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Anti-mycobacterial activity of secondary metabolites from marine sponge *Agelas sp*

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ABSTRACT

Marine sponge has become a significant source of natural products for the development of therapeutic agents. Therefore, this study aims to isolate anti-mycobacterial activity of marine sponge *Agelas sp*. During the experiment, ethanol was used for sponge extraction, and the resulting ethanol extract was separated into 7 fractions using open column chromatography. The fractions were subsequently purified by preparative high-performance liquid chromatography, and ^{1}H-NMR and mass spectroscopy were used to identify and characterize the compounds that were obtained. Anti-mycobacterial activity testing was carried out using the disc diffusion method with *Mycobacterium smegmatis* bacteria. The results showed that the extract of *Agelas sp.* yielded 10 known compounds, namely 2-cyano-4,5-dibromo-1*H*-pyrrole (1), 4,5-dibromo-2-methyl carboxylate (2), 4,5-dibromo pyrrole-2-carboxylic acid (3), 4,5-dibromopyrrole-2-carboxamide (4), 5-bromopyrrole-2-carboamide (5), longamide (6), oroidin (7), keramadine (8), cyclooroidin (9), and manzacidin A (10). Compound 1 (2-cyano-4,5-dibromo-1*H*-pyrrole) inhibited *Mycobacterium smegmatis* bacteria, with an inhibition zone diameter of 7 mm at a dose of 50 µg/disc. However, compounds 2-10 did not show any anti-mycobacterial activity at a dose of 50 µg/disc. This study presented the first report on anti-mycobacterial activity of compound 1 (2-cyano-4,5-dibromo-1*H*-pyrrole).

***Keywords*:** Anti-mycobacterial, Alkaloids, Secondary metabolites, Marine sponge, *Agelas* sp.

Introduction

Tuberculosis is an infectious disease, affecting 7.5 million individuals globally in 2022, which was the highest number since the World Health Organization (WHO) began monitoring in 1995.[1] Several studies have shown that Indonesia currently ranks second in the number of cases, following India. In addition, Indonesia was reported to be the third most affected country globally in 2020.[1] The main factor contributing to the disease's rising incidence is the growing issue of drug-resistant tuberculosis, which makes treatment more difficult. This suggests that investigating natural anti-tuberculosis compounds with novel modes of action is imperative.

In line with these results, *Agelas* sp. is known to possess various chemical structures, such as bromopyrrole alkaloids,[2-10] pyrrole alkaloids,[11,12] pyrrole-imidazole alkaloids,[13] terpenoids alkaloids, carotenoids, sterol, and glycosphingolipids.[14] Bioactive compounds from *Agelas* sp are also known to have potential pharmacological activities, including antiangiogenic,[6] cytotoxic,[15] Protein Tyrosine Phosphatase 1B inhibitor (PTP1B),[16] antihistaminic, and antimalarial.[14] However, bioactive compounds, such as anti-mycobacteria have not been widely reported.

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Several studies have reported the presence of bioactive chemicals, particularly anti-mycobacterial compounds in underwater organisms. The crude ethanol extract has been shown to possess inhibitory effects against *Mycobacterium smegmatis*. An inhibitory zone measuring 12 mm in diameter was formed by the extract at a dosage of 50 µg/disc. In order to report on the isolation, identification, and anti-mycobacterial activity of alkaloid compounds from *Agelas sp*., this study was conducted.

Materials and Methods

General experimental procedure

HPLC preparation was conducted using the Hitachi Ltd. L-6200 machine. Using a JNM-AL-400 NMR spectrometer (JEOL), 400 MHz ^{1}H and ^{13}C-NMR spectra were recorded. Chemical shifts were measured using the residual solvent signals (DMSO-d_6; δ_H 2.71 and CD_3OD δ_H 3.31). For the EIMS, a JMS-MS 700 mass spectrometer (JEOL, Tokyo, Japan) was utilized. For column chromatography (CC), ODS (Wakogel®100C18) was employed.

Wako Pure Chemical Industries was the source of all other chemical compounds, that includes organic solvents.

Sample collection

Sampling was conducted using scuba diving equipment in Manado waters in March 2024 with GPS positioning 1°27'50.6″N 124°46'53.6″E. These samples were transferred to the laboratory for further analysis, where the sample with code number 137 (Figure 1) was identified as *Agelas sp*.

Extraction and Isolation

Agelas sp., weighing 550 gr (dry weight), was divided into small pieces and extracted 3 times using ethanol. After evaporating the ethanol, the resulting extract weighed 22.05 gr, which was divided into 7 part using

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open-column chromatography with a 100 g stationary phase of octadecyl silica (ODS) and a mobile phase of methanol and water in a graded manner.

Compound **1** (3.2 mg) as well as compound **2** (8.0 mg) were obtained from fraction 5 (414.9 mg) and eluted with 75% MeOH using repeated preparative HPLC on a PEGASIL ODS column. Furthermore, the flow rate was set at 2.0 mL/min while UV (Ultraviolet) detection was performed at 210 nm. Fraction 4 (552.1 mg) was eluted with 55% MeOH and separated using a PEGASIL ODS 10 mm × 250 mm HPLC column with the same flow rate and UV detection settings. This analysis yielded compounds **1** (1.5 mg), **3** (50 mg), **4** (2.5 mg), and **7** (3.5 mg). However, compounds **5** (2.5 mg), **6** (3.0 mg), and **10** (10 mg) were obtained from fraction 3 (1040.0 mg). This was eluted with 50% MeOH and separated using a PEGASIL ODS 10mm × 250 mm HPLC column with the aforementioned flow rate and UV detection at 210 nm. In addition, compounds **5** (1.3 mg), **8** (4.7 mg), and **9** (4.0 mg) were isolated from fraction 3.2 using HPLC on a PEGASIL ODS column 10 mm × 250 mm with a flow rate of 2.0 mL/min and UV detection at 210 nm, which was eluted with 30% MeOH.

Anti-mycobacterial assay

Using *Mycobacterium smegmatis* bacteria from a laboratory culture supplies, anti-mycobacterial activity was evaluated in comparison to chemicals derived from *Agelas sp*. For 2 days, the bacteria were cultivated in 10 mL of 7H9 broth medium at 37°C. Then, at 40°C, 1 mL of the inoculum was dissolved into 100 mL of Middlebrook 7H9 agar medium and placed into a square plate. After being dissolved in MeOH, the samples were put on a 6 mm paper disc. In addition, the paper disc was placed on a plate and incubated for two days at 37°C after methanol was evaporated in a desiccator. The positive control was streptomycin (2 µg/disc), whereas the negative control was methanol.[17,18]

Figure 1: Marine sponge *Agelas* sp.[40,41]

Results And Discussion

In this study, marine sponge weighing 550 g was extracted 3 times using ethanol. Furthermore, 22.05 g of ethanol extract was separated into 7 parts using an ODS column (100 gr). Purification of the compound used HPLC (ODS) with a mobile phase of methanol and H_2O in stages and produced a total of 10 known natural products as displayed in Figure 2, namely 2-cyano-4,5-dibromo-1H-pyrrole, [14,19] 4,5-dibromo-2- methyl carboxylate,[7,19] 4,5-dibromo pyrrole-2-carboxylic acid, [7,19] 4,5-dibromopyrrole-2-carboxamide,[7,19] and 5-bromopyrrole-2-carboamide.[14,19] Others included longamide, [14,19] oroidin, [3,20] keramadine,[3] cyclooroidin, [14,21] and manzacidin A. [14,21,22] The structures of all compounds were determined mainly based on ^{1}H-NMR, mass spectroscopy data and by comparison with literature values for isolated compounds.

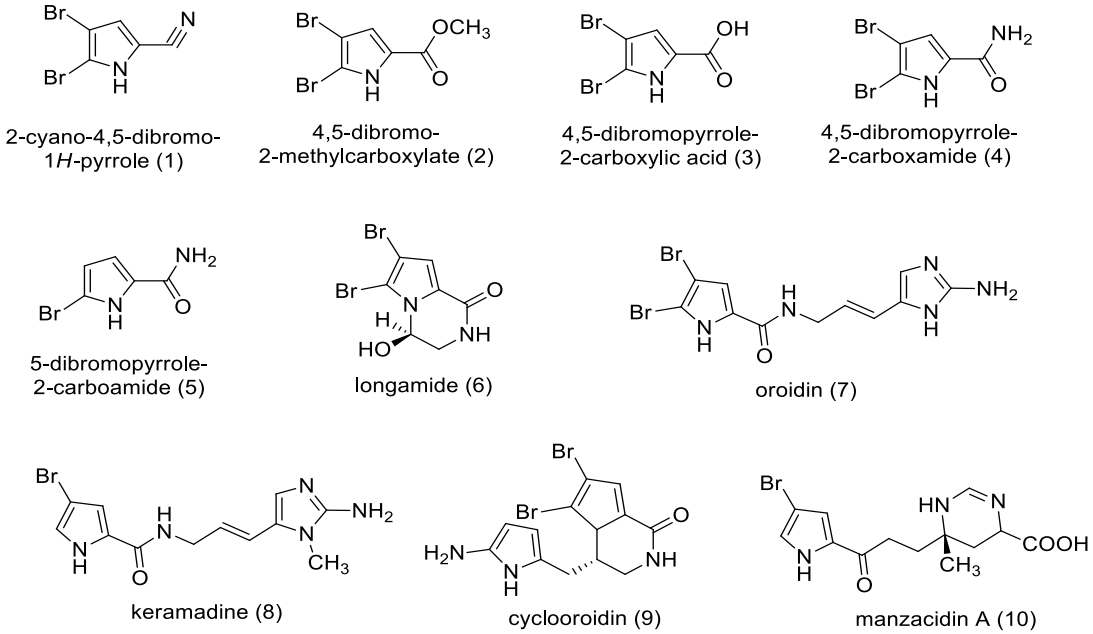

Figure 2: Isolated compounds **1-10** from marine sponge *Agelas* sp.

Characterization of the isolated compounds

2-cyano-4,5-dibromo-1*H*-pyrrole (compound **1**): crystalline solid; ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H (ppm) 7.07 (1H, brs, NH-1), 6.97 (1H, brs, H-3); FAB-MS: *m/z* 248, 250, 252 $[M+H]^+$.

4,5-dibromopyrrole-2-methylcarboxylate (compound **2**): crystalline solid, ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H (ppm) : 6.83 (1H, s), 3.81, (3H, s, OCH_3-7); FAB-MS *m/z* 281, 283, and 285 $[M+H]^+$.

4,5-dibromopyrrole-2-carboxylic acid (compound **3**): crystalline solid; ^{1}H-NMR (400 MHz, CD_3OD) δ_H (ppm) : 12.98 (1H, s, NH-1), 6.81 (1H, s, H-3); FAB-MS: *m/z* 267, 269, and 271 $[M+H]^+$.

4,5-dibromopyrrole-2-carboxamide (compound **4**): crystalline solid; ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H (ppm) : 12.61, (1H, brs, NH-1), 7.58, (1H, brs, NH-7a), 7.20, (1H, brs, NH-7b), 6.91, (1H, s, H-3); FAB-MS *m/z* 266, 268, and 270 $[M+H]^+$.

5-bromopyrrole-2-carboamide (compound **5**): crystalline solid ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H (ppm) : 12.09, (1H, brs, NH-1), 7. 09, (1H, brs, NH-7a), 6.72, (1H, brs, NH-7b), 6.72, (1H, brs, H-3); FAB-MS *m/z* 189, 191 $[M+H]^+$.

Longamide (compound **6**): amorphous white solid; ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H (ppm) : ^{1}H-: 7.87 (1H, brs, NH-7), 6.97, (1H, brs, H-3), 5.59, (1H, brs, OH-9), 3.70, (1H, dd, 13.0, 2.0, H-8a), 3.68, (dd, 13.9, 1.0, H-8b); EIMS *m/z* 308, 310, 312 $[M]^+$.

Oroidin (compound **7**): amorphous white solid; ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H (ppm) : 12.77, brs, (1H, brs, NH-1), 12.11, (1H, brs, NH-14), 8.44, (1H, brs, NH-7), 7. 67, (2H, brs, NH2-16), 6.96,(1H, br s, H-3), 6.89 (1H, brs, H-15), 6.22, (1H, d, 16.4 Hz, H-10), 6.10, (1H, m, H-9), 3.94, (2H, m, H-8); EI-MS *m/z* 388, 390, 392 [M]+.

Keramadine (compound **8**): pale yellow amorphous solid; ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H (ppm) : 11.84, (1H, brs, NH-3), 11.84 brs, (1H, brs, NH-12), 8.44, (1H, m, NH-9), 7.68, (2H, s, NH_2-2), 7.00, (1H, s, H-4), 6.98, (1H, m, H-13), 6.82, (1H, m, H-15), 6.20, (1H, m, H-6), 5.82, (1H, dt, 11.0, 5.6, H-7), 4.00, (2H, t, 5.6, H-8), 3.38, (3H, s, CH_3-16). FAB-MS *m/z* 324 and 326 $[M+H]^+$.

Cyclooroidin (compound **9**): colorless oil; ^{1}H-NMR (400 MHz, CD_3OD) δ_H (ppm) : 6.94, (1H, s, H-4), 6.35, (1H, s, H-12), 4.64, (1H, m, H-9), 3.88, (1H, dd, 13.0, 3.0, H-8a), 3.52, (1H, dd, 13.0, 1.2, H-8b), 2.98, (2H, bd, 6.0, H-10); FAB-MS *m/z* 388, 390, 392 $[M+H]^+$.

Manzacidin A (compound **10**): colorless oil; ^{1}H-NMR (400 MHz, CD_3OD) δ_H (ppm): 12.34, 1H, brs, NH-1), 10.27, (1H, brs, NH-14), 8.20, (1H, brs, H-13), 7.23, (1H, brs, H-2), 6.98, (1H, brs, H-4), 4. 27, (1H, d, 11.5 Hz, H-8a), 4.14, (1H, d, 11.5 Hz, H-8b), 2.25, (1H, dd, 13.5, 4.0 Hz H-10a), 2.12, (1H, dd, 13.5, 9.0 Hz, H-10b), 1.34, (H3, s, CH_3-15); FAB-MS *m/z* 344, 346 $[M+H]^+$.

Structure elucidation

Compound **1** was isolated as a crystalline solid with molecular formula $C_5H_2N_2Br_2$ based on FABMS at *m/z* 250. Two bromine grubs in compound **1** were identified based on FABMS data at *m/z* 248, 250, and 252 with a ratio of 1:2:1. ^{1}H-NMR data (in DMSO-d_6) shows spectra at NH signal at δ_H 7.07 (s), aromatic proton δ_H 6.96, (brs). Compound **1** was identified as 2-cyano-4,5-dibromo-1*H*-pyrrole (**1**).[23]

Compound **2** was formed as a crystalline solid, and the FABMS of **2** suggested pseudo molecular ion peaks at *m/z* 281, 283, and 285 with a ratio of 1:2:1, confirming that compound **2** contained two grub bromine with molecular formula $C_5H_3Br_2NO_2$. ^{1}H-NMR (in CD_3OD) data of compound **2** showed an aromatic signal at δ_H 6.83 (s) and methoxy group δ_H 3.81 (s). Compound **2** was identified as 4,5-dibromopyrrole-2-methylcarboxylate by analysing the data provided above.[7,19]

The FABMS spectrum **3** suggested the molecular ion peak at *m/z* 269 and the molecular formula $C_5H_3Br_2NO_2$. The two bromine grubs in compound **3** were confirmed in the FABMS spectrum at *m/z* 267, 269, and 271 with a ratio of 1:2:1. ^{1}H-NMR (in DMSO-d_6) data shows an aromatic proton spectrum at δ_H 6.81 (s) and an NH proton at δ_H 12.9 (s). Compound **3** was identified as 4,5-dibromopyrrole-2-carboxylic acid.[7,19]

FABMS of **4** exhibited pseudo molecular ion peaks at *m/z* 266, 268, and 270 with a ratio of 1:2:1. This confirms that compound **4** has two bromine grubs with the molecular formula $C_5H_4Br_2N_2O$. Based on the ^{1}H-NMR data (in DMSO-d_6), there are NH signals δ_H 12.61 (s), aromatic signal δ_H 6.72 (s), NH_2 signals δ_H 7.58 (s), 7.20 (s). These findings led to the identification of component **4** as 4,5-dibromopyrrole-2-carboxamide.[7,19]

The spectrum of FABMS data at *m/z* 189, 191 with a 1:1 ratio of compound **5** suggests that there is one bromine grub. The ^{1}H-NMR data (in DMSO-d_6) reveal NH signals δ_H 12.09, (br s) aromatic signals δ_H 7.09 (s), and δ_H 6.72 (s). Compound **5** was identified as a 5-bromopyrrole-2-carboamide with the molecular formula $C_5H_5BrN_2O$.[14,19]

Compound **6,** based on ^{1}H-NMR (in DMSO-d_6) data, contained one NH signal δ_H 7.87 (s), one aromatic signal δ_H 6.97 (s), one hydroxyl δ_H 5.59 (s), and methylene signal δ_H 3.68 (dd, *J*=13.0, 2.0), 3.71 (dd, *J*=13.9, 1.0). Two bromine groups in compound **6** were identified based on EIMS data at *m/z* 308, 310, 312 with a ratio of 1: 2:1. Compound **6** was identified as longamide with molecular formula $C_7H_5Br_2N_2O_2$.[19,20]

The data from ^{1}H-NMR (in DMSO-d_6) revealed a signal of NH groups at δ_H 12.77 (br s), 12.11 (br s), 8.44 (brs), 7.67 (brs), sp^2 methines δ_H 6.22 (d, *J*=16.4 Hz), δ_H 6.10 (m), two aromatic signals at δ_H 6.96 (br s), 6.89 (br s), and one sp^3 methylene 3.94 (t, *J*=5.0 Hz). EIMS ion peak at *m/z* 388, 390 and 392 confirmed that compound **7** had the molecular formula $C_{11}H_{11}Br_2N_5O$. The data provided given led to the identification of compound **7** as oroidin.[23, 24]

Based on positive FABMS data at *m/z* 324 and 326, compound **8** had the molecular formula $C_{12}H_{14}N_5Br$. ^{1}H-NMR data showed signals NH δ_H 11.84 (brs), 8.44, (brs) 7.68 (brs); signals aromatic protons δ_H 7.01 (brs), 6.98 (m); 6.82 (m); sp^2 methines δ_H 6.20, (m), 5.82, dt, 11.0, 5.6 Hz), one sp^3methylene signals δ_H 4.00 (t, *J* =5.6 Hz) and methyl signal δ_H 3.38 (s). These data identified compound **8** as a keramadine.[25]

The spectrum FABMS data for compound **9** showed molecular ion peaks at *m/z* 388, 390, 392 with a ratio of 1:2:1, indicating that compound **9** contained two bromine atoms. The molecular structure of **9** was $C_{11}H_{11}Br_2N_5O$ based on FABMS data at *m/z* 390. ^{1}H-NMR (in DMSO-d_6) data of compound **9** revealed the existence of an aromatic signal δ_H 6.94 (brs) ; δ_H 6.35 (brs), sp^3 methylene signals δ_H 3.88 (dd, *J* =13.0, 3.0 Hz), 3.52 (dd, *J* = 13.0, 3.0 Hz), and δ_H 2.96 (brd, *J* = 6.0 Hz). The data above led to the identification of compound **9** as cyclooroidin.[26]

Spectrum FABMS at *m/z* 344 clarified that the molecular formula of compound **10** was $C_{12}H_{15}BrN_3O_4$. Compound **10** contained a bromine molecule. This can be clarified based on FABMS data at *m/z* 344 and 346 with a ratio of 1:1. ^{1}H-NMR data of compound **10** showed signal NH at δ_H 12.34 (brs), 10.27 (brs), aromatic signal δ_H (8.20, brs), (7.23, brs), (6.98, brs), ; and sp^3 methylene signals δ_H 4.27 (d, *J* = 11.5 Hz) 4.14 (d, *J* = 11.5 Hz) and 2.25 (dd, *J* = 13.5, 4.0 Hz) 2.12 (dd, *J* =13.5, 9.0 Hz), and one methyl signal 1.34 (s). Compound **10** was identified as the compound manzacidin A.[27]

Anti-Mycobacterial Activity

The isolation of compounds (**1** to **10**) from *Agelas sp*. were examined for their efficacy against *M. smegmatis* bacteria using the disc diffusion method. According to Table 1, only 2-cyano-4,5-dibromo-1*H*-pyrrole (**1**) could inhibit *M. smegmatis* bacteria, leading to an inhibition zone diameter of 7 mm at a dose of 50 µg/disc. This study was the first to report the inhibitory potential of the compound 2-cyano-4,5-dibromo-1H-pyrrole against *M. smegmatis*. Meanwhile, compounds **2** to **10** displayed no anti-mycobacterial activity at a dose of 50 µg/disc. Therefore, the presence of the nitrile group in compound **1** emerged as a significant factor contributing to anti-mycobacterial activity.

Marine sponge genus *Agelas* was a prolific source of bioactive secondary metabolites, characterized by their diverse chemical structures and potential applications in drug development.[12,14] *Agelas oroides*, for instance, had been found to possess a range of secondary metabolites, including pyrrole derivatives, sterols, terpene alkaloids, glycosphingolipids, and carotenoids. Some of these metabolites had demonstrated anti-microbial activity. *Agelas nakamurai* has produced the biactive compound agelasines and is reported to be able to inhibit *M. smegmatis* bacteria at a dose of 20 µg/disc.[16]

Furthermore, leucettamol A from Agelas sp. showed ability to inhibit M. smegmatis at a dose of 50 µg/disc.[21] The bioactive compound longamide from *Agelas longissima* was reported to exhibit anti-microbial activity. In addition, longamide B from *Agelas dispar* could inhibit the growth of *B. subtilis* and *S. aureus* bacteria. Nagelamides A to H from Okinawa *Agelas sp* are reported to be able to inhibit pathogenic bacteria. Agelasidin A, C, and D from the Caribbean *Agelas clathodes* were reported to exhibit antibacterial activity.[14]

Mycobacterium smegmatis was a species of acid-fast bacteria in the phylum Actinomycetota and the genus Mycobacterium. It was characterized by its bacillus shape, measuring 3.0 to 5.0 µm in length.[28] *M. smegmatis* was renowned for its ability to move around its environment using a sliding mechanism, which was a unique feature among bacteria. This phenomenon was facilitated by bacteria that formed monolayered sheets and exhibited slow movement, without

relying on extracellular structures like flagella or pili. Furthermore, it was commonly used as a valuable model organism for the study of *M. tuberculosis* and other mycobacterial pathogens due to its ease of cultivation in several synthetic or complex laboratory media.[29] These bacteria formed visible colonies in 3 to 5 days and were used for the cultivation of mycobacteriophages. The genome of *M. smegmatis* had been sequenced and exhibited approximately 2000 homologous genes shared with *M. tuberculosis*, making it an exceptional model for the study of mycobacteria in general. It was also used in studies due to its genetic similarity and analogous functional characteristics to *M. tuberculosis*, particularly in investigations of the Snm secretion system which served as the main virulence factor in *M. tuberculosis*. In addition, specific genes essential for Snm secretion were identified by deleting genes in the RD1 locus of *M. smegmatis* and assessing the efficiency of Snm secretion, which could facilitate their application to the Snm secretion system of *M. tuberculosis*.[29]

The results of the experiment on the secondary metabolites extracted from *Agelas sp* against *M. smegmatis* bacteria demonstrated that alkaloid compound 2-cyano-4,5-dibromo-1H-pyrrole (**1**) could inhibit the growth of *M. smegmatis* bacteria, as evidenced by an inhibition zone diameter measuring 7 mm per disc. According to the observed zone of inhibition, antibacterial activity could be classified as follows, a diameter greater than 20 mm was considered to be very strong, 15-20 mm was categorized as strong, 10-14 mm was under the medium category, 9 to 7 mm was classified as weak, and any diameter less than 7 mm indicated no antibacterial activity.[30] Improving anti-mycobacterial activity required the optimization of the compound structure of 2-cyano-4,5-dibromo-1*H*-pyrrole. Compound **1** possessed a structure similar to that of compounds 2 to 4. Consequently, the nitrile group present in compound **1** played a crucial role in conferring anti-mycobacterial activity. The methoxy (CH_3OH), hydroxyl (OH), and amino (NH_2) groups in compounds **2** to **4** were found to have no impact on anti-mycobacterial activity.

Marine organisms were renowned for their production of numerous bioactive compounds containing nitrile groups, exhibiting intriguing pharmacological properties including antibacterial, antiprotozoal, cytotoxic, and anti-tuberculosis activities.[31] The compound 3-dodecyl pyridine, which contained a terminal nitrile group, was found in the Indonesian sponge and demonstrated cytotoxic activity.[32] Sponges belonging to the genus Mycale were found to have bioactive chemicals containing nitrile groups, such as 5- (19-cyanononadecanyl) pyrrole-2-carboxaldehyde, which had antiprotozoal action against Leishmania mexicana, with an LD_{50} of 12 µg/mL.[31] The substance axinynitrile-A, which was created by sponge Axinyssa Isabela, was shown to have cytotoxic action against several types of human cancer cell lines, including those from the breast, lung, and colon.[33] Furthermore, another sponge, *Hamigera tarangaensis*, produced alkaloid compound hamigeran R, which contained a nitrile group and exhibited cytotoxic activity.[34]Alkaloid compounds with nitrile groups were also present in the nudibranch *Jorunna funebris*. [35]

Each compound had a distinctive mechanism for inhibiting bacteria. Alkaloid compounds, in particular, interfere with bacterial energy metabolism or block toxins in the cell structure, which ultimately stopped bacterial growth. Alkaloid compounds primarily target adenosine triphosphate (ATP) in their efforts to inhibit bacteria. ATP was synthesized during the process of respiration and served as a crucial energy source for various cellular activities. In addition, ATP played a vital role in respiration, and primary metabolism, and functioned as an energy source for numerous enzyme reactions. As a result, blocking ATP synthase caused disturbances in several regular metabolic functions in microorganisms, which could ultimately result in their demise.[36] Alkaloids could impact DNA topoisomerase and respiration, as well as bacterial protease activity. Furthermore, these substances interacted with intestinal flora and protected the intestinal mucosa. An enzyme called DNA topoisomerase located in the nucleus was responsible for controlling DNA superhelical state. Consequently, it affected the overall topological organization of DNA by influencing the breakage and binding of its strands.[37]Alkaloids could suppress bacteria through several methods, such as the inhibition of bacterial metabolism, damaging bacterial cell walls and membranes, alteration of bacterial cell membrane permeability, and the production of nucleic acids and proteins. Alkaloids possessed broad-spectrum antibiotic properties and caused minimal side effects. Therefore, the main focus in drug candidate discovery was on isolating alkaloid compound for use as anti-microbial.[38]

Compounds **2** to **10** did not exhibit any anti-mycobacterial action in this investigation. However, earlier studies on the pharmacological effects and genesis of this substance had been documented. Particularly, it has been reported that the sea *Agelas oroides* contained the chemical 4,5-dibromopyrrole-2-methylcarboxylate (**2**), which inhibited PTP1B enzyme.[19] 4,5-dibromopyrrole-2-carboxylic acid (**3**) was identified from *Agelas* sponges, which had been shown to have immunosuppressive properties.[14] In addition, the chemical dibromopyrrole-2-carboxamide (**4**), which was produced from *Agelas oroides*, was reported to exhibit inhibitory effects against *P. aeruginosa*[3] and cytotoxic activity.[14] *Agelas nakamurai hoshino* yielded compound 5-bromopyrrole-2-carboxamide (**5**), which had been shown to suppress the growth of gram-positive bacteria and fungus.[14] *Agelas longissimi*, *A. nakamurai*, and *Homaxinella sp*. had all been found to contain longamide (**6**) [14]. Sponges *Agelas* produced oroidin (**7**) and were reported to exhibit several biological activities, such as the inhibition of multidrug-resistant *Saccharomyces cerevisiae*, anti-protozoa activity, antifouling properties, antibacterial effects, inhibition of bacterial biofilm formation, and larval *Balanus amphitrite inhibition*.[14,38,39] Therefore, *Agelas sp*. yielded keramadine (**8**), which was described as a serotonergic receptor antagonist.[4,14] *Agelas oroides*, a sponge, yielded cyclooroidin (**9**).[14] An isolated chemical from sponge *Hymeniacidon sp*. had been identified as manzacidin A (**10**).[14] It had been reported that 2-cyano-4,5-dibromo-1*H*-pyrrole (**1**), obtained from sponges *Agelas oroides* and *Acanthostylotella sp*., possessed cytotoxic properties.[14]

Table 1: Anti-mycobacterial activity against *M. smegmatis* of compounds (**1**-**10**) from Marine Sponge *Agelas sp*.

Compound (50 µg/*disc*)	**Diameter of inhibition zone (mm)**
2-cyano-4,5-dibromo-1*H*-pyrrole (1)	7
4,5-dibromopyrrole-2-methylcarboxylate (2)	-
4,5-dibromopyrrole-2-carboxylic acid (3)	-
4,5-dibromopyrrole-2-carboxamide (4)	-
5-bromopyrrole-2-carboamide (5)	-
longamide (6)	-
oroidin (7)	-
keramadine (8)	-
cyclooroidin (9)	-
manzacidin A (10)	-
Streptomycine sulfate (2 µg)	25
Negative control (5 µl)	-

Diameter of paper disc=6 mm

Conclusion

In conclusion, *Agelas* sp. produced bromopyrrole alkaloids with interesting pharmacological effects, including antituberculosis. *Agelas* sp yielded 10 known substances 2-cyano-4,5-dibromo-1H-pyrrole (**1**), 4,5-dibromo-2 methyl carboxylate (**2**), 4,5-dibromopyrrole-2-carboxylic acid (**3**), 4,5-dibromopyrrole-2-carboxamide (**4**), 5-bromopyrrole-2-carboamide (**5**), longamide (**6**), oroidin (**7**), keramadine (**8**), cyclooroidin (**9**), and manzacidin A (**10**). Among these, only compound **1** (2-cyano-4,5-dibromo-1*H*-pyrrole) could inhibit *Mycobacterium smegmatis* with an inhibition zone diameter of 7 mm at a concentration of 50 µg/disc. Meanwhile, compounds **2** to **10** did not reveal any inhibition on *Mycobacterium smegmatis* at the same concentration.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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