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*Original Research Article*

# Inhibitory Effect of the Marine Sponge *Amphimidon chloros* Extracts Against Multidrug-Resistant Bacteria

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ABSTRACT

Antibiotic misuse has led to the development and spread of multidrug-resistant (MDR) microorganisms. This study evaluated the antibacterial activity of n-hexane, ethyl acetate, and aqueous extracts of the marine sponge *Amphimidon chloros*. The antibacterial activity of n-hexane, ethyl acetate, and aqueous extracts was evaluated using broth microdilution and disc diffusion. The chemical composition of the extracts was analysed using Liquid Chromatography-Mass spectrometry (LC-MS). The antibacterial potency of the extracts was in the following order: aqueous > n-hexane > ethyl acetate extracts. The most significant inhibitory activity of the aqueous extract was against *Shigella flexneri*, with an inhibition zone of 20.6 mm. *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and *Listeria monocytogenes* were utterly resistant to ampicillin and chloramphenicol; however, the aqueous extract efficiently inhibited these bacteria. The extract MIC ranged from 0.0195–2.5 mg/mL. With maximal inhibition against *Staphylococcus aureus* (0.0195 mg/mL), *Bacillus cereus* (0.0390 mg/mL), *Shigella flexneri* (0.0390 mg/mL), and *Salmonella enterica* (0.0781 mg/mL). While L. monocytogenes (0.3125 mg/mL) and S. aureus (0.625 mg/mL) were the only Gram-positive bacteria inhibited, the ethyl acetate extract was equally effective (MIC of 0.3125 mg/mL) against Gram-negative bacteria, including *K. pneumonia*, *S. typhi*, *S. enterica*, *Enterobacter aerogenes*, and *E. coli*. The n-hexane extract inhibited *S. aureus*, *S. flexneri*, and *S. enterica* with a MIC value of 0.1562/mL. The most prevalent phytochemicals in the aqueous and n-hexane extracts from the LC-MS study were nakinadine and kermaphidine. The study revealed that the aqueous extract of *A. chloros* possesses both Gram-positive and Gram-negative antibacterial activity.

***Keywords*:** *Amphimedon chloros*, Antibacterial activity, Ethyl acetate, n-Hexane, Aqueous extract.

## Introduction

The open waters and deep seas comprise roughly 70% of the earth's surface and are home to about 80% of all plant and animal species.[1,2] It's well-known that prokaryotic bacteria, marine invertebrates, and multicellular complex organisms coexist with sharks and whales in the ocean.[3] Modern technological advancements like computerised closed-circuit mixed gas rebreathers, remote-controlled vehicles, manned submersibles, and scuba diving gear have made it possible for scientists to extract novel substances from marine animals at different depths in the ocean.[4] Lately, various aquatic species, such as sea slugs, sponges, and soft corals, have been the main source of naturally occurring bioactive chemicals.[5,6] Of the nearly 40,000 natural products that have been isolated to date, sponge, cnidarian, mollusc, tunicate, bryozoan, echinoderm, red algae, green algae, brown algae, and microbial natural products are included.[7,8] From the start of marine research on natural products to the present day of drug discovery, marine sponges have been a major source of the naturally occurring substances under investigation.[9] One of the most abundant sources is marine sponges, from which hundreds of compounds are discovered annually.[3,10,11]

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The cell layer of a marine sponge is composed of two thinner layers and one layer that resembles jelly. Perhaps in this strata is the spicule structure, made of calcium carbonate, silica, and a protein known as spongin. Molluscs is the scientific name used to refer to marine invertebrates.[12] Sponge varieties are classified based on their spicules' and spicule structures' ratios.[12] Sea sponges operate as filter feeders, removing the need for hazardous particles to be consumed because they produce bioactive compounds that neutralise their toxicity. This adaptation guarantees their survival in potentially particle-rich environments. About eleven different kinds of sponges have been connected to identifying bioactive substances. It is generally recognised that three of these families (Petrosia, Discodemia, and Haliclona) produce strong anti-inflammatory and anticancer substances.[13,14] Sponge metabolism yields a wide variety of classifiable bioactive substances based on their molecular makeup. These compounds include alkaloids, cyclic peptides, terpenoids, sterols, and nucleosides.[10] The microbial symbionts of the sponges, not the sponges themselves, are solely responsible for producing this vast array of physiologically active compounds.[3,12] Sponge-secreting mucus-containing poisons are cytotoxic compounds used to defend against predatory marine organisms. For this reason, sponges can outgrow crammed reefs or rocks and keep up with quickly-growing species. They might be able to employ these toxins without risk, though moderately.[15] According to statistics on marine natural products, sponges contain a remarkably high concentration of biologically active molecules that need to be extracted. Over 28 years, from 1985 to 2012, biologically active compounds from marine organisms were identified. Hu *et al*. have determined these compounds' chronological trend, chemical structure distribution, bioactivity groups, and species distribution.[16] It was discovered that

75% of the chemicals were obtained from marine invertebrates, with sponges having the highest number of bioactive compounds. About 48.8% of the 9,812 marine natural products separated from invertebrates between 1990 and 2009 were unique to sponges.[17] Numerous studies have demonstrated the antibacterial efficacy of sponge extracts. None of these studies included sponge species from the Gulf of Aqaba. Thus, this study aimed to evaluate the antibacterial activity of the various extract sections (polar, semi-polar, and non-polar) of *Amphimedon chloros* sponge recovered from Aqaba Gulf sponge species.

## Materials and Methods

*Study location*

The Gulf of Aqaba served as the research site. At the northernmost point of the Red Sea, the semi-enclosed Gulf of Aqaba is located in a subtropical dry zone bounded by latitudes 28° 00' to 29° 33' N and longitudes 34° 25' to 35° 00' E. The Gulf's average depth is about 800 meters, making it remarkable for having a greater depth (over 1830 meters) than breadth (maximum 25 kilometres). Most of the Gulf region's rainfall occurs between November and May. An average of 35 mm of rain falls in Aqaba each year. The daily temperature varies from 14°C in January to 45°C in summer. The seawater evaporates at a rate of 200–365 cm/year.[18] There is little annual change in salinity, which ranges from 40.3 to 40.8 PSU.[19]

*Sponge collection and identification*

Specialists from the Marine Science Station (MSS) in the Gulf of Aqaba (29°27′ N, 34°58′ E) gathered a large number of sponge samples in September 2021. The sponge was obtained by divers between one and eighteen metres below the surface. *Amphimedon chloros*, a sponge species, was identified from the specimen (Figure 1) using morphological characters from Systema Porifera[20,21] and the latest update to the World Porifera Database.[22] Debris was removed and frozen at -80°C before being sent to Aqaba International Laboratories for extraction and freeze-drying in sterile polypropylene containers submerged in seawater.

**Figure 1:** A sample of marine sponge, *Amphimedon Chloros* obtained from the Gulf of Aqaba, Red Sea.

*Extraction of active metabolites*

The sponge fragments were weighed, freeze-dried, and pulverised into a powder. The freeze-dried material (45.0–350.0 g) was then macerated for 48 hours in a 1:1 combination of methanol and dichloromethane. The fluid was then dehydrated using the lyophiliser to produce a crude extract (9.6 g). The non-polar, semi-polar, and polar components were separated by partitioning the mixture with n-hexane (0.5 L) and ethyl acetate (0.5 L) while it was diluted in distilled water. The extraction solvents were dried, and the pure extract from each solvent was labelled appropriately and refrigerated at -20°C.[23–27]

*Determination of the antibacterial activity of different extracts*
*Bacteria strains used.*

The following bacterial strains were used in this research: *Escherichia coli* 0157:H7 (ATCC 43888), *Staphylococcus aureus* (ATCC 25923), *Klebsiella pneumonia* (ATCC 13883), *Salmonella typhi* (ATCC 14028), *Bacillus cereus* (ATCC 11778), *Salmonella enterica* (ATCC 13076), *Shigella flexneri* (ATCC 12022), *Pseudomonas aeruginosa* (ATCC 27853), *Enterobacter aerogenes* (ATCC 13048), and *Listeria monocytogenes* (ATCC 7644). Isolates were stored on nutrient agar slants at 4°C. The strains were first activated by culturing them at 37°C for 24 hours in ***Muller Hinton Broth (***MHB) before any antimicrobial assays were performed.[28]

*Antibacterial agar disc diffusion assay*

Mueller-Hinton agar and the disc diffusion method were used to quantify the antibacterial activity of marine extracts. The widths of inhibitory zones were expressed in millimetres.[29] Sterile filter paper discs were impregnated with twenty (20) µL of sponge extracts and placed on infected Petri dishes containing 0.1 mL of a bacterial solution containing $1.5 \times 10^8$ colony forming units per millilitre. Positive controls were discs pre-dosed with 50 µg/mL of ampicillin and chloramphenicol, whereas negative controls were discs containing 5% dimethyl sulfoxide (DMSO). The extract-impregnated disc's inhibition zone diameter (IZD) and the positive and negative controls were assessed following a 24-hour incubation period at 37°C. All tests were done in triplicates.[23,24,27,29,30]

*Determination of Minimum inhibitory concentration (MIC)*

The MIC of sponge extracts that showed antimicrobial action (IZD > 8 mm) was calculated using the microdilution technique. The samples were diluted with 5% dimethyl sulfoxide to yield concentrations of 0.0195 to 2.5 mg/mL. Each bacterial strain was introduced at a density of 0.5 McFarland ($1.5 \times 10^8$ CFU/mL) in a 96-well microplate with 100 µL of extract dilutions. One hundred µL of each bacterium strain was added to one hundred µL of Muller Hinton broth in a microplate and used as a positive control. The microplate was kept at 37°C for a total of 24 hours. The turbidity formation indicated the occurrence of bacterial growth. The minimum inhibitory concentration (MIC) is the amount of extract needed to prevent further development of a given bacteria strain. The test was done in triplicates.[31,32]

***Minimum Bactericidal Concentration (MBC)***

For sponge preparations with MIC below 100 µg/mL, the MBC was determined. The lowest drug dose that eliminates 99.9% of the microorganisms in a trial is known as the MBC. To ascertain the MBC, 20 µL of non-growing culture medium from each well was plated on MHA. The quantity of living organisms (based on the CFU) was determined following a 24-hour incubation period at 37°C. The action is regarded as bactericidal when MIC and MBC are equal or when MBC is 1, 2, or 3 times greater than MIC.[33]

*Liquids chromatography-mass spectrometry (LC-MS)*

The LC-MS (LC-8030, Shimadzu, Japan) analysis was conducted using gradient solvents as the mobile phases. Solvent A consisted of 0.1% (v/v) formic acids diluted in water, and solvent B was 0.1% (v/v) formic acids dissolved in acetonitrile. In this procedure, Agilent Zorbax Eclipses XDB-C18 columns (2.1x150 mm x 3.5 m), a temperature of 25 °C, injection volumes of 1 L, and ethyl acetate sample concentration of 18 mg/mL were utilised. The mass detector was equipped with SIL-30AC autosamplers with cooler, LC-30AD pumps, a CTO-30 columns oven, Electrospray ion-mass spectrometers (ESI-MS) with a Shimadzu LC-MS 8030, skimmer voltages of 65 V, and a fragmentors voltage of 125 V. The extracts from the sponge (10 mg/mL) were injected into the detectors. A drying agent with a purity level of 99.99% and a flow rate of 10 L/min was used under positive ions modes. This part of the research used nebulisers operating at 45 pounds per square inch (psi) with capillary temperatures of 350 °C. The eluents were run through mass/charge of ions (m/z) analyses measuring 100 to 1000 times. Results were confirmed with the use of actual, industry-standard chemicals.

## Results and Discussion

Over the past few decades, a great deal of studies have led to the identification of marine creatures containing chemicals and extracts with potential antibacterial properties.[9,11,34] The antibacterial activity of *A. chloros* sponge extracts was tested. The results showed a discernible variance in the extracts' ability to inhibit a particular category of bacteria. Six of the seven sponge crude extracts and fractions displayed considerable activity against at least two bacterial strains in a weak to vigorous spectrum. The inhibitory activities of weak (6-8 mm), moderate (8-12 mm), good (12-16 mm), and potent (> 16 mm) were measured.[33] The aqueous extract comprehensively impacted all ten species of bacteria tested. The efficiency of this extract varied between 8.2 mm for *L. monocytogenes* and 20.6 mm for *S. flexneri*, depending on the size of the inhibitory zones. It's worth noting that this extract particularly targets *S. enterica, P. aeruginosa, E. coli* 0157:H7, and *K. pneumoniae*. These findings were based on the diameters of the inhibitory zones, which were 12.2 mm, 18.5 mm, 14.3 mm, and 12.6 mm, respectively. Furthermore, the same extract showed 10.2 and 8.2 mm IZD values against Gram-positive bacteria, including *S. aureus* and *L. monocytogenes*, with *B. cereus* being the most sensitive (13 mm) (Table 1). In recent years, there has been an increase in the number of antibacterial screening studies conducted all over the globe, specifically focusing on sea sponges.[35,36] The results confirm that *A. chloros*, a red sea sponge, contains potent antibacterial agents. The ethyl acetate extract had little effect on the bacteria, inhibiting nine of the ten tested. The available data shows that the ethyl acetate extract is less effective than the aqueous extract. Even though the ethyl acetate extract exhibited a less significant effect on *S. flexneri* and *P. aeruginosa*, the aqueous extract outperformed the ethyl acetate extract in all aspects. The ethyl acetate extract had no noticeable impact on *B. cereus*. Although *K. pneumoniae, P. aeruginosa,* and *L. monocytogenes* were utterly resistant to ampicillin and chloramphenicol, the aqueous extract effectively inhibited their growth. Conversely, the diameter of the inhibitory zone of n-hexane was 15.6 mm for *S. flexneri* and 8.1 mm for *P. aeruginosa*, suggesting that it was only effective against these two species. Chloramphenicol was only effective against six of the ten different species of bacteria against which ampicillin was found to be effective. Notably, this extract primarily targets three Gram-negative pathogenic bacteria: *S. enterica, P. aeruginosa,* and *E. coli* 0157:H7. The majority of pathogens classified by the World Health Organization are gram-negative bacteria. Gram-negative bacteria are more resistant than Gram-positive bacteria due to their unique structure, and they are a major global cause of disease and mortality.[26,30,37,38] To manage and combat resistance, many documented strategies can be employed, such as the development of antimicrobial adjuvants, structural modification of currently licensed antibiotics, and research and screening of novel materials such as natural compounds with novel mechanisms of action and susceptible targets of resistant Gram-negative bacteria.[39,40] Global outbreaks related to food have been linked to *L. monocytogenes*. The former may flourish and endure in unfavourable environments with high salt, low pH, and high temperatures.[39] The food and waterborne bacteria *E. coli* O157:H7 produces the Shiga toxin, responsible for the hemolytic uremic syndrome (HUS), diarrhoea, and hemorrhagic colitis in humans.[41,42] Therefore, when combined with the appropriate antibiotics, employing ethyl acetate extract in a novel way against these two kinds of pathogenic bacteria may be promising. The microdilution technique was used further to evaluate the antibacterial activity of the sponge extract and ascertain its minimum inhibitory concentration (MIC). Antibacterial activity was demonstrated by the sponge extracts at concentrations ranging from 0.0195 to 2.5 mg/mL. The analysis results carried out using the disc diffusion technique, particularly the aqueous extract, were corroborated by the overall MIC values of the sponge extracts. *A. chloros* aqueous extracts were found to have antibacterial activity against *E. coli* 0157:H7, *S. aureus, K. pneumonia, S. typhi, B. cereus, S. enterica, S. flexneri, P. aeruginosa,* and *E. aerogenes* based on the results of the MIC test. These bacteria had minimum inhibitory concentrations (MICs) ranging from 0.0390 to 0.3125 mg/mL; for *L. monocytogenes*, the value of MIC exceeded 2.5 mg/mL (Table 2). The aqueous component of *A. chloros* exhibited bactericidal efficacy against *S. flexneri, S. enterica,* and *S. aureus* at concentrations of 0.1562 mg/mL, as shown by the MBC test findings. None of the ten studied bacteria showed signs of being susceptible to the bactericidal effects of the ethyl-acetate extract. The MIC test revealed the antibacterial effectiveness of ethyl-acetate extracts of *A. chloros* against *E. coli* 0157:H7, *S. aureus, K. pneumonia, S. typhi, S. enterica, E. aerogenes,* and *L. monocytogenes.* These extracts exhibit MICs ranging from 0.3125 to 0.625 mg/mL; other bacteria have shown tolerance to extract concentrations of more than 2.5 mg/mL. The MBC test revealed that the aqueous component of *A. chloros* inhibited *S. flexneri, S. enterica*, and *S. aureus* at 0.1562 mg/mL (Table 2). There was no evidence that the ethylacetate extract inhibited any of the 10 bacteria tested.

Furthermore, Gram-positive bacteria with the lowest susceptibility level were *L. monocytogenes* and *S. aureus*. Nine of the 10 bacteria studied were inhibited by the ethyl acetate extract, which was less effective on the bacteria than the aqueous extract. The aqueous extract only had a minimal impact on Gram-positive bacteria, while the ethyl acetate extract showed better inhibitory activity. Even though *K. pneumonia, P. aeruginosa,* and *L. monocytogenes* were utterly resistant to ampicillin and chloramphenicol, the aqueous extract could still suppress these bacteria. The ethyl acetate and aqueous extracts were usually efficient against all ten bacterial species tested. Ampicillin and chloramphenicol affected 6-7 of the 10 bacterial species studied. In medical practice, infectious diseases are one of the leading causes of death, and their prevalence is rising. Despite the availability of numerous treatments, they are useless against newly emerging multidrug-resistant (MDR) infections. This implies that searching for novel compounds will require effort, resources, and time. MDR bacteria are on the rise due to antibiotic overuse, therefore, it's critical to monitor their development and spread.[43,44]

The chemical composition of the sea sponge *A. chloros* was examined using liquid chromatography-mass spectrometry (LC-MS) in extracts of water, n-hexane, and ethyl acetate (Tables 3 and 4). With the discovery of eighteen distinct compounds, the n-hexane extract displayed the highest diversity, whereas the water extract only revealed six distinct compounds. Significant concentrations of alkaloids were present in the n-hexane (36.2%) and aqueous (50.6%) extracts, with the former also showing notable levels of fat and lipid derivatives (28.2%). It was found that both extracts contained a fair amount of alkaloids. It was found that kermaphidine was the most abundant component in the n-hexane extract, accounting for 12.6% of the total. Trichosenoic acid, pentacosenal, pyrinodemin, zamamidine, and pentadecenoic acid were other constituents identified. The chemical makeup of *A. chloros* has not been thoroughly studied, and the only known component in this species with broad-spectrum antibacterial activity is the pyridinium alkaloid amphitoxin.[45] The identification of the chemical compositions of the sponge species in this genus has shown that they share a large number of compounds, such as tricosenoic acid, pentacosenal acid, zamamidine, nakinadine, pyrinodemins, and ircinol.[46] It was also demonstrated that significant concentrations of hydroxytricosanoic acid, keramaphidine B, and methoxyhexadecanoic acid were present in the ethyl acetate extract. These components showed distinct chemical profiles when compared to the other solvent extractions. On the other hand, the most prevalent phytoconstituents in the water extract was nakinadine (21.2%), which was closely followed by esterastin, ircinol, tricosenal, carotene, and keramamine. Compared to the other solvent extractions, these components displayed different chemical profiles. Additionally, cyclic bis-1,3-dialkylpyridinium alkaloids and cyclostellettamines, which exhibit moderate cytotoxic and antibacterial activity against A 549 cell-line and Gram-positive pathogens, respectively, were extracted from the Korean sponge *Halicona sp. In vitro*, some alkaloids derived from the marine sponge *Agelas mauritiana* demonstrated antibacterial and antifungal effects against methicillin-resistant strains of *S. aureus* and *Cryptococcus neoformans*.[47–55] Gram-positive and Gram-negative organisms showed the same level of sensitivity to the extract, making it difficult to distinguish which is more sensitive, especially to the aqueous extract of *A. chloros*. The results of this investigation indicate that *A. chloros*'s aqueous extract may have broad-spectrum antibacterial activity. Aqueous components of some red sea sponges, such as *A. chloros*, contain fractions and crude extracts that can boost drug antibacterial activity and effectiveness. Additionally, regarding its ability to inhibit

both bacteria, *A. chloros* aqueous extract exhibited better antibacterial activity than control antibiotics, the n-hexane and ethyl-acetate extracts. The results align with a prior investigation[56], demonstrating that *Halyclona* sponges and extracts possess extensive and potent antibacterial capabilities against Gram-positive and Gram-negative bacteria. As shown by Song *et al*.[57] study employing ketoconazole and trichodermastone A-D, generated from the marine fungus *Trichoderma koningii*, which exhibit antifungal activity against *Candida albicans*, the usage of marine natural materials has synergistic effects with antimicrobial medicines. One of the animal kingdoms that produces the most significant amount of bioactive metabolites is the marine sponge family; however, occasionally, microorganisms rather than sponges create the molecules.[58] *A. donnani* lectin extracts have been shown to exhibit antibacterial activity against biofilm-forming isolates of *P. aeruginosa*, *K. pneumoniae*, and *E. coli*, three Gram-negative bacteria.[59] *A. chloros* displayed efficacy against three Gram-negative bacteria that form biofilms in our investigation, namely *E. coli*, *K. pneumoniae*, and *P. aeruginosa*.[59–63]

**Table 1:** Inhibition zone diameters of *Amphimedon chloros* crude extracts tested against ten species of bacteria

| Bacteria species | Inhibition zone diameters (mm) | | | | |
|---|---|---|---|---|---|
| | **Aqueous** | ***n*-hexane** | **ethyl-acetate** | **Amp.** | **Chl.** |
| *E. coli 0157:H7* | 14.3 | 0.0 | 10.6 | 15.0 | 15.0 |
| *Staph aureus* | 10.2 | 0.0 | 12.4 | 14.5 | - |
| *Klebsiella pneumonia* | 12.6 | 0.0 | 10.2 | - | - |
| *Salmonella typhi* | 12.2 | 0.0 | 12.4 | 16.0 | 16.0 |
| *Bacillus cereus* | 13.0 | 0.0 | 0.0 | 13.0 | 15.0 |
| *Salmonella enterica* | 14.2 | 0.0 | 9.0 | 21.5 | 20.0 |
| *Shigella flexneri* | 20.6 | 15.6 | 7.3 | 13.0 | 22.0 |
| *pseudomonas aeruginosa* | 18.5 | 8.1 | 7.0 | - | - |
| *Enterobacter aerogenes* | 13.4 | 0.0 | 9.1 | 12.5 | 22.0 |
| *Listeria monocytogenes* | 8.2 | 0.0 | 10.4 | - | - |

Inhibition zones are in mm ± SE (n = 3). –: no inhibition zone. Amp. = ampicillin, Chl.= chloramphenicol

**Table 2:** Minimum Inhibitory Concentration (MIC) and Minimum bactericidal concentration (MBC) of *Amphimedon chloros* crude extracts (mg/mL)

| Bacteria species | **Aqueous** | | ***n*-hexane** | | **ethyl-acetate** | |
|---|---|---|---|---|---|---|
| | **MIC** | **MBC** | **MIC** | **MBC** | **MIC** | **MBC** |
| *E. coli 0157:H7* | 0.1562 | >2.5 | >2.5 | >2.5 | 0.625 | >2.5 |
| *Staph aureus* | 0.0390 | 0.1562 | >2.5 | >2.5 | 0.625 | >2.5 |
| *Klebsiella pneumonia* | 0.1562 | >2.5 | >2.5 | >2.5 | 0.3125 | >2.5 |
| *Salmonella typhi* | 0.3125 | >2.5 | >2.5 | >2.5 | 0.3125 | >2.5 |
| *Bacillus cereus* | 0.0195 | >2.5 | >2.5 | >2.5 | >2.5 | >2.5 |
| *Salmonella enterica* | 0.0781 | 0.1562 | >2.5 | >2.5 | 0.3125 | >2.5 |
| *Shigella flexneri* | 0.0390 | 0.1562 | 0.1562 | >2.5 | >2.5 | >2.5 |
| *pseudomonas aeruginosa* | 0.3125 | >2.5 | >2.5 | >2.5 | >2.5 | >2.5 |
| *Enterobacter aerogenes* | 0.1562 | >2.5 | >2.5 | >2.5 | 0.3125 | >2.5 |
| *Listeria monocytogenes* | >2.5 | >2.5 | >2.5 | >2.5 | 0.3125 | >2.5 |

(-); not detected based on extract concentration up to 2.5 mg/mL.

**Table 3:** Chemical composition of *Amphimedon chloros* hexane extract

| | **Compounds** | **Chemical formula** | **MW (g/mol)** | **%** |
|---|---|---|---|---|
| 1 | Glycine | $C_2H_5NO_2$ | 75.07 | 7.1 |
| 2 | Purine | $C_5H_4N_4$ | 120.11 | 9.3 |
| 3 | Methoxy hexadecanoate | $C_{17}H_{34}O_3$ | 286.4 | 3.2 |
| 4 | Hachijodine | $C_{19}H_{34}N_2O$ | 306.5 | 4.4 |
| 5 | Tricosenal | $C_{23}H_{44}O$ | 336.6 | 1.5 |
| 6 | Keramamine | $C_{23}H_{33}N_3$ | 351.5 | 2.2 |
| 7 | Tricosenoic acid | $C_{23}H_{44}O_2$ | 352.6 | 11.6 |
| 8 | Pentacosenal | $C_{25}H_{48}O$ | 364.6 | 10.4 |
| 9 | Hydroxytricosanoic acid | $C_{23}H_{46}O_3$ | 370.6 | 4.4 |
| 10 | Kermaphidine | $C_{26}H_{40}N_2$ | 380.6 | 12.6 |

| | | | | |
|---|---|---|---|---|
| 11 | Pentadecenoic acid | $C_{25}H_{50}O_2$ | 382.7 | 6.2 |
| 12 | Ircinol | $C_{26}H_{40}N_2O_2$ | 412.6 | 3.6 |
| 13 | Nakinadine | $C_{27}H_{40}N_2O_2$ | 424.6 | 1.2 |
| 14 | Amphimedoside A | $C_{28}H_{46}N_2O_6$ | 506.7 | 2.5 |
| 15 | Esterastin | $C_{28}H_{46}N_2O_6$ | 506.7 | 1.4 |
| 16 | Carotene | $C_{40}H_{56}$ | 536.9 | 1.8 |
| 17 | Pyrinodemin | $C_{37}H_{57}N_3O$ | 559.9 | 9.2 |
| 18 | Zamamidine | $C_{49}H_{60}N_6O$ | 749 | 7.4 |

**Table 4:** Chemical composition of *Amphimedon Chloros* water extract

| | Compounds | Chemical formula | MW (g/mol) | % |
|---|---|---|---|---|
| 1 | Tricosenal | $C_{23}H_{44}O$ | 336.6 | 15.2 |
| 2 | Keramamine | $C_{23}H_{33}N_3$ | 351.5 | 10.6 |
| 3 | Ircinol | $C_{26}H_{40}N_2O_2$ | 412.6 | 18.8 |
| 4 | Nakinadine | $C_{27}H_{40}N_2O_2$ | 424.6 | 21.2 |
| 5 | Esterastin | $C_{28}H_{46}N_2O_6$ | 506.7 | 19.9 |
| 6 | Carotene | $C_{40}H_{56}$ | 536.9 | 13.3 |

## Conclusion

The marine sponge *A. chloros* exhibited broad-spectrum antibacterial activity against Gram-positive and Gram-negative bacteria. The activity appears to be correlated with the polar hydrophilic phytoconstituents since the most effective extract was the aqueous extract. The bacteriostatic and bactericidal activities of *A. chloros* extracts may suggest that they contain novel antibacterial phytoconstituents that can be developed to treat infections such as those caused by multidrug-resistant bacteria. Further investigations are required to isolate and elucidate the active compounds in *A. chloros* and to determine their cytotoxicity.

## 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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