

Evaluation and profiling of an anti-mycobacterium active compound derived from Indonesian marine sponge *Aptos* sp.

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Abstract. The sponge *Aptos* sp. from Morotai Island contains chemical compounds with pharmaceutical potential. To identify the specific substances active against *Mycobacterium smegmatis* a bioassay-guided isolation strategy was reproduced. This study aimed to profile the bioactive compounds from *Aptos* sp. and evaluate their anti-mycobacterial activity. A bioassay-guided fractionation approach was applied, partitioning the crude methanol extract with water, butanol, and ethyl acetate. The ethyl acetate phase demonstrated the most effective antibacterial activity. The active compound was purified using open-column chromatography, yielding a single band, as confirmed by thin-layer chromatography. The liquid chromatography-tandem mass spectrometry analysis identified the compound as aaptamine. The minimum inhibitory concentration value of isolated aaptamine against *M. smegmatis* was 250 $\mu\text{g mL}^{-1}$ higher than the positive control rifampicin of 62.5 $\mu\text{g mL}^{-1}$.

Key Words: aaptamine, fractionation, MIC, Morotai Island, tuberculosis.

Introduction. Marine ecosystems provide habitats for diverse life, including algae, coral reefs, sponges, and microorganisms (Carroll et al 2019). Marine organisms, especially invertebrates, are considered a valuable source for novel drug candidates because they produce biologically active compounds (Malve 2016; Carroll et al 2019). The secondary metabolites of marine sponges exhibit broad structural diversity and a range of biological activities, rendering them a promising source of drugs. Sponges are notable for their aspects of secondary metabolite chemistry and the diverse microbial biodiversity associated with sponges (Taylor et al 2007; Li et al 2020). In nature, secondary metabolites serve as a defense system to protect themselves from predators, combat microbial infections, shield against UV radiation, and maintain beneficial symbiotic partnerships with microbes (Webster & Taylor 2012; Kiran et al 2018). Sponges produce diverse secondary metabolites with potent biological activities, including antibacterial, antiviral, antifungal, antimalarial, anticancer, anti-fouling, and immunosuppressive effects, making them a valuable resource for developing therapeutic drugs (Mehub et al 2014; Anjum et al 2016).

Natural sources are a significant resource for developing medicines, especially those focused on treating bacterial infections (Martin et al 2014). Sponges contain metabolite compounds that are capable of counteracting and inhibiting pathogenic bacteria. This unique ability makes them an interesting source, especially as new antibacterial agents (Varijakzhan et al 2021). The diverse sponges found in Indonesian waters are a source of fascinating and potentially valuable bioactive compounds (Hanif et al 2019). Researchers have discovered promising common bioactive compounds in native Indonesian sponges, including manzamines (Rao et al 2003; Rao et al 2004), theonellapeptolides (Haedar et al 2004), and aaptamines (Hamada et al 2019).

Aptamine is a unique marine molecule with an interesting structure and belongs to the marine alkaloid group within the 1H-benzo[de]-1,6-naphthyridine. It has been isolated from various sponge genera, including *Aptos*, *Suberites*, *Luffariella*, *Hymeniacidon*, *Suberea*, and *Xestospongia*. The sponge *Aptos* are the principal origin of aptamine and its derivatives (Trang et al 2022).

The genus *Aptos* is frequently investigated as a source of cytotoxic agents (Fristiohady et al 2020). This study reveals the potential of *Aptos* sp. to produce antituberculosis compounds. To achieve the objectives, extraction, metabolite separation, preliminary chemical characterization, and confirmation of antimycobacterial activity was performed to identify the potential active compounds. This discovery is vital for the development of new tuberculosis treatments, which remain a critical global health challenge.

Material and Method

Sample collection, extraction, and partition of active compound. The marine sponge *Aptos* sp. (Figure 1) was collected using SCUBA at a depth of 5-10 m offshore of Morotai Island, North Maluku Province, Indonesia. The fresh samples were immediately stored at -18°C until the metabolites were extracted. The extraction process was carried out by cutting the sponge into small pieces, weighing 100 g, and macerating with methanol (1:1) in 3 repetitions. After filtration, the liquid was concentrated by rotary evaporation to obtain a crude extract (Fristiohady et al 2020). The crude methanol extract was then separated into several fractions using ethyl acetate, butanol, and water as organic solvents. Each phase was then concentrated using an evaporator. A part of the partition extract was resuspended with dimethyl sulfoxide (DMSO) at 50 µg mL⁻¹ for anti-mycobacterium screening.



Figure 1. *Aptos* sp.

Anti-mycobacterium assay. To assess the anti-mycobacterial activity of each fraction, the selected *M. smegmatis* NCTC 8159 was evaluated by using a diffusion assay (Hossain 2024). *M. smegmatis* cell cultures were diluted with Mueller-Hinton Broth to achieve a final concentration of 5×10⁵ CFU mL⁻¹. Sterile discs (6 mm) embedded with 50 µg mL⁻¹ of active fraction were placed on the plates. The rifampicin discs acted as positive controls. After incubation at 37°C for 24 hours, the plates were observed for a clear zone of inhibition around the discs in triplicate, and then the inhibition was measured (Hudzicki 2016).

Fractionation analysis. Open-column chromatography was employed to separate potential bioactive fractions with a gradient mobile phase of hexane, dichloromethane, ethyl acetate, and methanol. As the fractions eluted from the column, the trapped fractions in each band were collected, and the solvent was removed. Furthermore, an in vitro antimycobacterial test was carried out for each fraction.

Liquid Chromatography-Mass Spectrometry (LC-MS) analysis. The LC-MS analysis was performed using a liquid chromatography system from Waters (USA) under the following conditions: Solvent A (formic acid (FA)+ H₂O) and solvent B (acetonitrile + 0.1% FA) with run conditions from 95% A to 100% B in 17 minutes. The flow rate was set at 0.3 mL min⁻¹, and as much as 1 µL of the active fraction was injected into a column (ACQUITY UPLC - BEH C8, 1.7 µm 2.1×100 mm).

Thin-layer chromatography (TLC). The TLC was employed to monitor the metabolite profile. TLC analysis was performed on a GF254 silica gel plate (Merck, Germany) using dichloromethane: methanol (9:1) as the mobile phase. Metabolites were visualized under UV light at the wavelengths of 254 and 365 nm. To confirm the active fraction, the TLC plate was then stained with 10% sulfuric acid (Utami et al 2024).

Determination of Minimum Inhibitory Concentration (MIC) by microtiter plate resazurin assay. A Resazurin microtiter plate assay is used to evaluate the active fraction in 96-well plates. A stock solution of the active fraction ($1 \mu\text{g mL}^{-1}$) was prepared in DMSO solution, diluted serially by adding 100 μL to the first well of the microplate, followed by the addition of 50 μL of sterile Mueller-Hinton broth and then transferring a portion of the fraction from the first well to the second well (second row). 10 μL of stock bacterial suspension of *M. smegmatis* with a 0.5 McFarland of final concentration $\sim 1 \times 10^8$ CFU mL^{-1} was added to each well. The microtiter plate for a positive control (Rifampicin stock) and a negative control (sterile nutrient broth) was also prepared. The plates were reclosed and incubated at 37°C for 24 hours. After incubation, 10 μL of resazurin indicator (0.5 mg mL^{-1}) was added to each well, and the cultures were incubated again for 24 hours at 37°C. The minimum inhibitory concentration (MIC) was defined as the lowest drug/active fraction concentration that prevented a color change of resazurin from occurring, meaning that it inhibits bacterial growth (Jorgensen et al 2009). The assay was performed in triplicate and subjected to colony counting, after which fluorescence was determined using Varioscan (excitation 530 nm and emission 570 nm) (Sarker et al 2007). The values obtained are the average \pm standard deviation (SD) of each measurement.

Results and Discussion

The evaluation of Aaptos extracts activity against Mycobacterium smegmatis. Sponges are major players in marine pharmaceuticals, contributing 30% of marine natural medicinal compounds (He et al 2020). The characterization of the marine sponge *Aaptos* sp. from the island of Morotai, North Maluku Province, Indonesia (Figure 1) is irregularly massive in shape with a maximum size of 20-30 cm, bright yellow transversely with a slightly slick surface, usually quite soft and easily squeezed, mainly when dry (Murniasih et al 2025). Subsequently, the sponge (100 g, wet weight) was extracted by maceration with MeOH to obtain a wider compound group. The right solvent is crucial for getting the valuable bioactive compounds from marine sponges (Ebada et al 2008). Further, the antibacterial effect of the methanol extract of *Aaptos* (MEA) was flash-screened through agar diffusion assay, and it showed good antimicrobial activity against *M. smegmatis* at 37°C for 24 hours. Based on the MEA results, *Aaptos* crude extract was partitioned with semipolar solvents to compare the potential of compounds, such as ethyl acetate, water, and butanol, which generated three fractions. Most of the active compound in the crude extract of a sponge was found in the ethyl acetate fraction (8 mm, at 100 μg per disc in dimethyl sulphoxide), while in the n-butanol fraction it was not detected (Table 1). The ethyl acetate fraction was selected for further purification based on its bioactivity.

Table 1
The fractions activity against *Mycobacterium smegmatis*

No	Fractions	Diameter zone (mm)
1	Ethyl acetate	8.00 \pm 0.10
2	n-butanol	-
3	Water	6.50 \pm 0.05

Separation of ethyl acetate extract using open-column chromatography. The ethyl acetate fraction (12.5 mg) was further separated using normal-phase open column chromatography on silica gel-60 and found to be a brown solid. The fraction was eluted

from the column on four subfractions: hexane (1.62 mg), dichloromethane (31.42 mg), ethyl acetate (178.1 mg), and methanol (54.11 mg). The antibacterial activity of each subfraction was evaluated using *M. smegmatis*. The results of their inhibitory effects are presented in Table 2. All sub-fractions inhibited *M. smegmatis* bacterial cells at 50 µg per disc with varied strengths. Based on inhibitory activity, the fractions ranked were methanol>dichloromethane>ethyl acetate>hexane. The methanol sub-fraction displayed the most potent effect on *M. smegmatis* cells with an inhibition zone value of 14±0.06 mm. It was more effective than the positive control, rifampicin, which inhibited growth at 12±0.32 mm with 5 µg per disc. The antibacterial activity was found to be specific to the sponge *Aptos* sp. extracts, as the negative controls, the DMSO used for extract dilution, had not exhibited any antibacterial effects. This study suggests that sponges make a range of secondary metabolites with various spectra. These spectra might depend on which organic solvent compound is used for extraction. The choice of solvent itself is influenced by how easily the chemicals dissolve in water (polarity) and how well the extracts dissolve in the chosen solvent (Utami et al 2024).

Table 2

The result of EtOAc fractionation and anti-*Mycobacterium tuberculosis* assay from open-column chromatography normal phase

Fractions	Solvents	Weight (mg)	Concentration (µg)	Inhibition zone (mm)
F1.	Hexane	1.62	50	7.0±0.02
F2.	CH ₂ Cl ₂	31.42	50	12.0±0.32
F3.	EtOAc	178.10	50	9.0±0.14
F4.	MeOH	54.11	50	14.0±0.06
Control (+)	Rifampicin	-	5	12.0±0.14
Control (-)	DMSO	-	-	-

TLC detection. To identify the main bioactive compound from the sponge *Aptos* sp. based on the highest result in the methanol sub-fraction, we performed a thin-layer chromatography (TLC) analysis to isolate and identify its key bioactive component (Figure 2). The rapid test by TLC supported us in detecting and confirming the presence of a single active compound. The TLC results using a silica gel GF254 plate revealed distinct, separated bands, indicating the absence of other interfering compounds or impurities once purified. This implied that an active MeOH sub-fraction, purified using open column chromatography and analyzed by TLC, had reached a single spot on a mobile phase ratio of dichloromethane (DCM) and methanol (9:1) with a 10% H₂SO₄ stain. The TLC technique was used to analyze the extract's presence of alkaloids and terpenoids, where this type is a common chemical compound discovered in the sponge *Aptos suberitoides* (Utami et al 2024).



Figure 2. Separation of the EtOAc fraction of *Aptos* sp. and its bio-profile spot.

LC-MS analysis. Using LC-MS, this section examined the active methanol sub-fraction within the sponge *Aaptos* sp. (Figure 3). The spectral data suggested that the sponge *Aaptos* sp. compound presented a relatively uncomplicated chemical fingerprint. Based on the LC-MS spectra, the active fraction showed a major peak at a retention time of 3.16 minutes (bottom) in contrast to the control solvent (MeOH), which exhibited a minor peak at 3.93 minutes. The main peak indicated the primary compound within the MeOH sub-fraction, which is suspected to be the bioactive component responsible for causing antibacterial effects. Nevertheless, numerous spectral peaks in another active MeOH fraction were also detected in the control solvent, indicating maybe an issue with insufficient column cleaning.

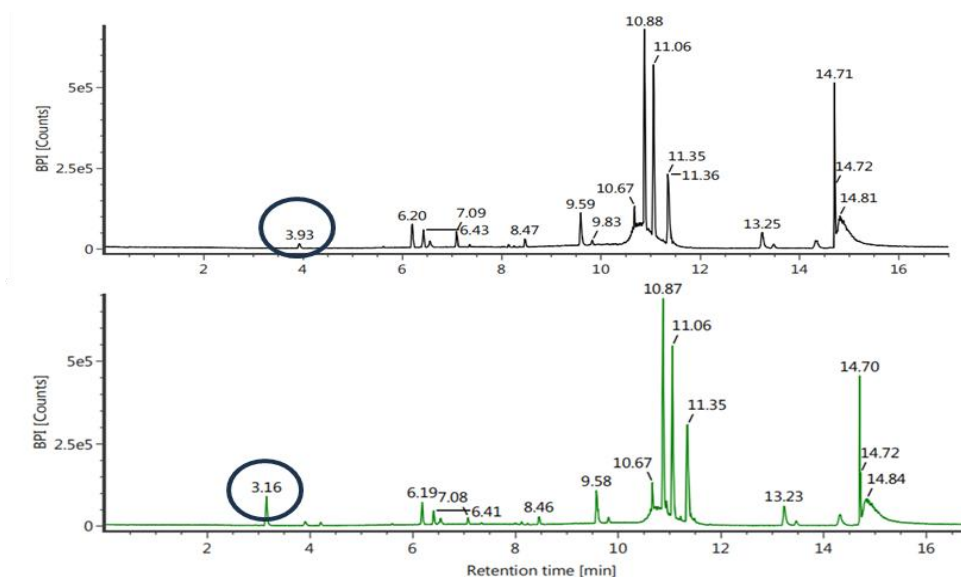


Figure 3. Chromatogram of active fraction from sponge *Aaptos* sp. by LC-MS, MeOH as solvent control (top); the *Aaptos* sp. active fraction (bottom).

The analysis of the main peak using MassLynx shows a molecular ion with an m/z ratio of 229.0968 [M-H⁺], with chemical formula C₁₃H₁₂N₂O₂ and possibly similar to the compound Aaptamine (based on reference) (Figure 4).

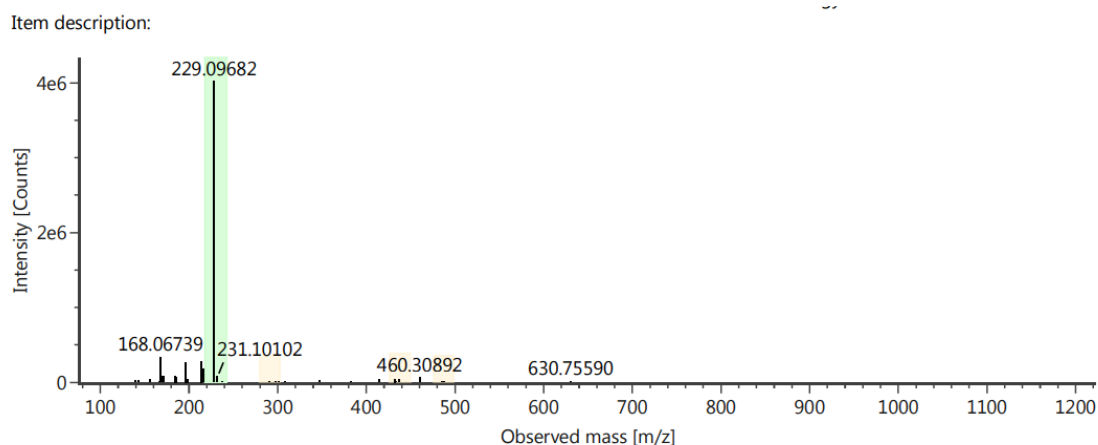


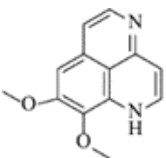
Figure 4. The mass fragmentation peak at the corresponding peak at 3.16 of the active MeOH compound.

Table 3 details the LC-MS results for the EtOAc fraction in the subfraction of methanol. The results showed that the sponge *Aaptos* sp. from Morotai island waters, was the main producer of aaptamine compound without another component, based on the reference. Previous research reported that the presence of aaptamine with a molecular weight of

229.0928 and its derivatives was produced by sponge *A. suberitoides* collected from Tulamben, Bali (Cahyani et al 2023). A study by Ebada et al (2008) has shown that mass spectrometry (MS) analysis is necessary for obtaining a comprehensive understanding of the compounds detected in the chromatograms. This tool would reveal the exact chemical fingerprint of the compounds, allowing scientists to identify them definitively and understand their potential effects on living organisms.

Table 3

LC-MS data from sponge MeOH fraction

Retention time (min.)	Neutral mass (Da)	Prediction compound	Structure	References
3.160	229.0968	Aaptamine		Instrument library of Waters Acquity UPLC I-Class and XEVO G2-XS QToF
4.624	229.0968	Aaptamine		Cahyani et al (2023)

MIC of active compound. The increasing prevalence of bacteria resistant to existing antibiotics poses a significant threat, prompting extensive research toward discovering novel and effective antimicrobial agents (Moloney 2016). In this study, we assessed resazurin to evaluate minimum inhibitory concentration of active compound (aaptamine) from sponge *Aptos* sp. to examine their antibacterial efficacy against *M. smegmatis* using a rapid assay (Table 4, Table 5). The lowest concentration of aaptamine compound at which resazurin remained in the original blue color was 250 $\mu\text{g mL}^{-1}$ against *M. smegmatis* for six repetitions. The aaptamine compound showed activity against the test bacteria with a MIC of 250 $\mu\text{g mL}^{-1}$ by an inhibition rate of 42.679%. In comparison, a positive control (Rifampicin) had a MIC of 62.5 $\mu\text{g mL}^{-1}$ with an inhibition rate of 52.2016% in four repetitions. Rapid screening based on the resazurin assay successfully confirmed the antibacterial screening at the lowest dose through the MIC assay. Marine products have been shown to exhibit broad-spectrum antibacterial properties against Gram-positive and Gram-negative bacteria in the presence of *Aptos* sp. extract (Fajarningsih et al 2018). A previous study revealed that the marine sponge extract *Haliclona* sp. from Manado, North Sulawesi, Indonesia has promising antibacterial properties (Maarisit et al 2017). Those extracts inhibited the growth of *M. smegmatis* bacteria within 10 μg per disc and were identified as cyclostelletamine 1 alkaloid through bioassay-guided purification (Maarisit et al 2022).

Table 4

The minimum inhibitory concentration of the *Aptos* sp. methanol fraction – active fraction

Conc. ($\mu\text{g mL}^{-1}$)	S1-1	S1-2	S1-3	S1-4	S1-5	S1-6	Mean
500	73.8619	74.0713	73.2844	84.0797	83.6935	76.6482	77.606
250	49.0376	45.5005	22.7985	55.1372	60.2623	23.3398	42.679
125	-6.86946	-6.1115	-10.876	-6.40026	-0.87813	-8.1688	-6.5506
62.5	-1.92481	-0.2285	-2.4301	-0.0841	-0.40893	-5.2814	-1.7263
31.25	-1.13077	1.8288	0.24074	3.34468	0.24074	-6.4003	-0.3127
15.625	1.43178	0.85431	-6.7612	2.98375	1.50397	-3.9821	-0.6616
7.812	0.85431	11.3572	3.99434	-8.1678	-16.5783	-11.273	-3.3023

Sample: The active fraction of *Aptos* sp. (six replicates)

Table 5

The minimum inhibitory concentration of the *Aaptos* sp. methanol fraction – control (rifampicin)

Conc. ($\mu\text{g mL}^{-1}$)	R1	R2	R3	Mean
500	73.0246	72.6167	73.3566	72.9993
250	85.1155	84.6319	85.0578	84.9351
125	90.5979	89.3058	90.1287	90.0108
62.5	39.0039	63.258	54.3432	52.2017
31.25	1.72052	5.29367	-2.68274	1.44381
15.625	14.4972	-8.13269	1.32351	2.56268
7.812	5.07711	1.25132	2.33409	2.88751

Control: Rifampicin (three replicates)

Conclusions. This study successfully characterized the anti-mycobacterial potential of the marine sponge *Aaptos* sp. collected from Morotai Island waters, Indonesia. Through bioassay-guided fractionation, a methanol sub-fraction was identified as the most potent component, exhibiting an inhibition zone of 14.0 ± 0.06 mm against *M. smegmatis*. Notably, this activity exceeded the potency of the positive control, rifampicin (12.0 ± 0.32 mm), with a MIC of $250 \mu\text{g mL}^{-1}$. These findings underscore the *Aaptos* genus as a promising source of bioactive secondary metabolites and provide a strong foundation for the development of novel anti-mycobacterial therapeutics.

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Conflict of interest. The authors declare that there is no conflict of interest.

References

- Anjum K., Abbas S. Q., Shah S. A., Akhter N., Batool S., Hassan S. S., 2016 Marine sponges as drug treasure. *Biomolecules and Therapeutics* 24(4):347-362.
- Cahyani N. K. D., Kasanah N., Kurnia D. S., Hamann M. T., 2023 Profiling prokaryotic communities and aptamines of sponge *Aaptos suberitoides* from Tulamben, Bali, *Marine Biotechnology* 25:1158-1175.
- Carroll A. R., Copp B. R., Davis R. A., Keyzers R. A., Prinsep M. R., 2016 Marine natural products. *Natural Product Reports* 36(1):122-173.
- Ebada S. S., Edrada R. A., Lin W., Proksch P., 2008 Methods for isolation, purification and structural elucidation of bioactive secondary metabolites from marine invertebrates. *Nature Protocols* 3(12):1820-1831.
- Eajarningsih N. D., Munifah I., Zilda D. S., 2018 Evaluation of antibacterial assays for screening of marine invertebrate extracts. *Squalen Bulletin of Marine and Fisheries. Postharvest and Biotechnology* 13(1):1-8.
- Fristiohady A., Sadarun B., Wahyuni W., Malaka M. H., Ahmad F., Malik F., Purnama L. O. M. J., Sahidin I., 2020 Isolation and identification of secondary metabolite acetone extract *Aaptos* sp. and its antioxidant properties and acute toxicity. *Journal of Applied Pharmaceutical Sciences* 10(6):81-89.
- Haedar J. R., Uria A. R., Lallo S., Dibwe D. F., Wakimoto T., 2022 New theonellapeptolides from Indonesian marine sponge *Theonella swinhoei* as anti-austerity agents. *Marine Drugs* 20(11):661.
- Hamada T., Matsumoto Y., Phan C. S., Kamada T., Onitsuka S., Okamura H., Iwagawa T., Arima N., Tani F., Vairappan C. S., 2019 Aaptamine-related alkaloid from the marine sponge *Aaptos aaptos*. *Natural Product Communications* 14(9):1-3.
- Hanif N., Murni A., Tanaka C., Tanaka J., 2019 Marine natural products from Indonesian waters. *Marine Drugs* 17(6):364.

- He Q., Miao S., Ni N., Man Y., Gong K., 2020 A review of the secondary metabolites from the marine sponges of the genus *Aaptos*. *Natural Product Communications* 15(9):1-12.
- Hossain T. J., 2024 Methods for screening and evaluation of antimicrobial activity: A review of protocols, advantages, and limitations. *European Journal of Microbiology and Immunology* 14(2):97-115.
- Hudzicki J., 2016 Kirby-Bauer disk diffusion susceptibility test protocol. *American Society for Microbiology*, pp. 1-23.
- Jorgensen J. H., Ferraro M. J., 2009 Antimicrobial susceptibility testing: A review of general principles and contemporary practices. *Medical Microbiology* 49(11):1749-1755.
- Kiran G. S., Sekar S., Ramasamy P., Thinesh T., Hassan S., Lipton A. N., Ninawe A. S., Selvin J., 2018 Marine sponge microbial association: towards disclosing unique symbiotic interactions. *Marine Environmental Research* 140:169-179.
- Li Z., Hentschel U., Webster N., Olson J., Häggblom M., 2020 Editorial: special issue on sponge microbiome. *FEMS Microbiology Ecology* 96(5):1-3.
- Maarisit W., Abdjul D. B., Yamazaki H., Kato H., Rotinsulu H., Wewengkang D. S., Sumilat D. A., Kapojos M. M., Ukai K., Namikoshi M., 2017 Anti-mycobacterial alkaloids, cyclic 3-alkyl pyridinium dimers, from the Indonesian marine sponge *Haliclona* sp. *Bioorganic & Medicinal Chemistry Letters* 27(15):3503-3506.
- Maarisit W., Untu S. D., Lengkey Y. K., Mongi J., Kanter J. W., Pareta D. N., Sambou C. N., Tumbel S. L., Montolalu F. M., Korua S. A., Tulungen F. R., 2022 Isolation and evaluation of anti-mycobacterial activity of alkaloid compounds from marine invertebrate sponge *Haliclona* sp. *Tropical Journal of Natural Product Research* 6(10):1622-1625.
- Malve H., 2016 Exploring the ocean for new drug developments. *Journal of Pharmacy and Bioallied Sciences* 8(2):83-91.
- Mehub M. F., Lei J., Franco C., Zhang W., 2014 Marine sponge-derived natural products between 2001 and 2010: Trends and opportunities for discovery of bioactives. *Marine Drugs* 12(8):4539-4577.
- Martin A., Vieira H., Gaspar H., Santos S., 2014 Marketed marine natural products in the pharmaceutical and cosmeceutical industries: Tips for success. *Marine Drugs* 12(2):1066-1101.
- Moloney M. G., 2016 Natural products as a source for novel antibiotics. *Trends in Pharmacological Sciences* 37(8):689-701.
- Murniasih T., Hadi T. A., Ahmadi P., Sari M., Bustanussalam, Hadisaputri Y. E., Putra M. Y., Rahmawati S. I., Indriani D. W., 2025 Assessments of anti-breast cancer activity and profiling of active compounds using LC-MS/MS from the Indonesian *Agelas nakamura*. *Journal of Applied Pharmaceutical Science* 15(4):204-209.
- Rao K. V., Kasanah N., Wahyuono S., Tekwani B. L., Schinazi R. F., Hamann M. T., 2004 Three new manzamine alkaloids from a common Indonesian sponge and their activity against infectious and tropical parasitic diseases. *Journal of Natural Product* 67(8):1314-1318.
- Rao K. V., Santarsiero B. D., Mesecar A. D., Schinazi R. F., Tekwani B. L., Hamann M. T. 2003 New manzamine alkaloids with activity against infectious and tropical parasitic diseases from an Indonesian sponge. *Journal of Natural Product* 66(6):823-828.
- Sarker S. D., Nahar L., Kumarasamy Y., 2007 Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. *Methods* 42(4):321-324.
- Taylor M. W., Radax R., Steger D., Wagne M., 2007 Sponge-associated microorganisms: Evolution, Ecology, and biotechnological potential. *Microbiology and Molecular Biology Reviews* 71(2):295-347.
- Trang D. T., Tai B. H., Hang D. T. T., Yen P. H., Nhiem N. X., Kiem P. V., 2022 Four new aaptamine alkaloids from marine sponge *Aaptos aaptos*. *Natural Product Research* 36:5022-5031.
- Utami S. T., Paramita N. S. P., Agustina K., Prayoga M. B., Maulidia F. N., Apsari C. N., Putro E. W., Aji Nugroho A., Wibowo K., Harahap S. A., Handayani I., Gusnaniar N.,

- Santosa D., Atikana A., 2024 Identification of the secondary metabolite derived from *Aptos* spp. and its antibacterial activity. Indonesian Journal of Pharmacy 35(2):325-339.
- Varijakzhan D., Loh J. Y., Yap W. S., Yusoff K., Seboussi R., Lim S. H. E., Lai K. S., Chong C. M., 2021 Bioactive compounds from marine sponges: Fundamentals and applications. Marine Drugs 19(5):246.
- Webster N. S., Taylor M. W., 2012 Marine sponges and their microbial symbionts: love and other relationships. Environmental Microbiology 14(2):335-346.

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