infections, particularly in immunocompromised populations including infants, young children, the elderly, and hospitalized patients [1]. Transmission is primarily associated with fecal contamination and poor environmental sanitation [2]. Globally, diarrheal diseases remain a major public health burden, with an estimated 1.8 billion cases reported annually, many of which are linked to pathogenic *E. coli* infections [3].

Clinically, diarrheal infections are frequently treated with synthetic -lactam antibiotics such as chloramphenicol [4]. However, prolonged and indiscriminate use of chloramphenicol has been associated with serious adverse effects, including aplastic anemia, granulocytopenia, gastrointestinal disturbances, and hypersensitivity reactions [5]. More critically, sustained exposure to antibiotics has accelerated the emergence of resistant *E. coli* strains, complicating treatment strategies and increasing therapeutic failure rates [6]. The growing threat of antimicrobial resistance underscores the urgent

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need for alternative antibacterial agents derived from natural sources.

On the other hand, mangrove plants represent a promising reservoir of bioactive secondary metabolites due to their adaptation to extreme coastal environments. *Rhizophora mucronata* L. (Rhizophoraceae), a widely distributed mangrove species, has been reported to contain diverse phytochemical constituents, including alkaloids, flavonoids, phenolics, peptides, saponins, and terpenoids, many of which exhibit antimicrobial activity [7,8]. Previous studies have demonstrated inhibitory effects of *R. mucronata* leaf extracts against bacterial pathogens such as *E. coli*, *Aeromonas* sp., *Streptococcus* sp., and *Edwardsiella* sp., as well as antifungal activity against *Penicillium digitatum* [9].

Despite these findings, limited information is available regarding the antibacterial activity of specific chromatographic fractions, particularly those with retention factor (R_f) values greater than 0.5. Fraction-based investigation is essential to narrow down bioactive constituents and enhance the likelihood of isolating potent antibacterial compounds. Therefore, this study aims to evaluate the in vitro antibacterial activity of the methanolic leaf extract fraction ($R_f > 0.5$) of *R. mucronata* against antibiotic-resistant *Escherichia coli*, contributing to the ongoing search for plant-derived alternatives to conventional antibiotics.

2 Methodology

2.1 General

The instruments used in this study included an analytical balance (Kern), rotary evaporator (Eyela N-1000), incubator (Memmert INB 500), autoclave (Tommy SX-300/500/700), laminar airflow cabinet (Safe Fast Elite 212 SD), UV lamp (UVGL-25), hot plate (Akebono), drying oven (Jouan), refrigerator (LG), and a thin-layer chromatography (TLC) system. Standard laboratory glassware consisted of Pyrex Petri dishes, beakers, test tubes, separating funnels, graduated cylinders, Erlenmeyer flasks, volumetric pipettes, dropper pipettes, and 1.5 mL cuvettes. Micropipettes included a Pipetteman P20 (2–20 µL) and an Eppendorf micropipette (100–1000 µL).

Additional laboratory materials comprised calipers, aerators, sterile cotton swabs, inoculation loops, spirit lamps, TLC capillary tubes, aluminum foil, filter paper, forceps, sterile gauze, tissue paper, gloves, labeling materials, microwave oven, paper discs, sample containers, and other routine laboratory consumables required for extraction, chromatographic separation, and antibacterial assays.

2.2 Extraction and Isolation

Fresh leaves of *Rhizophora mucronata* were air-dried for 3–5 days and subsequently cut into small pieces to enhance solvent penetration and extraction efficiency [1]. The dried material was macerated in 70% methanol for 3 × 24 h. The extract was filtered and concentrated at 60 °C using a rotary evaporator to yield 1.98 g of crude extract (coded A17A01). The crude extract was partitioned using a chloroform:methanol:water (1:1:1, v/v) solvent system, resulting in two fractions: a semipolar fraction (F1B16, 0.02 g) and a polar fraction (F1B17, 1.96 g).

Based on bioactivity screening, F1B17 was selected for further purification. Fraction F1B17 was subjected to elution using a methanol:ethyl acetate solvent system (10:90, v/v), followed by separation using Thin Layer Chromatography (TLC). Compounds exhibiting retention factor (Rf) values greater than 0.5 were collected for further evaluation. The selected fraction (Rf > 0.5) was subsequently partitioned using methanol:dichloromethane (1:1, v/v), yielding two sub-fractions: F2B10 (methanol fraction) and F2B11 (dichloromethane fraction). Both sub-fractions were subjected to phytochemical screening using Dragendorff's reagent and cerium sulfate to detect alkaloids and hydrocarbons, respectively.

2.3 Bacterial Strain and Culture Conditions

A clinical isolate of *Escherichia coli* O157:H7 was obtained from the Regional General Hospital (RSUD) Dr. Zainoel Abidin, Banda Aceh, Indonesia, and maintained at the Microbiology Laboratory, Faculty of Medicine, Universitas Syiah Kuala prior to antibacterial testing. Bacterial colonies grown on Nutrient Agar (NA) were aseptically transferred using a sterile inoculation loop into a tube containing 0.9% NaCl solution and homogenized with a vortex mixer for 15 s. The turbidity of the suspension was adjusted to McFarland standard No. 3 (approximately 10 CFU/mL) and subsequently diluted with sterile 0.9% NaCl to obtain a final concentration of 10 CFU/mL [14], consistent with the standard aerobic bacterial sensitivity range (10–10 CFU/mL) [14]. The optical density was measured at 625 nm using a spectrophotometer, and suspensions with absorbance values between 0.08 and 0.13 were considered standardized and suitable for antibacterial assays [14].

2.4 Bioactivity Assay Procedure

The antibacterial activity of the F2B10 fraction was evaluated using the disc diffusion method against *Escherichia coli* O157:H7. A standardized bacterial suspension was uniformly inoculated onto sterile Nutrient Agar (NA) plates using the spread plate technique. The suspension was evenly distributed across the agar surface in three directions, rotating the Petri dish by 60° between streaking steps to ensure homogeneous bacterial coverage. Sterile paper discs were impregnated with the F2B10 fraction at concentrations of 20, 40, 60, 80, and 100 µg/mL and carefully placed onto the inoculated agar surface using sterile forceps, with gentle pressure applied to ensure full contact. Discs containing 2% dimethyl sulfoxide (DMSO) served as the negative control, while chloramphenicol discs (30 µg/mL) were used as the positive control [4,5]. All plates were incubated at 37 °C for 12–24 h. After incubation, antibacterial activity was assessed by measuring the diameter of the inhibition zones (mm) using a digital caliper [6].

3 Results

Partitioning of the crude methanolic extract (A17A01) of *Rhizophora mucronata* leaves using chloroform:methanol:water (1:1:1, v/v) yielded two fractions: a semipolar fraction (F1B16, 0.02 g) and a polar fraction (F1B17, 1.96 g). Bioactivity screening against resistant Escherichia coli demonstrated that F1B17 exhibited a larger inhibition zone (8.25 mm), identical to that of chloramphenicol, and greater than both F1B16 and the crude extract (Tables 1 and 2). These findings indicate enrichment of antibacterial constituents in the polar fraction following solvent partitioning.

Table 1. Inhibition zone diameters of crude extract A17A01 against antibiotic-resistant *Escherichia coli*.

Sample	Concentration	Inhibition Zone Diameter (mm)
A17A01	100 μg/mL	7.25
DMSO (–)	2%	0
Chloramphenicol (+)	30 μg/mL	7.75

Table 2. Inhibition zone diameters of crude extract and fractions against antibiotic-resistant *Escherichia coli*.

Sample	Concentration	Inhibition Zone Diameter (mm)
A17A01	100 μg/mL	7.75
F1B16	100 μg/mL	7.25
F1B17	100 μg/mL	8.25
DMSO (–)	2%	0.0
Chloramphenicol (+)	30 μg/mL	7.75

Further fractionation of F1B17 by elution and thin-layer chromatography (TLC) revealed compounds with retention factor ($R_f > 0.5$). Subsequent partitioning of this fraction produced two sub-fractions: F2B10 (methanol fraction) and F2B11 (dichloromethane fraction). Phytochemical screening confirmed the presence of alkaloids in F2B10 and hydrocarbons in F2B11 (Table 3).

Table 3. Phytochemical screening results of fractions F2B10 and F2B11.

Name of Compound	Reagent	Discoloration	Remark	
			F2B10	F2B11
Hydrocarbons	Cerium sulfate	Blackish color spots	++	-
Alkaloid	Dragendorff	Orange colored spots	++	+

Remark: (++) Moderate; (+) Weak; (–) None.

Interestingly, since antibacterial assays showed that F2B10 exhibited a larger inhibition zone (8.50 mm) compared to F2B11 (7.50 mm) and the standard antibiotic chloramphenicol (7.00 mm). These results demonstrate that F2B10 possessed the strongest antibacterial activity among the tested fractions. Dose-response evaluation of F2B10 at concentrations of 20, 40, 60, 80, and 100 µg/mL showed measurable antibacterial effects across all concentrations (Table 4).

Table 4. Inhibition zone diameters of fractions F2B10 and F2B11 against antibiotic-resistant *Escherichia coli*.

Sample	Concentration	Inhibition Zone Diameter (mm)
F2B10	100 μg/mL	8.50
F2B10	80 μg/mL	7.70
F2B10	60 μg/mL	7.60
F2B10	40 μg/mL	7.55
F2B10	20 μg/mL	7.50
F2B11	100 μg/mL	7.50
DMSO (–)	2%	0.0
Chloramphenicol (+)	30 μg/mL	7.75

4 Discussion

The initial bioactivity screening demonstrated that the crude methanolic extract (A17A01) exhibited an inhibition zone comparable to chloramphenicol (7.25 mm), while the negative control (2% DMSO) showed no inhibitory effect. This confirms that the observed antibacterial activity was attributable to bioactive constituents within the extract rather than solvent interference. The presence of antibacterial compounds in *Rhizophora mucronata* leaves is consistent with previous reports identifying diverse secondary metabolites, including alkaloids, flavonoids, phenolics, terpenoids, peptides, and saponins, as contributors to antimicrobial activity [2–4].

Bioactivity-guided fractionation via Thin Layer Chromatography (TLC) enabled the enrichment of active constituents, particularly within fractions

exhibiting retention factor (R_f) values greater than 0.5. The selection of fractions based on chromatographic mobility reflects differential polarity and affinity of bioactive compounds toward the stationary and mobile phases. Visualization using cerium sulfate and Dragendorff's reagents further supported the presence of alkaloid-type compounds in the active fraction.

Among the sub-fractions obtained, F2B10 displayed superior antibacterial activity compared to F2B11 and even surpassed chloramphenicol at equivalent concentrations. The dose–response evaluation of F2B10 revealed a clear concentration-dependent increase in inhibition zone diameter, reaching 8.50 mm at 100 µg/mL. This pattern suggests a pharmacologically relevant interaction between the active compounds and bacterial cellular targets, where higher concentrations enhance disruption of essential microbial processes such as membrane integrity, nucleic acid synthesis, or protein biosynthesis [10–13].

The stronger activity observed in F2B10 relative to F2B11 is consistent with phytochemical screening results indicating the presence of alkaloids in F2B10. Alkaloids are well-documented antimicrobial agents that can intercalate with DNA, inhibit topoisomerase activity, alter membrane permeability, and disrupt enzymatic pathways critical for bacterial survival [1]. In contrast, the hydrocarbon-rich fraction (F2B11) exhibited weaker antibacterial activity, suggesting that non-polar constituents may contribute less significantly to antimicrobial efficacy in this system. The polarity of F2B10 and its methanol solubility further support the hypothesis that polar alkaloid compounds are primarily responsible for the observed antibacterial effect.

These findings align with previous studies reporting antibacterial properties of secondary metabolites from *R. mucronata* [2,3]. Notably, fractions with higher chromatographic mobility ($R_f > 0.5$) may contain moderately polar compounds with enhanced biological activity. Given the escalating global challenge of antibiotic resistance, the identification of plant-derived fractions capable of inhibiting resistant Escherichia coli is of considerable therapeutic relevance.

However, while inhibition zone measurements provide preliminary evidence of antibacterial potential, further investigations are required to determine minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), cytotoxicity profiles, and structural elucidation of the active compounds. Comprehensive spectroscopic analyses (e.g., NMR, MS) would be essential to characterize the alkaloid constituents responsible for the activity observed in F2B10. Such studies would strengthen the potential of *R. mucronata*–derived metabolites as candidates for novel antimicrobial development.

5 Conclusion

The present study demonstrates that the methanolic leaf extract of *Rhizophora mucronata* and its chromatographic fractions exhibit measurable antibacterial activity against resistant *Escherichia coli* O157:H7. Bioactivity-guided fractionation identified F2B10 ($R_f > 0.5$) as the most active fraction, displaying a clear dose-dependent response and achieving a maximum inhibition zone of 8.50 mm at 100 µg/mL. Notably, this activity exceeded that of the reference antibiotic chloramphenicol under the tested conditions.

Phytochemical screening confirmed the presence of alkaloid constituents in F2B10, suggesting that polar, alkaloid-rich compounds are primarily responsible for the observed antibacterial effect. These findings highlight the therapeutic potential of *R. mucronata*–derived secondary metabolites as promising candidates for alternative antimicrobial development, particularly in addressing antibiotic-resistant pathogens. Further investigations involving compound purification, structural elucidation, and mechanistic studies are necessary to validate their pharmacological potential and explore their suitability for future drug development.

Data Availability Statement

Data will be made available on request.

Author Contributions

N.N. conceptualized and designed the study and was responsible for methodology development, formal analysis, laboratory investigation, data curation, visualization, and preparation of the original manuscript draft. S.A. conducted validation of the research findings. All authors have read and approved the final version of the manuscript prior to publication.

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Conflicts of Interest

The authors declare no conflicts of interest.

Ethical Approval and Consent to Participate

Not applicable.

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