SCIENTIA NATURALIS LETTERS

SCIENTIFIA

Article

Academic Editor: Katherine Maloney

Received: 3 June 2025
Revised: 18 June 2025
Accepted: 19 June 2025
Published: 25 June 2025

Citation: *Sci. Nat. Lett.* 2025, 1, 1, 1–6

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

Andria Mauliza [1,*], Sri Agustina [1] and Sofyatuddin Karina [1]

[1] Department of Marine Science, Faculty of Marine and Fisheries, Syiah Kuala University, Banda Aceh 23111, Indonesia
* Correspondence: andriamauliza@hotmail.com.

Abstract: This study investigated the antibacterial potential of secondary metabolites from the Indonesian marine sponge *Callyspongia* sp. against antibiotic-resistant *Escherichia coli*. Conducted between November 2017 and January 2018, the research involved extraction, isolation, and characterization of bioactive compounds at Syiah Kuala University's Marine Chemistry, Microbiology, and Instrumentation Laboratories. Isolation was guided by bioactivity, using chromatographic techniques. FTIR spectral analysis identified functional groups indicative of alkaloids, with imine (C=N) absorption at 1637.4 cm^{-1} and fingerprint confirmation at 1407.2 cm^{-1}. Bioactivity testing showed inhibition zones of 7 mm and 8.25 mm at concentrations of 20 µg/mL and 100 µg/mL, respectively. The positive control, chloramphenicol (35 µg/mL), exhibited a 7.5 mm inhibition zone. These findings suggest that alkaloid compounds from *Callyspongia* sp. possess promising antibacterial activity comparable to conventional antibiotics. This highlights the potential of *Callyspongia* sp. as a source of novel antibacterial agents against resistant bacterial strains.

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

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 significant public health concern, with nearly 1.7 billion cases reported annually among children, leading to approximately 525,000 deaths in children under five years of age [2]. The widespread use and misuse of antibiotics have led to the emergence of antibiotic-resistant strains of *E. coli*, complicating treatment strategies and increasing morbidity and mortality rates. Resistance mechanisms such as the production of extended-spectrum β-lactamases (ESBLs), efflux pumps, and biofilm formation enable these bacteria to survive antibiotic treatments [3]. Consequently, there is an urgent need to discover and develop new antimicrobial agents that can effectively combat resistant bacterial strains. 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 alkaloids, which exhibit significant antimicrobial activities [5]. Despite the potential of sponge-derived compounds, the development of resistance by target bacteria remains a challenge. Bacteria can adapt to bioactive compounds through various

mechanisms, such as modifying drug targets, reducing drug uptake, or increasing efflux [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. By isolating, characterizing, and evaluating the bioactivity of these compounds, we hope to contribute to the development of new antimicrobial agents derived from marine natural products.

2. Results

2.1. Phytochemical screening

Phytochemical screening of the methanolic crude extract (A1B12) from the marine sponge *Callyspongia* sp. revealed the presence of several classes of secondary metabolites, including alkaloids, peptides, steroids, terpenoids, and hydrocarbons. The alkaloids were indicated by an orange precipitate with Dragendorff's reagent, while the presence of peptides was confirmed by purple coloration with ninhydrin, suggesting primary and secondary amine groups. Steroids and terpenoids were detected via Salkowski's and Liebermann-Burchard's tests, respectively, based on characteristic color reactions. Hydrocarbons were identified by dark UV-active spots on TLC plates. Flavonoids were not detected in the crude extract (Table 1).

Table 1. The results of phytochemical screening.

Secondary Metabolite	Reagent	Remark
Hydrocarbon	Cerium Sulphate	+++
Alkaloid	Dragendroff	+++
Peptide	Ninhydrin	+++
Terpenoid	Lieberman-Burchard	+++
Steroid	Salkowski	+++
Flavonoid	Base reagent	-

2.2. Primary Bioactivity test

The antibacterial activity of the crude extract A1B12 was tested against antibiotic-*resistant Escherichia coli* using the disk diffusion method. Inhibition zone of 9.5 mm was observed at extract concentration of 100 µg/mL while the positive control showed the Inhibition zone of 9.0 mm (Table 2). Therefore, we used A1B12 for the next steps.

Table 2. The primary bioactivity test.

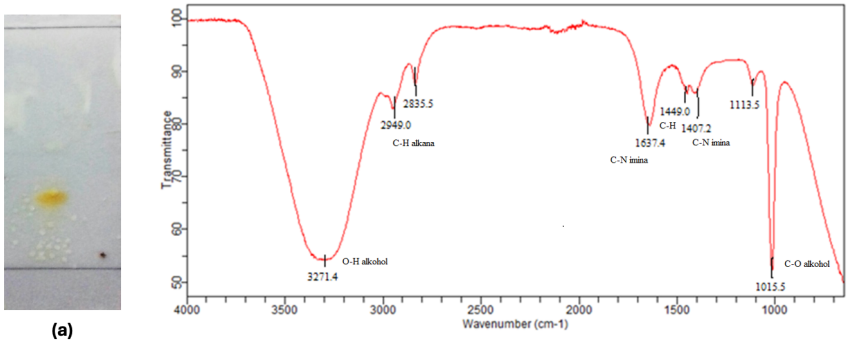
Sample	Concentration	Inhibition Zone (mm)
Chloramphenicol (+)	35 µg/mL	9.0
DMSO (-)	2%	0
A1B12	100 µg/mL	9.5

2.3.Isolation of active compound

Since the crude extract that showed activity, it was subjected to solvent partitioning using a mixture of chloroform, methanol, and water (1:1:1 v/v). This process yielded two fractions, a semi-polar fraction (A1B17, 0.07 g) and a polar fraction (A1B18, 1.41 g). Phytochemical screening of the two fractions revealed that A1B18 contained a higher abundance of alkaloids and peptides compared to A1B17, which was richer in non-polar hydrocarbons. Antibacterial testing of the two fractions against resistant *E. coli* showed that A1B18 exhibited a larger inhibition zone (8.5 mm) than A1B17 (7.5 mm), indicating stronger antibacterial activity in the polar fraction. As a result, A1B18 was selected for further purification using column chromatography.

2.1. Characterization of the active compound

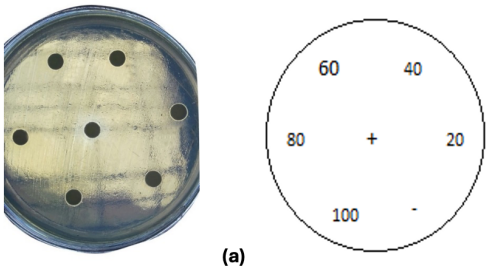
The silica-gel column chromatography of A1B18 has applied by a gradient system of dichloromethane-methanol yielded two sub-fractions: A12B03 (0.11 g) and A12B04 (0.3 g). TLC analysis showed that A12B04 contained a single major compound (Fig. 1a). The characterization of the compound by Fourier Transform Infrared (FTIR) spectroscopy of A12B04 revealed characteristic absorbance peaks at 3271.4 cm^{-1} (O–H stretching), 2949.0 and 2835.0 cm^{-1} (C–H stretching), 1637.4 cm^{-1} (C=N stretching), and 1407.2 cm^{-1} (C–N stretching), suggesting the presence of a hydroxylated alkaloid (Fig. 1b).



(a) (b)

Figure 1. Visualization of TLC by dragendroff's reagent. The orange spot on TLC indicate the presence of nitrogen-containing compound (a); FTIR spectra of active compound (b).

On the other hand, the bioactivity of A12B04 was evaluated at various concentrations (20, 40, 60, 80, and 100 µg/mL) to determine its dose-dependent antibacterial activity. The results showed that the inhibition zones of 7.0 mm, 7.15 mm, 7.25 mm, 8.10 mm, and 8.25 mm were observed, respectively. These results indicated increasing antibacterial activity with higher concentrations of the isolated compound (Fig.2).



(a)

Compound	Concentration	Inhibition Zone (mm)
Chloramphenicol (+)	35 µg/mL	7.52
DMSO (-)	2%	0.0
A12B04	100 µg/mL	8.27
	80 µg/mL	8.13
	60 µg/mL	7.25
	40 µg/mL	7.15
	20 µg/mL	7.06

(b)

Figure 2. Bioactivity results of A12B04. The inhibitor zone with various concentration (a-b).

3. Discussion

Authors The phytochemical screening of the methanolic crude extract (A1B12) of *Callyspongia* sp. revealed a diverse array of secondary metabolites, including alkaloids, peptides, steroids, terpenoids, and hydrocarbons. These classes of compounds are widely recognized for their biological activities, particularly in marine sponges, which are known producers of pharmacologically active natural products. The absence of flavonoids is consistent with other marine sponge studies, as flavonoids are predominantly associated with terrestrial plants [7].

Among the detected compounds, alkaloids and peptides have been frequently reported as potent antimicrobial agents. Alkaloids are known to inhibit bacterial growth through multiple mechanisms, including disruption of cell wall

synthesis, interference with nucleic acid functions, and protein biosynthesis inhibition [8]. Similarly, bioactive peptides can exert antimicrobial effects by interacting with bacterial membranes, leading to pore formation and subsequent cell lysis [9].

The crude extract A1B12 exhibited moderate antibacterial activity against antibiotic-resistant *E. coli*, with an inhibition zone of 9.5 mm, slightly higher than the positive control (chloramphenicol, 9.0 mm). Although this activity is categorized as weak to moderate based on standard classification [10]. it is significant considering the extract was unpurified and effective against a resistant strain. These results support the presence of active constituents in the crude extract and justify further purification. Partitioning of A1B12 into polar (A1B18) and semi-polar (A1B17) fractions enhanced our understanding of the active components. A1B18 demonstrated stronger antibacterial activity (8.5 mm inhibition) and contained higher concentrations of polar metabolites, particularly alkaloids and peptides. This finding suggests that these polar compounds are the principal contributors to the antibacterial effect, aligning with previous reports on sponge-derived alkaloids [11].

Further fractionation of A1B18 via silica-gel column chromatography led to the isolation of a major compound, A12B04. The FTIR spectrum of A12B04 confirmed the presence of hydroxylated alkaloid functionalities, including O–H, C=N (imine), and C–N groups, which are frequently observed in bioactive marine natural products. This molecular profile supports the hypothesis that A12B04 is a key antibacterial constituent of the extract. Dose-response analysis of A12B04 revealed a clear concentration-dependent increase in antibacterial activity. Inhibition zones expanded from 7.0 mm at 20 µg/mL to 8.25 mm at 100 µg/mL. This trend suggests that A12B04 acts in a dose-dependent manner, possibly via membrane disruption or interference with vital bacterial processes. The comparable activity of A12B04 at higher concentrations to chloramphenicol demonstrates its potential as a candidate for further pharmacological development.

Overall, these findings highlight *Callyspongia* sp. as a promising source of marine-derived alkaloids with antibacterial activity, particularly against antibiotic-resistant E. coli. Further studies, including structural elucidation (e.g., NMR, MS) and mechanism-of-action assays, are warranted to advance the therapeutic potential of compound A12B04.

4. Materials and Methods

4.1 Materials

The instruments and laboratory equipment used in this study included an analytical balance (Kern), Fourier-Transform Infrared (FTIR) spectrometer, rotary evaporator (Eyela N-1000), incubator (Memmert Type INB 500), 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 including Petri dishes, beakers, test tubes, and separatory funnels (all Pyrex brand). Micropipettes used were Pipetteman P20 F123563 (2–20 µL) and Eppendorf micropipette (100–1000 µL). Other standard laboratory tools and consumables were also utilized as required.

4.2 Bacterial Strain and Culture Conditions

A clinical isolate of antibiotic-resistant *Escherichia coli* O157:H7 was obtained from a patient at Dr. Zainoel Abidin General Hospital, Banda Aceh, Indonesia. The strain was maintained and cultured at the Microbiology Laboratory, Faculty of Medicine, Syiah Kuala University. To prepare the bacterial culture, 2.8 g of Nutrient Broth (NB) was dissolved in 100 mL of distilled water, sterilized by autoclaving at 121 °C for 2 hours, and cooled under aseptic conditions in a laminar airflow cabinet. The medium was supplemented with chloramphenicol at a concentration of 30 µg/mL, poured into sterile Petri dishes, and allowed to solidify at 30 °C for 24 hours. The resistant *E. coli* strain was inoculated onto the surface of the medium using the streak plate method in a zigzag pattern for cultivation [12].

4.3 Biomaterial Bacterial Strain and Culture Conditions

Specimens of the marine sponge *Callyspongia* sp. were collected from the coastal waters of Sabang, Indonesia, in March 2017. Sponge material was identified morphologically, then washed, chopped into small pieces, and air-dried. Dried samples were macerated in 96% methanol for 72 hours (3 × 24-hour cycles). The extract was filtered and concentrated under reduced pressure using a rotary evaporator. Phytochemical screening of the crude extract was conducted at the Marine Chemistry Laboratory, while subsequent antibacterial assays were performed at the Microbiology Laboratory, Faculty of Medicine, Syiah Kuala University.

4.4 Extraction and Isolation

A total of 1.48 g of the crude methanolic extract was subjected to solvent partitioning using a mixture of chloroform, methanol, and water (1:1:1, v/v). The mixture was shaken vigorously and allowed to separate for 10 minutes. Two distinct phases were obtained: a semi-polar fraction (chloroform-rich, designated A1B17) and a polar fraction (methanol-water-rich, designated A1B18). Each fraction was evaporated to dryness and tested for antibacterial activity against the resistant E. coli strain. The fraction that exhibited the most prominent zone of inhibition was selected for further fractionation and compound isolation. The most active fraction (A1B18) was subjected to gradient column chromatography using silica gel as the stationary phase and a dichloromethane-methanol mobile phase in increasing polarity. Two sub-fractions were collected: A12B03 and A12B04. Thin-layer chromatography (TLC) was used to monitor compound separation. A12B04, which showed a single major compound on TLC, was selected for further characterization.

5. Conclusions

This study demonstrated that the methanolic extract of the marine sponge *Callyspongia* sp., collected from the waters of Sabang, contains several classes of bioactive secondary metabolites, including alkaloids, peptides, terpenoids, steroids, and hydrocarbons. Among these, alkaloids and peptides were identified as the most likely contributors to the observed antibacterial activity against antibiotic-resistant *Escherichia coli* O157:H7.

Through bioactivity-guided fractionation, the polar fraction (A1B18) was found to exhibit stronger antibacterial effects than the semi-polar fraction. Subsequent purification yielded a single major compound, A12B04, characterized as a hydroxylated alkaloid based on FTIR spectral data. The compound exhibited dose-dependent antibacterial activity, with an inhibition zone comparable to that of the standard antibiotic chloramphenicol. These findings support the potential of *Callyspongia* sp. as a promising source of novel antimicrobial agents, particularly in the ongoing search for effective treatments against multidrug-resistant bacterial infections. Further research, including compound structure elucidation and mechanism-of-action studies, is recommended to develop these sponge-derived compounds into therapeutic candidates.

Supplementary Materials: The following supporting information can be downloaded at: https://www.scientifia.com/article/doi.

Author Contributions: S.A and S.K contributed to the conceptualization of the study. Methodology, formal analysis, investigation, data curation, visualization, and original draft preparation were performed by A.M. 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.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable. No ethical approval was required for this study.

Data Availability Statement: The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgments: The authors would like to thank the Faculty of Marine and Fisheries and the Faculty of Medicine at Universitas Syiah Kuala for providing research facilities and institutional support. The technical support provided by the Marine Chemistry and Microbiology Laboratory staff is greatly appreciated. The authors also acknowledge RSUD Dr. Zainoel Abidin, Banda Aceh, for granting access to the Escherichia coli O157:H7 clinical isolate utilized in this study.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Diarrhoeal disease. World Health Organization: https://www.who.int/news-room/fact-sheets/detail/diarrhoeal-disease (accessed on 15 April 2025).
2. *E. coli*. World Health Organization: https://www.who.int/news-room/fact-sheets/detail/e-coli (accessed on 18 April 2025).
3. Álvarez-Martínez, F.J.; Barrajón-Catalán, E.; Micol, V. Tackling antibiotic resistance with compounds of natural origin: A comprehensive review. *Biomedicines* **2020**, *8*(10), p.405.
4. Varijakzhan, D.; Loh, J.Y.; Yap, W.S.; Yusoff, K.; Seboussi, R.; Lim, S.H.E.; Lai, K.S.; Chong, C.M. Bioactive compounds from marine sponges: Fundamentals and applications. *Marine drugs* **2021**, *19*(5), p.246.
5. de Sousa, L.H.N.; de Araújo, R.D.; Sousa-Fontoura, D.; Menezes, F.G.; Araújo, R.M. Metabolities from marine sponges of the genus *Callyspongia*: Occurrence, biological activity, and NMR data. *Marine Drugs* **2021**, *19*(12), p.663.

6. Poirel, L.; Madec, J.Y.; Lupo, A.; Schink, A.K.; Kieffer, N.; Nordmann, P.; Schwarz, S. Antimicrobial resistance in *Escherichia coli*. *Microbiology spectrum* **2018**,*6*(4), pp.10-1128.
7. Laport, M.S.; Santos, O.C.S.; Muricy, G. Marine sponges: potential sources of new antimicrobial drugs. *Current pharmaceutical biotechnology* **2009**,*10*(1), pp.86-105.
8. Elissawy, A.M.; Soleiman Dehkordi, E.; Mehdinezhad, N.; Ashour, M.L.; Mohammadi Pour, P. Cytotoxic alkaloids derived from marine sponges: A comprehensive review. *Biomolecules* **2021**, *11*(2), p.258.
9. Hancock, R.E.; Haney, E.F.; Gill, E.E. The immunology of host defence peptides: beyond antimicrobial activity. *Nature Reviews Immunology* **2016**, *16*(5), pp.321-334.
10. Alksne, L.E.; Projan, S.J. Bacterial virulence as a target for antimicrobial chemotherapy. *Current opinion in Biotechnology* **2000**, *11*(6), pp.625-636.
11. Hong, L.L.; Ding, Y.F.; Zhang, W.; Lin, H.W. Chemical and biological diversity of new natural products from marine sponges: a review (2009–2018). *Marine Life Science & Technology* **2022**, *4*(3), pp.356-372.
12. Bian, C.; Wang, J.; Zhou, X.; Wu, W.; Guo, R. Recent advances on marine alkaloids from sponges. *Chemistry & Biodiversity* **2020**, *17*(10), p.e2000186.
13. Mbah, J.A.; Ngemenya, M.N.; Abawah, A.L.; Babiaka, S.B.; Nubed, L.N.; Nyongbela, K.D.; Lemuh, N.D.; Efange, S.M. Bioassay-guided discovery of antibacterial agents: in vitro screening of Peperomia vulcanica, Peperomia fernandopoioana and Scleria striatinux. *Annals of Clinical Microbiology and Antimicrobials* **2012**, *11*, pp.1-10.