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RESEARCH ARTICLE

Bioactive Alkaloids from *Callyspongia* sp.: A Marine Source of Antibacterial Agents Against Drug-Resistant *Echerichia coli*

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Abstract

This study evaluated the antibacterial potential of secondary metabolites isolated from the Indonesian marine sponge *Callyspongia* sp. against antibiotic-resistant *Escherichia coli*. Sponge specimens collected from Sabang waters were subjected to extraction, bioassay-guided fractionation, and chromatographic purification. Fourier transform infrared (FTIR) analysis of the active compound revealed absorption bands at 1637.4 cm¹ (C=N) and 1407.2 cm¹ (C–N), consistent with a hydroxylated alkaloid structure. Antibacterial activity was determined using the disk diffusion method. The isolated compound exhibited dose-dependent inhibition, producing inhibition zones of 7.0 mm at 20 µg/mL and 8.25 mm at 100 µg/mL. Chloramphenicol (35 µg/mL) showed a 7.5 mm inhibition zone. These findings suggest that *Callyspongia* sp. represents a promising source of alkaloid-based antibacterial agents against resistant pathogens.

Keywords: Alkaloid, *Callyspongia* sp., *Escherichia coli*, Antibiotic-resistant bacteria.

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1 Introduction

Escherichia coli (*E. coli*) is a gram-negative bacterium commonly found in the gastrointestinal tract of humans and animals. While most strains are harmless, certain pathogenic variants can cause severe illnesses, including diarrhea, urinary tract infections, and sepsis [1]. Globally, diarrheal diseases remain a major public health concern, with nearly 1.7 billion cases reported annually among children, resulting in approximately 525,000 deaths among children under five years of age [2].

The widespread use and misuse of antibiotics have led to the emergence of antibiotic-resistant E. coli strains, complicating treatment strategies and increasing morbidity and mortality rates. Resistance mechanisms including the production of extended-spectrum -lactamases (ESBLs), activation of efflux pumps, and biofilm formation enable these bacteria to survive antibiotic exposure [3]. Consequently, there is an urgent need to discover and develop new antimicrobial agents capable of effectively combating resistant bacterial strains. In this context, marine ecosystems have emerged as a promising source of novel bioactive compounds. Marine sponges, in particular, are prolific producers of secondary metabolites with diverse biological activities, including antibacterial, antiviral, antifungal, and anticancer properties [4]. The genus *Callyspongia*, widely distributed in tropical and subtropical marine environments, has been reported to produce various bioactive compounds, notably

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alkaloids, which exhibit significant antimicrobial activities [5].

However, despite the therapeutic potential of sponge-derived compounds, the ability of bacteria to develop resistance remains a challenge. Bacteria can adapt to bioactive compounds through several mechanisms, such as modifying drug targets, decreasing drug uptake, or increasing efflux activity [6]. Therefore, continuous exploration and characterization of novel compounds from marine sponges are essential to stay ahead in the fight against antibiotic-resistant pathogens. This study aims to investigate the antibacterial activity of alkaloid compounds isolated from the Indonesian marine sponge *Callyspongia* sp. against antibiotic-resistant *E. coli* strains. Through isolation, characterization, and bioactivity evaluation, this research seeks to contribute to the development of new antimicrobial agents derived from marine natural products.

2 Methodology

2.1 General

The instruments and laboratory equipments used in this study included an analytical balance (Kern), a Fourier-transform infrared (FTIR) spectrometer, a rotary evaporator (Eyela N-1000), an incubator (Memmert Type INB 500), an autoclave (Tommy SX-300 / 500/700), and a laminar airflow cabinet (Safe Fast Elite 212 SD). Additional equipment included a UV lamp (UVGL-25), hot plate (Akebono), oven (Jouan), refrigerator (LG), thin-layer chromatography (TLC) apparatus, column chromatography setup, and standard glassware such as Petri dishes, beakers, test tubes, and separatory funnels (Pyrex). Micropipettes used were Pipetteman P20 F123563 (2–20 µL) and Eppendorf micropipettes (100–1000 µL). Other standard laboratory tools and consumables were utilized as required.

2.2 Bacterial Strain and Culture Conditions

A clinical isolate of antibiotic-resistant *Escherichia coli* O157:H7 was obtained from a patient at the Zainoel Abidin General Hospital, Banda Aceh, Indonesia. The strain was maintained and cultured at the Microbiology Laboratory, Faculty of Medicine, Univesitas Syiah Kuala. For the preparation of bacterial cultures, 2.8 g of nutritional broth (NB) was dissolved in 100 mL of distilled water and sterilized by autoclaving at 121 °C for 15 minutes. After cooling under aseptic conditions in a laminar airflow cabinet, the medium was supplemented with chloramphenicol (30 µg/mL), poured into sterile Petri dishes and allowed to solidify at room temperature. The resistant *E. coli* strain was inoculated onto the medium using the streak plate method in a zigzag pattern and incubated at 37°C for 24 hours [12].

2.3 Biomaterial

Specimens of the marine sponge Callyspongia sp. were collected from the coastal waters of Sabang, Indonesia, in March 2017. The sponge samples were morphologically identified, thoroughly washed with seawater to remove debris, chopped into small pieces, and air-dried. The dried material was macerated in methanol P.A. for 72 hours (3 × 24-hour cycles). The extract was filtered and concentrated under reduced pressure using a rotary evaporator to obtain the crude methanolic extract. Phytochemical screening was performed at the Marine Chemistry Laboratory, while antibacterial assays were conducted at the Microbiology Laboratory, Faculty of Medicine, Universitas Syiah Kuala.

2.4 Extraction and Isolation

A total of 1.48 g of the crude methanolic extract was subjected to solvent partitioning using chloroform, methanol, and water (1:1:1, v/v/v). The mixture was vigorously shaken and allowed to stand for phase separation. Two fractions were obtained: a semi-polar chloroform-rich fraction (A1B17) and a polar methanol–water-rich fraction (A1B18). Each fraction was evaporated to dryness and evaluated for antibacterial activity against the resistant E. coli strain. The fraction exhibiting the largest inhibition zone was selected for further purification. The most active fraction (A1B18) was subjected to gradient column chromatography using silica gel as the stationary phase and dichloromethane–methanol mixtures of increasing polarity as the mobile phase. Two subfractions, A12B03 and A12B04, were collected. Separation was monitored by thin-layer chromatography (TLC). Subfraction A12B04, which displayed a single major spot on TLC, was selected for further characterization.

3 Results

3.1 Phytochemical screening

Qualitative phytochemical profiling of the methanolic crude extract (A1B12) derived from the marine sponge *Callyspongia* sp. revealed a chemically diverse metabolite composition, encompassing alkaloids, peptides, steroids, terpenoids, and hydrocarbons.

The presence of alkaloids was evidenced by the formation of an orange precipitate following treatment with Dragendorff's reagent, indicative of nitrogen-containing heterocyclic compounds. Peptidic constituents were confirmed by the development of a characteristic purple coloration upon reaction with ninhydrin, suggesting the presence of primary and secondary amine functionalities. Steroidal and terpenoid metabolites were identified through Salkowski's and Liebermann–Burchard assays, respectively, based on their diagnostic color transitions. Hydrocarbon constituents were inferred from the observation of dark, UV-active spots on thin-layer chromatography (TLC) plates. Notably, flavonoids were not detected in the crude extract (Table 1), consistent with the predominantly marine origin of the sample and its characteristic secondary metabolite profile.

Table 1. Qualitative phytochemical screening of methanolic extract (A1B12) from *Callyspongia* sp.

Secondary Metabolite	Reagent	Remark
Hydrocarbon	Cerium sulfate	+++
Alkaloid	Dragendorff	+++
Peptide	Ninhydrin	+++
Terpenoid	Liebermann–Burchard	+++
Steroid	Salkowski	+++
Flavonoid	Base reagent	–

3.2 Primary Bioactivity test

The antibacterial activity of the crude extract A1B12 was evaluated against antibiotic-resistant *Escherichia coli* using the disk diffusion assay. An inhibition zone of 9.5 mm was observed at an extract concentration of 100 µg/mL, whereas the positive control exhibited an inhibition zone of 9.0 mm (Table 2). Based on these results, extract A1B12 was selected for further fractionation and subsequent analyses.

Table 2. Antibacterial activity of crude extract A1B12 against antibiotic-resistant *Escherichia coli* determined by disk diffusion assay.

Sample	Concentration	Inhibition Zone (mm)
Chloramphenicol (+)	35 μg/mL	9.0
DMSO (–)	2%	0
A1B12	100 μg/mL	9.5

3.3 Isolation of active compound

Since the crude extract exhibited antibacterial activity, it was subjected to liquid–liquid partitioning using a chloroform–methanol–water system (1:1:1, v/v/v) to achieve polarity-based fractionation. This procedure afforded two fractions: a semi-polar fraction (A1B17, 0.07 g) and a polar fraction (A1B18, 1.41 g). Qualitative phytochemical profiling indicated that A1B18 was enriched in nitrogen-containing constituents, particularly alkaloids and peptidic compounds, whereas A1B17 predominantly contained non-polar hydrocarbon components. Antibacterial evaluation against resistant Escherichia coli demonstrated that A1B18 produced a larger inhibition zone (8.5 mm) than A1B17 (7.5 mm), suggesting that the antibacterial activity is primarily associated with polar, nitrogenous metabolites. On the basis of these findings, fraction A1B18 was prioritized for further purification by silica gel column chromatography to isolate the active constituent(s).

3.4 Characterization of the active compound

Silica gel column chromatography of fraction A1B18, employing a gradient elution system of dichloromethane–methanol, afforded two subfractions: A12B03 (0.11 g) and A12B04 (0.30 g). Thin-layer chromatography (TLC) analysis indicated that A12B04 contained a single predominant component, as evidenced by a single major spot under UV detection (Figure 1a). Structural characterization of A12B04 by Fourier transform infrared (FTIR) spectroscopy revealed diagnostic absorption bands at 3271.4 cm^{1} (O–H stretching), 2949.0 and 2835.0 cm^{1} (aliphatic C–H stretching), 1637.4 cm^{1} (C=N stretching), and 1407.2 cm^{1} (C–N stretching) (Figure 1b). The presence of imine (C=N) and C–N functionalities, together with hydroxyl stretching, is consistent with a hydroxylated alkaloid framework.

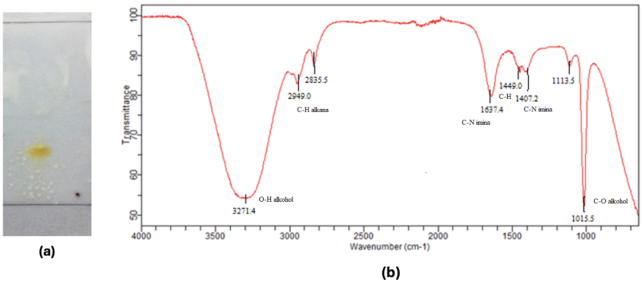

Figure 1. Draggendorf Visualization (a), FTIR spectra (b).

The antibacterial activity of subfraction A12B04 was further evaluated across a concentration range of 20–100 µg/mL to assess dose-dependent effects. As summarized, inhibition zones of 7.0, 7.15, 7.25, 8.10, and 8.25 mm were observed at concentrations of 20, 40, 60, 80, and 100 µg/mL, respectively (Figure 2). The progressive increase in inhibition zone diameter

with increasing concentration indicates a clear dose-dependent antibacterial response, supporting the bioactivity of the isolated compound against resistant *E.coli*.

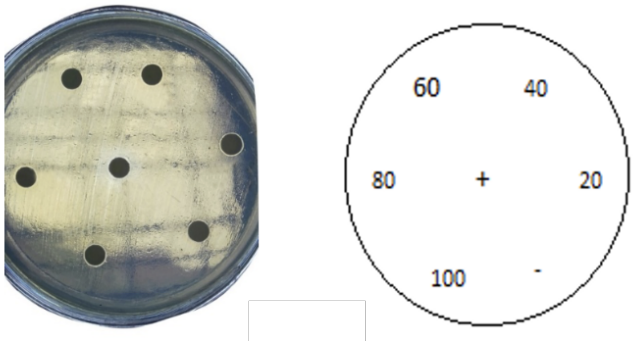

Figure 2. Bioactivity results

Table 3. Dose-dependent antibacterial activity of subfraction A12B04 against antibiotic-resistant *Escherichia coli* determined by disk diffusion assay.

Compound	Concentration	Inhibition Zone (mm)
Chloramphenicol (+)	35 μg/mL	7.52
DMSO (–)	2%	0.00
A12B04	100 μg/mL	8.27
A12B04	80 μg/mL	8.13
A12B04	60 μg/mL	7.25
A12B04	40 μg/mL	7.15
A12B04	20 μg/mL	7.06

4 Discussion

Phytochemical profiling of the methanolic extract (A1B12) of *Callyspongia* sp. revealed a chemically diverse metabolite composition, including alkaloids, peptides, steroids, terpenoids, and hydrocarbons. Marine sponges are well established as prolific producers of structurally unique secondary metabolites with pronounced biological activities, particularly nitrogen-containing compounds. The absence of flavonoids is consistent with previous reports, as these metabolites are predominantly biosynthesized by terrestrial plants rather than marine invertebrates [7]. Among the detected constituents, alkaloids and peptides are particularly noteworthy due to their documented antimicrobial properties. Marine-derived alkaloids have been shown to exert antibacterial effects through multiple mechanisms, including disruption of cell wall biosynthesis, inhibition of nucleic acid replication, and interference with protein synthesis pathways [8]. Antimicrobial peptides, in contrast, typically act via membrane-targeting mechanisms, leading to pore formation, membrane destabilization, and eventual bacterial cell lysis [9]. The coexistence of these metabolite classes in A1B12 suggests a multifactorial basis for the observed antibacterial activity.

The crude extract A1B12 demonstrated moderate inhibitory activity against antibiotic-resistant *E.coli*, producing an inhibition zone of 9.5 mm, slightly exceeding that of chloramphenicol (9.0 mm). Although classified as weak to moderate according to standard interpretative criteria [10], this level of activity is notable given that the extract represents a complex and unrefined mixture tested against a resistant clinical strain. These findings support the presence of bioactive constituents and justify subsequent bioassay-guided fractionation. Liquid–liquid partitioning of A1B12 yielded a polar fraction (A1B18) and a semi-polar fraction (A1B17), with A1B18 exhibiting superior antibacterial activity (8.5 mm inhibition zone). Phytochemical analysis indicated enrichment of alkaloids and peptides in the polar fraction, whereas A1B17 was dominated by non-polar hydrocarbons. The enhanced activity of A1B18 suggests that polar nitrogen-containing metabolites are the principal contributors to the antibacterial effect, consistent with previous studies on sponge-derived alkaloids [11].

Further purification of A1B18 via silica gel column chromatography afforded subfraction A12B04 as the major constituent. FTIR analysis of A12B04 revealed characteristic absorption bands corresponding to O–H, C=N (imine), and C–N functionalities, supporting its classification as a hydroxylated alkaloid. These structural features are commonly associated with bioactive marine alkaloids and are often implicated in hydrogen-bonding interactions and nucleophilic reactivity relevant to antibacterial mechanisms. Dose–response evaluation of A12B04 demonstrated a concentration-dependent increase in antibacterial activity, with inhibition zones ranging from 7.0 mm at 20 µg/mL to 8.25 mm at 100 µg/mL. The observed trend indicates pharmacological responsiveness and supports the hypothesis that A12B04 acts through direct interaction with bacterial cellular targets. The comparable activity of A12B04 at higher concentrations to chloramphenicol further underscores its potential as a lead scaffold for antibacterial development.

Collectively, these findings identify *Callyspongia* sp. as a promising source of bioactive alkaloids with activity against antibiotic-resistant *E. coli*. Comprehensive structural elucidation using advanced spectroscopic techniques (e.g., NMR and MS) and

mechanistic investigations are warranted to confirm the molecular identity of A12B04 and to further define its antibacterial mode of action.

5 Conclusion

This study demonstrates that the methanolic extract of the marine sponge *Callyspongia* sp., collected from the coastal waters of Sabang, Indonesia, contains multiple classes of bioactive secondary metabolites, including alkaloids, peptides, terpenoids, steroids, and hydrocarbons. Among these, alkaloids and peptidic constituents appear to be the primary contributors to the observed antibacterial activity against antibiotic-resistant *Escherichia coli* O157:H7.

Bioassay-guided fractionation revealed that the polar fraction (A1B18) exhibited greater antibacterial potency than the semi-polar fraction, supporting the role of polar, nitrogen-containing metabolites in mediating the bioactivity. Subsequent chromatographic purification afforded a predominant compound, A12B04, which was tentatively characterized as a hydroxylated alkaloid based on FTIR spectral features. The compound displayed a concentration-dependent antibacterial response, with inhibition zones comparable to those of chloramphenicol at higher concentrations.

Therefore, these findings highlight *Callyspongia* sp. as a promising marine source of alkaloid-based antibacterial scaffolds. Comprehensive structural elucidation (e.g., NMR and MS analyses) and mechanistic investigations are necessary to confirm the molecular identity of A12B04 and to further evaluate its potential as a lead compound for the development of therapeutics targeting multidrug-resistant bacterial pathogens.

Data Availability Statement

Data will be made available on request.

Author Contributions

S.A. and S.K. contributed to the conceptualization of the study. A.M. performed the methodology, formal analysis, investigation, data curation, visualization, and preparation of the original draft. Validation was carried out by A.M., S.A., and S.K. All authors have read and agreed to the published version of the manuscript.

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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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