BIODIVERSITAS
Volume 24, Number 12, December 2023
Pages: 6675-6683

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d241229

Antibacterial activity of sea hare (*Dolabella auricularia*) egg string extracts against potentially pathogenic bacteria

CHENCY GRACE O. LIGUEZ[1], SHARON ROSE TABUGO[1,2,♥]

[1]Department of Biological Sciences, Mindanao State University-Iligan Institute of Technology. Iligan City 9200, Lanao del Norte, Philippines. Tel./fax.: +63-917-1100599, ♥email: sharonrose.tabugo@g.msuiit.edu.ph

[2]Molecular Systematics and Conservation Genomics Laboratory, Center for Biodiversity Studies and Conservation, Premier Research Institute of Science and Mathematics, Mindanao State University-Iligan Institute of Technology. Iligan City 9200, Lanao del Norte, Philippines

Manuscript received: 7 November 2023. Revision accepted: 23 December 2023.

Abstract. *Liguez CGO, Tabugo SR. 2023. Antibacterial activity of sea hare (*Dolabella auricularia*) egg string extracts against potentially pathogenic bacteria. Biodiversitas 24: 6675-6683.* Pharmaceutical industries now recognize the vast variety of ocean organisms, each possessing distinct biological characteristics. Sea hares, for example, are marine organisms that use bioactive chemicals to defend against predators, including their eggs. To explore the potential antibacterial properties of sea hare species (*Dolabella auricularia*) found in Pujada Bay, Philippines, egg strings were collected and extracted using hexane and methanol solvents. The antibacterial activity of each fraction was then determined through Minimum Inhibitory Concentration (MIC) testing against four potentially pathogenic bacteria, two gram-positive strains (*Staphylococcus aureus*, *Bacillus subtilis*) and two gram-negative strains (*Escherichia coli*, *Pseudomonas aeruginosa*). The broth microdilution method was employed to assess the antibacterial activity of *Dolabella auricularia* egg strings. The results revealed the MIC values for the four bacterial strains. The hexane extracts of both extract 1 and extract 2 exhibited a MIC of 0. 23 and 0.33 mg/mL and a MIC of 0.52 and 0.125 mg/mL against *P. aeruginosa*, respectively. The methanolic extracts (1 and 2) displayed a MIC of 1.46 and 2.83 mg/mL against *E. coli* and an even more potent MIC of 1.33 and 0.79 mg/mL against *P. aeruginosa*. In the case of *B. subtilis*, the hexane extracts (1 and 2) had a MIC of 1.5 and 0.54 mg/mL, while the methanolic extracts (1 and 2) exhibited a MIC of 1.17 mg/mL and 0.83 mg/mL. Lastly, against *S. aureus*, hexane extracts (1 and 2) suppressed the growth with a MIC of 0.77 mg/mL and 0.25 mg/mL, respectively, while both methanolic extracts (1 and 2) demonstrated a MIC of 3.33 mg/mL. These findings showcase the promising antibacterial activity of *Dolabella auricularia* egg string extracts and highlight their potential for further investigation and development in the pharmaceutical field.

Keywords: Antibacterial, bioactive chemicals, broth microdilution method, Pujada Bay, sea hare

INTRODUCTION

Oceans encircle more than 70% of the surface of our globe. Marine life is more diverse than its terrestrial counterparts because it is home to 80% of all living forms (Srilekha et al. 2017). Compounds with unique structures and biological activity are abundant in the ocean (Petersen et al. 2020). Over the past three decades, marine creatures like echinoderms, sea anemones, tunicates, and mollusks have produced various metabolites with biological activity and biomedical potential (Mashjoor and Yousefzadi 2017). Marine species produce chemicals with an ecological benefit as they struggle for existence in the harsh ocean environment (Tayone 2017). One of these marine species is the sea hare.

Sea hares are mollusks without shells that move slowly and eat a variety of algae and sponges. The marine molluscs of the genus *Aplysia* have been the focus of some of the most extensive studies on the neurological underpinnings of learning, memory, behavior, development, and growth (Lee et al. 2014; Aggio and Derby 2019). It has been hypothesized that sea hares, which other marine animals do not consume, have chemical defensive mechanisms. Sea hares are a source of marine bioactive chemicals, which have been employed in the food industry to improve the functional characteristics of foods or as food additives to increase specific food properties (stability, emulsification, texture improvement) (Huang et al. 2021).

Inking is an active chemical defense used as a last line of defense during an attack. Sea hare ink secretion is a sticky, purple mixture of gland secretions in the mantle cavity (Love-Chezem et al. 2013; Tayone 2020). Even though the identity of the bioactive compounds and the mechanisms underlying their effects are mostly unknown, it has been demonstrated that the secretion of ink shields sea hares from a variety of predators, particularly invertebrates like crustaceans and sea anemones, spiny lobsters, and fish (Prince and Johnson 2015). During their spawning season, sea hares lay yellow eggs in strings of gelatin (May and June). The egg masses of the wedge sea hare are known as "*lukot*" in Davao Oriental and neighboring locations. Its twisted structure, which resembles noodles, and unusual flavor, draws the attention of seafood enthusiasts. Egg masses are popular in the Philippines and other parts of the world, such as Samoa, Kiribati, and the Fiji Islands. The egg masses are gathered for food in the locations mentioned above, where they are consumed either raw or partially cooked (Delan et al. 2015; Pepito et al. 2015). Even though these eggs seem helpless,

they are not damaged by bacteria or seem to be consumed by predators. These findings imply that the eggs have other walls of defense, presumably substances created from scratch by sea hares. They contain a biologically active material to serve as a barrier between them and the environment. Studies show they have a lectin that can agglutinate marine microorganisms (Cheung et al. 2015; Filho et al. 2015; Hasan and Ozeki 2019; Swarna et al. 2021).

Pathogenic bacteria such as *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa* can cause diseases and spread in several ways. *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* can cause diseases and infect patients with cystic fibrosis (CF) lung infection, burns, cancer, bacteremia, mastitis, abortions, and upper respiratory infections (Khalid Abbas and Elsharbasy 2019; Magalhães et al. 2019; Sukmiwati et al. 2020; Qin et al. 2022). *Bacillus subtilis* is a common cause of infections and is susceptible to several existing medicines (Fang et al. 2014). Meningitis, neonatal sepsis, pneumonia, and wound infection by *B. subtilis* (Lampropoulos et al. 2021; Tokano et al. 2023). It occurs most frequently in patients with underlying diseases or immunocompromised (Kato et al. 2022). More research is required to determine whether sea hare egg strings may be used to treat infectious diseases brought on by common infections. Hence, this study assessed the potential of sea hare egg strings in inhibiting the growth of common potentially pathogenic bacteria.

MATERIALS AND METHODS

Sample collection and preparation

Around 2 kg of egg strings were hand-picked along the coast of Guang-guang, Pujada Bay, Mati City, Davao Oriental, Philippines (Figure 1). Pujada Bay is located in the southern portion of Mindanao between 6° 48'04" and 6° 54'25" N latitude and 126° 9'08" and 126° 19'33" E longitude. The sea hare samples were gathered manually from Pujada Bay in the intertidal zone at a depth of (10-150 m). The adherent sediments and contaminants were subsequently removed from the fresh samples at the sampling location by washing them in seawater before putting them in plastic bags. The samples were cleaned again, placed in a clean ice box container with seawater, and transported to the Molecular Systematics and Conservation Genomics Laboratory in the Premier Research Institute of Science and Mathematics (PRISM), MSU-IIT, for later experiments. On the same day, a quick rinse with tap water was performed in the laboratory to remove any epiphytes and contaminants that remained on the sea hare egg strings.

Preparation of bacteria and culture media

Two gram-positive bacteria (*Staphylococcus aureus* BIOTECH 1582 and *Bacillus subtilis* BIOTECH 1679), and two gram-negative bacteria (*Escherichia coli* BIOTECH 1634 and *Pseudomonas aeruginosa* BIOTECH 1335) were used as test strains. These strains were provided by PRISM obtained from the National Institute of Molecular Biology and Biotechnology, University of the Philippines Los Baños, Laguna, Philippines.

Two conventional media were employed to cultivate the test strains and assess the antibacterial efficacy of sea-hare egg string extracts. Nutrient Agar (NA) and Muller-Hinton Broth (MHB) were utilized for the actual bioassay.

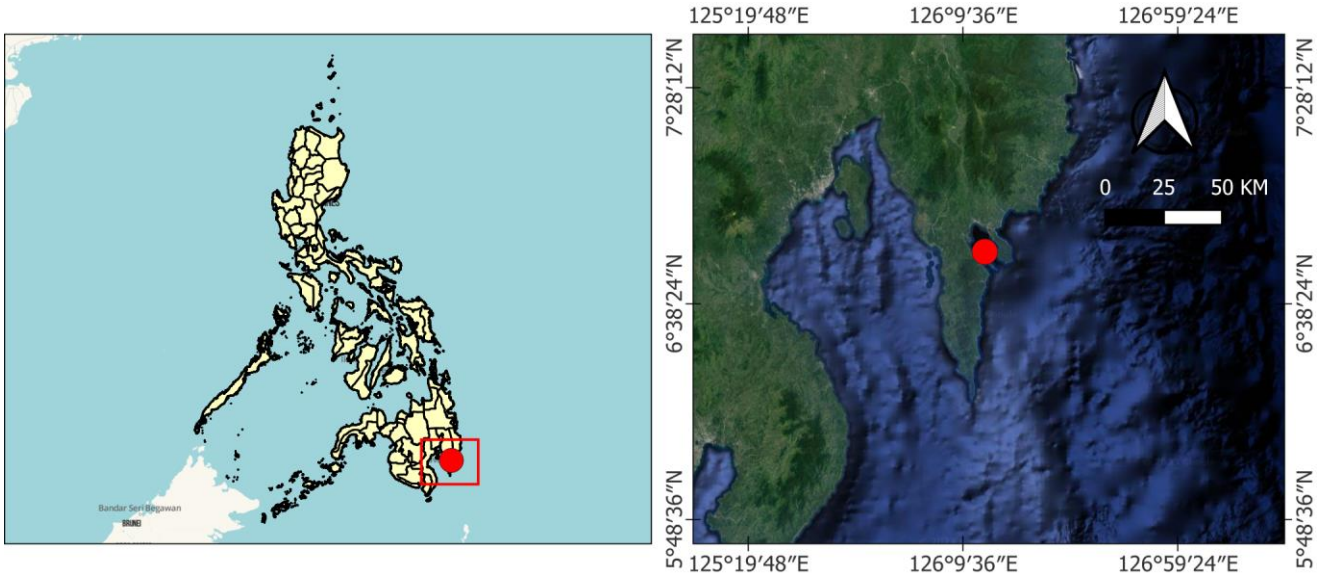

Figure 1. Map of collection site in the Pujada Bay, Pacific Seashore of Mindanao Island, Southern Philippines

Preparation of sea-hare egg string extracts

Sea hare egg strings were washed with tap water and followed by distilled water to remove the unwanted debris on the samples. About 200 g of sea-hare egg strings samples were immersed with hexane for a week, drained, and then transferred to methanol for another week. The mixtures were filtered using whatman no. 1 filter paper. The filtrates were labeled as sample 1 and the second as sample 2. Following the filtration procedure, the residue was removed, and the filtrates were concentrated in vacuo using a rotary evaporator at temperatures close to the boiling points of methanol and hexane. Approximately 4 mL of each extract was transferred to vials using a pipette, and the vials were covered using a foil with small holes to diffuse the remaining extraction solvents until the desired debris was obtained for the experiment.

Determination of antibacterial activity

All the materials used in the assay underwent thorough preparation and sterilization. Specifically, the medium was created by dissolving Nutrient Agar (NA) and Muller Hinton Broth (MHB) powder in 50 mL of sterile distilled water, and any remaining particles were dissolved by heating the mixture in a microwave. The entire assay was carried out under aseptic conditions. Test materials, including hexane and methanolic extracts and the positive control ciprofloxacin, were all prepared at 100 µg/mL concentration. The ciprofloxacin stock solution was prepared at a concentration 10 times higher than the maximum concentration needed for testing, with Dimethylsulfoxide (DMSO) serving as the solvent. The extracts were likewise prepared at the same concentration as the stock solution, with the dilutions made in test tubes using Muller Hinton Broth, as indicated in Tables 1 and 2.

A colony of bacteria was transferred in a 3 mL phosphate-buffered saline solution (PBS) using a cotton swab, capped, and vortexed. It was adjusted to 0.5 McFarland by visually comparing the 0.5 McFarland tube to the PBS tube, giving a 1 x 10^8 CFU mL^{-1} suspension. Suspensions were used within 30 minutes. In a micro-titre plate, the first 10 columns were used for antimicrobial solutions, column 11 for sterility control, and column 12 for growth control. One row was used per isolate being tested. The concentrations tested were: 0.06, 0.125, 0.25, 0.5, 1,2,4,8,16,32 mg L^{-1}. One hundred microliter of sterile broth was added to the sterility control wells, while 50 µL of sterile broth was added to the growth control wells.

Moreover, using the stock solutions made in step 2 (shown in row A, Table 2), quantities were shown (rows B and C) to ten 1.5 mL tubes, labeling each one with its final concentration (row D). Next, take 50 µL of antimicrobial stock solution/extract solution from the tubes and place it in the respective well in a 96-well plate, starting with the strongest concentration in the first well. The 1 x 10^8 CFU mL^{-1} bacterial suspensions were diluted to 1:100 by 990 µL of PBS and 10 µL of each bacterial suspension. Then, fifty microliters of the diluted suspensions were added to each well containing antibiotic/extract solutions and the growth control well. The final bacterial concentration is 5 x 10^5 CFU mL^{-1}. The final antimicrobial concentration is shown in Table 2, row E. From the growth control well, 10 µL was taken and added to 990 µL PBS in a 1.5 mL tube (dilution 10^{-2}) and vortexed. Then, 100 µL was taken from this tube, added to 900 µL PBS in another 1.5 mL tube (dilution 10^{-3}), and vortexed. This was done for all bacterial suspensions. From this tube, 100 µL was taken and inoculated onto 2 Muller Hinton plates, covered, and sealed by parafilm. Muller Hinton and micro-titre plates were incubated at room temperature for 16 to 24 hours.

Statistical analysis

The mean standard deviation was used to express all experimental measurements performed in triplicate. A One-way Analysis of Variance was performed to examine statistical differences between the concentrations (ANOVA). The data were examined using Paleontological Statistics (PAST) v.4.03 software. Tukey's pairwise test was performed to determine whether a statistically significant difference existed between the studied groups and the positive control.

Table 1. Stock solution preparation

	Antimicrobial solution	**Muller Hinton Broth**	**Final solution mg L^{-1}**
Tube 1	1 mL 1280 mg L^{-1}	9 mL	128
Tube 2	1 mL 128 mg L^{-1}	7 mL	16
Tube 3	1 mL 16 mg L^{-1}	7 mL	2
Tube 4	1 mL 2 mg L^{-1}	7 mL	0.25

Table 2. Plate preparation of antibacterial assay

Plate well	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Antimicrobial stock solutions (mg L^{-1})	128	128	128	16	16	16	2	2	2	0.25
Volume stock solution (µL)	250	125	62.5	250	125	62.5	250	125	62.5	250
Volume sterile broth (µL)	250	375	437.5	250	375	437.5	250	375	437.5	250
Antimicrobial concentration (mg L^{-1})	64	32	16	8	4	2	1	0.5	0.25	0.125
Final antimicrobial concentration in test (mg L^{-1})	32	16	8	4	2	1	0.5	0.25	0.125	0.06

RESULTS AND DISCUSSION

Antibacterial activities of *Dolabella auricularia* egg string extracts

Over the past few years, numerous investigations on the antibacterial properties of various sea species have been conducted in many different nations (Shakouri et al. 2017). The search and discovery of natural antibacterial compounds have been increasingly reported worldwide. Marine mollusks defend themselves from predators in an unusual way. With the number of marine species, numerous promising substances have been identified from marine macro-organisms. Marine environments are among the most abundant and diverse ecosystems in terms of biological and chemical variety. The marine environment is a rich source of bioactive chemicals, as evidenced by the approximately 1,241 novel biochemical compounds reported in 2012 (Afifi Khattab et al. 2016). In particular, bioactive peptides and compounds that are antibacterial, anti-inflammatory, and anti-cancer agents are among the biologically active components they represent as new reservoirs (Macedo et al. 2021; Zaky et al. 2022). Secondary metabolites from marine species exhibit a variety of bioactivities, including cytotoxic, antibacterial, and antifungal properties (Darya et al. 2020). Though many natural sources are suitable for the discovery of novel potentially bioactive compounds, the marine environment, which is home to a wide diversity of creatures with varying physiologies and capacities for adaptability, is quickly emerging as a top location for the identification of new therapeutic leads (Martins et al. 2014). The present investigation assessed the antibacterial activity of sea hare (*Dolabella auricularia*) egg string extracts against two gram-positive bacteria (*S. aureus* and *B. subtilis*) and two gram-negative bacteria (*E. coli* and *P. aeruginosa*).

The antibacterial activity was investigated using a standard technique. The results of tests on aqueous methanol and hexane extracts of *Dolabella auricularia* egg mass were analyzed (Table 3). A notable antibacterial activity has been seen in the current work against various bacterial strains. The MIC of each test substance was established in this experiment against opportunistic pathogen gram-positive bacteria, *B. subtilis* and *S. aureus*, and gram-negative bacteria, *P. aeruginosa* and *E. coli*. A significant number of bacterial strains were slightly resistant to the extract of *Dolabella auricularia*. However, in the methanolic first extract, bacterial growth was slightly increased in all tested bacterial strains. Firstly, is the bacteria *B. subtilis*, which had the highest antibacterial activity (MIC = 1.17 mg/mL), followed by *P. aeruginosa*, which exhibited (MIC = 1.33 mg/mL), and then *E. coli* with antibacterial activity (MIC = 1.46 mg/mL), and lastly by *S. aureus* with (MIC = 3.33 mg/mL) antibacterial activity. Methanolic extracts did not significantly influence potentially pathogenic bacteria, including *S. aureus*, compared to other test organisms. There is a slight resistance of the *S. aureus* to the methanolic extract of sea hare egg strings. There was a sudden decrease in the growth of bacterial strains for the methanolic second extract. The highest antibacterial activity was *P. aeruginosa* (MIC = 0.79 mg/mL), followed by *B. subtilis* with an MIC of (0.83 mg/mL). However, the third bacteria which was *E. coli*, exhibited antibacterial activity (MIC = 2.83 mg/mL), it increased when compared to the first methanolic extract of sea hare egg strings, and *S. aureus* with (MIC = 3.33 mg/mL) antibacterial activity remains unchanged from the first extract of methanol (Table 3, on methanol (E2)). Meanwhile, the first hexane extract showed a sudden decrease in bacterial growth from the methanolic extract, with *E. coli* as the leading bacteria among the four bacterial strains with the highest antibacterial activity (MIC = 0.23 mg/mL). Moreover, *P. aeruginosa* seconded this with a MIC of (0.52 mg/mL). Thirdly was *S. aureus* with a MIC of (0.77 mg/mL), and lastly was *B. subtilis*, which had the lowest antibacterial activity among the four (4) test organisms (MIC = 1.5 mg/mL). As for the second extract of hexane, there was an increase of minimum inhibitory concentrations among the four extracts of different concentrations of all test organisms except for *E. coli* as compared to the first extract of hexane. The highest antibacterial activity was *P. aeruginosa* (MIC = 0.125 mg/mL), followed by *S. aureus* (MIC = 0.25 mg/mL), and then followed by *E. coli* having an antibacterial activity of (MIC = 0.33 mg/mL), and finally by *B. subtilis* with (MIC = 0.54 mg/mL) (Table 3, on Hexane (E2)).

In addition, Table 4 shows the one-way ANOVA comparing the antibacterial activity of the sea hare egg strings aqueous extracts and the positive control. Since the p-value was greater than 0.05, the one-way ANOVA revealed no significant difference between groups. Thus, the antibacterial activity of extracts is comparable to each other and the positive control.

Table 5 shows Tukey's pairwise test comparing the antibacterial activity of extracts to that of the positive control, ciprofloxacin. It was shown that hexane extracts are more active than methanol extracts.

Table 3. The minimum inhibitory concentration of aqueous hexane, aqueous methanol, and the antibiotic, ciprofloxacin, against test organisms using a modified microtiter antimicrobial assay

Test materials	Average MIC values (mg/mL)			
	Staphylococcus aureus	***Bacillus subtilis***	***Escherichia coli***	***Pseudomonas aeruginosa***
Ciprofloxacin	0.06	0.06	0.06	0.06
Hexane (E1)	0.77	1.5	0.23	0.52
Hexane (E2)	0.25	0.54	0.33	0.125
Methanol (E1)	3.33	1.17	1.46	1.33
Methanol (E2)	3.33	0.83	2.83	0.79

Table 4. One-way Analysis of Variance (ANOVA) of sea-hare egg string extracts and positive control (Ciprofloxacin) against test organisms

Source of variation	Sum of squares	df	Mean square	F	P-value
Between groups	3.509829688	3	1.1699	0.8445	0.4955
Within groups	16.62304375	12	1.3852		
Total	20.13287344	15			

Table 5. Tukey's pairwise comparison of four sea-hare egg string extracts and the positive control (Ciprofloxacin)

	Hexane (1)	Hexane (2)	Methanol (1)	Methanol (2)	Ciprofloxacin
Hexane (1)					
Hexane (2)	1.883				
Methanol (1)	1.464	0.419			
Methanol (2)	2.542	0.6595	1.079		
Ciprofloxacin	3.569	1.686	2.105	1.027	

The hexane extract exhibits more antibacterial activity than the methanolic extract against potentially pathogenic bacteria. According to the results of the study of Zarrinmehr et al. (2022), the increased amount of lipids extracted by hexane and chloroform when compared to methanol and acetone may be explained by the polarity index of these organic solvents. Hexane, chloroform, acetone, and methanol have polarity indices of 0, 4.1, 5.1, and 5.1, respectively. These numbers indicate that methanol and acetone have greater polarity than chloroform and hexane. The degree to which a solvent is polar dictates its capacity to dissolve certain solutes. This suggests that hexane is more effective in extracting antibacterial components of sea hare egg strings. It can be speculated that the methanol and hexane extracts can inhibit the growth of gram-positive and gram-negative bacteria.

The production of natural products and medications has recently received much attention. One of the biggest causes of death is infectious disease. Human health is under threat all across the world. Most of them are caused by microbes like bacteria and viruses, rickettsia, and fungi. Bacteria are thought to cause up to 30% of all diseases, resulting in millions of deaths annually. Viruses or prions account for 37-44% of diseases caused by various microbial pathogens; bacteria or rickettsia cause 10-30%; protozoa account for 10.7%; and fungus account for 6.3% of diseases, and helminths account for 3.3% of illnesses, resulting in millions of fatalities annually. The greatest threat is the growth of drug- and multidrug-resistant pathogens; as a result, the pharmaceutical and medical industries urgently need innovative antimicrobial drugs derived from natural sources with novel modes of action (Ganesan et al. 2017; Shameem et al. 2017; Srikacha and Ratananikom 2020). Since the latter part of the 20th century, antimicrobial drugs have been further identified as the most significant clinical nutrient. As a result, many individuals have been spared from potentially fatal bacterial infections. It is still unclear how antimicrobial medication use will develop in the future due to the growing issue of bacterial resistance. Thus, several steps must be taken to lessen the issue, such as limiting the usage of antibiotics, conducting studies to comprehend the genetic pathways underlying resistance, and carrying out ongoing studies to develop novel natural or synthetic medications. Delivering suitable and effective antimicrobial medications to the patient is the ultimate objective. The creation of novel instruments to supplement or replace pharmaceutical antibiotics is necessary due to the rising incidence of antibiotic resistance. The introduction of medicinal antibacterial properties of sea hare egg mass has been prompted by the emergence of microorganism resistance to currently available medicines (Farhana et al. 2017; Asgharpour et al. 2020; Tam et al. 2021).

Numerous investigations into the chemical makeup and biological characteristics of sea hare secondary metabolites have revealed that they have cytotoxic, antibacterial, antifungal, and antiviral effects, as well as antifeedant actions (Ruaza 2022). Several studies revealed the presence of an antibacterial factor in sea hare egg strings. According to Swarna et al. (2021), the egg mass of the sea hare includes agglutinin, which could agglutinate the marine bacteria. The agglutinins found in the egg mass play an important function in the egg mass as a defense mechanism once it has been laid out. This contributes to its defensive mechanism by producing adverse environmental circumstances for microorganisms. Agglutinins have high-molecular-weight that resemble proteins and function as opsonins (Grinchenko and Kumeiko 2022). Several marine bacteria cause an initial decrease in agglutinin titer, which returns to normal levels once the marine bacteria are eliminated from the sea hare. It was discovered that marine bacteria agglutinated by sea hare serum are rapidly removed from animals. It has also been proposed that agglutinins contain a polysaccharide involved in agglutinating action and may function in egg fertilization or egg protection from pathogens (Ahmmed et al. 2022).

It was also reported that sea hare egg strings have lectins that suppress bacterial growth. Lectins are a type of protein that has the ability to bind to certain sugars in a noncatalytic manner. The antibacterial activity of lectins is caused by their unique identification of bacterial surface components. Some research showed that lectins could decrease biofilm development by interacting with bacterial cell components of the biofilm and altering the expression of genes involved with virulence and biofilm formation. Given that some lectins can identify, agglutinate, and prevent the formation of bacterial cells and biofilms, lectins play a role in the organism's inherent defense mechanism (Vasconcelos et al. 2014; Gardères et al. 2015; Marques et al. 2017). The precise role, processes, and distribution of lectin in a broader range of invertebrate eggs are of great interest. It appears to be broadly distributed in marine sea hare species and closely involved in organ creation in the early developing stages (Motohashi et al. 2017).

The results showed that the tested sea-hare egg string extracts are antibacterial against pathogenic bacteria. This observation backs up the findings of Ibrahim et al. (2020), who discovered that sea hare eggs have antibacterial action against the investigated microbial species. The extract of the egg string of the sea hare, *Dolabella auricularia*, was found to have effective inhibitory activity when compared to other gastropods and bivalves, inhibiting all gram-positive and gram-negative bacterial isolates (Tayone et al. 2021). Also, in the same recent study by Tayone et al. (2021), secondary metabolites exist in the sea hare's eggs, including phenols and flavonoids. It is thought that the secondary metabolites found in sea hare organisms come from the algae they eat. Sea hare ink and egg strings include flavonoids, phenolics, and other bioactive chemicals that may also be acquired from the same algal diet. Algae, accordingly, are among the richest sources of bioactive chemicals with a variety of medicinal uses. Flavonoids are regarded as constitutive antibacterial agents because they can cause damage to the cytoplasmic membrane, restrict energy metabolism, and prevent the formation of nucleic acids, among other mechanisms. As a result, the flavonoids' secondary metabolites, which include flavones, flavonols, and tannins, have antioxidant properties, a feature that is heavily influenced by the hydroxyl group, particularly the 3-OH. It has frequently been observed that phenolic compounds with no oxygen and a C3 side chain at a lower degree of oxidation are antimicrobials.

Some compounds with great promise in cancer treatment have been identified from algae. According to studies, among these compounds is dolastatin10, that can be found in sea hares. Three distinct amino acid residues can be found in dolastatin 10 (Dol-10), a well-known marine pentapeptide that was extracted from the Indian Ocean mollusk *Dolabella auricularia*, a source of very strong broad-spectrum anti-tubulin anti-cancer pentapeptide. Dol-10 can efficiently cause lung cancer cells and other tumor cells to undergo apoptosis at nanomolar concentrations. It has recently attracted a lot of interest as it has evolved into pharmaceuticals sold for treating certain types of lymphomas (Gao et al. 2021; Singh 2022; Tan et al. 2022; Zhou et al. 2023).

The same study was also observed by Desrini et al. (2018), which accordingly that the presence of various active substances with antibacterial activities, including flavonoids, tannins, and saponins, supports the potential of the methanol extract of *Dolabella auricularia* eggs to suppress and eliminate the bacteria. Despite the absence of alkaloids, these three secondary metabolites are strong enough to have antibiotic actions. Flavonoids are known to have antibacterial properties. Interfering with bacterial attachment to the substrate by disrupting the formation of protein complexes from covalent and hydrogen bonds is one example, interfering with the permeability of bacterial cell walls preventing bacteria from transporting essential nutrients and organic components. Furthermore, flavonoids have a subgroup known as flavones that can inhibit bacterial colony growth, as well as a ring B that can interfere with nucleic acids and hinder bacterial RNA and DNA synthesis (Kumar and Pandey 2013; Xie et al. 2014; Górniak et al. 2019).

Ciprofloxacin is the positive control used in the study for both solvents, hexane, and methanol, with average antibacterial activity of (MIC = 0.06 mg/mL) in all tested bacterial organisms. The drug used is an effective antibiotic against the growth of bacteria because of its synthetic composition. The chemical name of the antibiotic is one-cyclopropyl-6-fluoro-4-oxo-7-(piperazine-1-yl)-1, 4-dihydroquinoline-3-carboxylic acid. Its chemical formula is $C_{17}H_{18}FN_3O_3$, and its molecular weight is 331.34 g/mol. Both in vitro and in vivo studies have demonstrated that ciprofloxacin is effective against various gram-positive and gram-negative bacteria isolates. It is frequently used to treat serious digestive, respiratory, and urinary tract infections (Akhtar et al. 2016; Sharma et al. 2017). It inhibits DNA gyrase and topoisomerase IV activities, the two enzymes responsible and essential for bacterial viability (Mustaev et al. 2014; Al-Wahaibi et al. 2021). Topoisomerase II and Topoisomerase IV are two types of DNA gyrase inhibited by the broad-spectrum antibiotic ciprofloxacin; subunits A and B are found in DNA gyrase. Quinolones, such as ciprofloxacin, are thought to hinder subunit A from resealing the DNA double-strand, resulting in exonucleolytic destruction of single-stranded DNA (Shariati et al. 2022). Two extraction solvents were employed in the study namely, hexane and methanol. In the study of Borges et al. (2020), methanol is considered one of the best extraction solvents for bioactive compounds. Accordingly, this solvent did not destroy the phenols because no hydrogen peroxide or a substantial fraction of free radicals were produced owing to cavitation when exposed to sonication. It was stated that there could be an influence on the various yields by the polarity of the solvent, which may account for the diversity in the outcomes (Borges et al. 2020). Methanol, also known as methyl alcohol, is an organic substance with a molecular weight of 32.042 u.m.a. It is one of the most significant chemical raw materials and is only weakly soluble in fat and oil. Methanol is, in fact, primarily used in the chemical

industry as a feedstock, solvent, or cosolvent (Dalena et al. 2018).

The other extraction solvent used in the study is hexane. A family of compounds known as "hexane" has the molecular equation C_6H_{14}. N hexane, 2-methylpentane, 3-methylpentane, 2,2-dimethylbutane, and 2,3-dimethylbutane are among the five isomers that fall under this category (Cravotto et al. 2022). Dwivedi et al. (2020) stated that statistical analysis showed a strong and significant difference in total phenolics in different extraction solvents. Another study showed that the highest phenolic compound production was observed in the extract of hexane solvent. It was found that the hexane extract had the highest flavonoid content. For instance, papaya flower extract in hexane showed the highest level of antioxidant activity in the investigation, with methanol, chloroform, and aqueous extracts following. The variation in the extracted chemicals' polarity and solvents' polarity may cause the different activity (Adeshina et al. 2010). Yeo et al. 2014 showed in a study with leaves of *Alchornea cordifolia*, that among the three extraction solvents—hexane, following by ethylacetate, and methanol—ethylacetate was found to be the most effective in conferring antibiotic activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans.* The study's conclusions demonstrated that ethyl acetate, petroleum ether, and hexane—all non-polar and intermediate-polar solvents—were superior solvent systems for extracting antimicrobial chemicals. According to the data, the most active antimicrobial agents fall into the non-polar to intermediate polarity category (Grinchenko and Kumeiko 2022).

The positive control had a minimum inhibitory concentration of 0.06 mg/mL, higher than the two solvents of *D. auricularia* egg string extracts. The highest minimum inhibitory concentration is the hexane extracts compared to methanol extracts in different concentrations in all tested bacterial organisms. This may indicate that the hexane extract has a comparable inhibitory effect to ciprofloxacin. Thus, this was revealed a potential source of antibacterial compounds against tested microorganisms as shown in Table 3.

Sea hares are increasingly being evaluated as a natural source of antioxidants that benefit human health in the food sector. The presence of phenolics and flavonoids indicates a broad range of antimicrobial drug spectrum with high antibacterial action against gram-positive and gram-negative microorganisms. It is phenols' partly hydrophobic characteristic that causes this, which makes them antimicrobial. It either deactivates or inhibits hydrolytic enzymes or microbial adhesions as proteases (Baba and Malik 2015). The results of this study were found valid and serve as baseline data for future studies.

ACKNOWLEDGEMENTS

We would like to express our gratitude to DOST. Hon. Mayor Michelle N. Rabat and Ms. Veronica Tañan for their support and help in the data collection and preparation of the equipment. And also, to Michael Jeriel Bersaldo for creating the map of the study.

REFERENCES

Afifi R, Abdel-Nabi IM, El-Shaikh K. 2016. Antibacterial activity from soft corals of the Red Sea, Saudi Arabia. J Taibah Univ Sci 10 (6): 887-895. DOI: 10.1016/j.jtusci.2016.03.006.

Aggio JF, Derby CD. 2019. *Aplysia*. Animal Behavior. 2nd edition Vol 3 DOI: 10.1016/B978-0-12-809633-8.20857-1.

Ahmmed MK, Bhowmik S, Giteru SG, Zilani MNH, Adadi P, Islam SS, Kanwugu ON, Haq M, Ahmmed F, Ng CCW, Chan YS, Asadujjaman M, Chan GHH, Naude R, Bekhit AEDA, Ng TB, Wong JH. 2022. An update of lectins from marine organisms: Characterization, extraction methodology, and potential biofunctional applications. Mar Drugs 20 (7): 430. DOI: 10.3390/md20070430.

Akhtar R, Yousaf M, Naqvi SAR, Irfan M, Zahoor AF, Hussain AI, Chatha SAS. 2016. Synthesis of ciprofloxacin-based compounds: A review. Synth Commun 46 (23): 1849-1879. DOI: 10.1080/00397911.2016.1234622.

Al-Wahaibi LH, Amer AA, Marzouk AA, Gomaa HAM, Youssif BGM, Abdelhamid AA. 2021. Design, synthesis, and antibacterial screening of some novel heteroaryl-based ciprofloxacin derivatives as DNA gyrase and topoisomerase iv inhibitors. Pharmaceuticals 14 (5): 399. DOI: 10.3390/ph14050399.

Asgharpour F, Moghadamnia AA, Alizadeh Y, Kazemi S. 2020. Chemical composition and antibacterial activity of hexane extract of *Lycoperdon Pyriforme*. S Afr J Bot 131: 195-199. DOI: 10.1016/j.sajb.2020.01.044.

Baba SA, Malik SA. 2015. Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of *Arisaema jacquemontii* Blume. J Taibah Univ Sci 9 (4): 449-454. DOI: 10.1016/j.jtusci.2014.11.001.

Borges A, José H, Homem V, Simões M. 2020. Comparison of techniques and solvents on the antimicrobial and antioxidant potential of extracts from *Acacia dealbata* and *Olea europaea*. Antibiotics 9 (2): 48. DOI: 10.3390/antibiotics9020048.

Cheung RCF, Wong JH, Pan W, Chan YS, Yin C, Dan X, Ng TB. 2015. Marine lectins and their medicinal applications. Appl Microbiol Biotechnol 99 (9): 3755-3773. DOI: 10.1007/s00253-015-6518-0.

Cravotto C, Fabiano-Tixier A-S, Claux O, Abert-Vian M, Tabasso S, Cravotto G, Chemat F. 2022. Towards substitution of hexane as extraction solvent of food products and ingredients with no regrets. Foods 11 (21): 3412. DOI: 10.3390/foods11213412.

Dalena F, Senatore A, Marino A, Gordano A, Basile M, Basile A. 2018. Methanol production and applications: An overview. Methanol 2018: 3-28. DOI: 10.1016/B978-0-444-63903-5.00001-7.

Darya M, Sajjadi MM, Yousefzadi M, Sourinejad I, Zarei M. 2020. Antifouling and antibacterial activities of bioactive extracts from different organs of the sea cucumber *Holothuria leucospilota*. Helgol Mar Res 74: 1-13. DOI: 10.1186/s10152-020-0536-8.

Delan GG, Pepito AR, Asakawa M, Yasui K, Cunado VD, Maningo AG, Gonzales AA, Rica RLV. 2015. Partial biochemical characterization of egg masses of the wedge seahare *Dolabella auricularia* (Lightfoot, 1786). Trop Technol J 19 (1): 1-6. DOI: 10.7603/s40934-015-0001-5.

Desrini S, Mashita AI, Rosary AN, Hidayah UN, Fitria A. 2018. Antibacterial activity screening of *Muntingia calabura* L leaves methanol extract on three bacterial pathogens. Pharmacologyonline 2: 1-10.

Dwivedi MK, Sonter S, Mishra S, Patel DK, Singh PK. 2020. Antioxidant, antibacterial activity, and phytochemical characterization of *Carica papaya* flowers. J Basic Appl Sci 9: 1-11. DOI: 10.1186/s43088-020-00048-w.

Fang C, Stiegeler E, Cook GM, Mascher T, Gebhard S. 2014. *Bacillus subtilis* as a platform for molecular characterisation of regulatory mechanisms of *Enterococcus faecalis* resistance against cell wall antibiotics. PLoS ONE 9 (3): e93169. DOI: 10.1371/journal.pone.0093169.

Farhana JA, Hossain F, Mowlah A. 2017. Antibacterial effects of guava (*Psidium guajava* L.) extracts against food borne pathogens. Intl J Food Sci Nutr 6 (1): 1-5. DOI: 10.11648/j.ijnfs.20170601.11.

Filho SMG, Cardoso JD, Anaya K, do Nascimento ES, De Lacerda JTJG, Mioso R, Gadelha TS, De Almeida Gadelha CA. 2015. Marine

sponge lectins: Actual status on properties and biological activities. Molecules 20 (1): 348-357. DOI: 10.3390/molecules20010348.
Ganesan P, Reegan AD, David RHA, Gandhi MR, Paulraj MG, Al-Dhabi NA, Ignacimuthu S. 2017. Antimicrobial activity of some actinomycetes from Western Ghats of Tamil Nadu, India. Alexandria J Med 53 (2): 101-110. DOI: 10.1016/j.ajme.2016.03.004.
Gao G, Wang Y, Hua H, Li D, Tang C. 2021. Marine antitumor peptide dolastatin 10: Biological activity, structural modification and synthetic chemistry. Mar Drugs 19 (7): 363. DOI: 10.3390/md19070363.
Gardères J, Bourguet-Kondracki M-L, Hamer B, Batel R, Schröder HC, Müller WEG. 2015. Porifera lectins: Diversity, physiological roles and biotechnological potential. Mar Drugs 13 (8): 5059-5101. DOI: 10.3390/md13085059.
Górniak I, Bartoszewski R, Króliczewski J. 2019. Comprehensive review of antimicrobial activities of plant flavonoids. Phytochem Rev 18 (1): 241-272. DOI: 10.1007/s11101-018-9591-z.
Grinchenko AV, Kumeiko VV. 2022. Bivalves humoral immunity: Key molecules and their functions. Russ J Mar Biol 48: 399-417. DOI: 10.1134/S1063074022060062.
Hasan I, Ozeki Y. 2019. Histochemical localization of N-acetylhexosamine-binding lectin HOL-18 in *Halichondria okadai* (Japanese black sponge), and its antimicrobial and cytotoxic anti-cancer effects. Intl J Biol Macromol 124: 819-827. DOI: 10.1016/j.ijbiomac.2018.11.222.
Huang Y-Z, Jin Z, Wang Z-M, Qi L-B, Song S, Zhu B-W, Dong X-P. 2021. Marine bioactive compounds as nutraceutical and functional food ingredients for potential oral health. Front Nutr 8: 686663. DOI: 10.3389/fnut.2021.686663.
Ibrahim HAH, Amer MS, Ahmed HO, Hassan NA. 2020. Antimicrobial activity of the sea hare (*Aplysia fasciata*) collected from the Egyptian mediterranean sea, Alexandria. Egypt J Aquat Biol Fish 24 (4): 233-248. DOI: 10.21608/ejabf.2020.98019.
Kato A, Yoshifuji A, Komori K, Aoki K, Taniyama D, Komatsu M, Fujii K, Yamada K, Ishii Y, Kikuchi T, Ryuzaki M. 2022. A case of *Bacillus subtilis* var. natto bacteremia caused by ingestion of natto during COVID-19 treatment in a maintenance hemodialysis patient with multiple myeloma. J Infect Chemother 28 (2022): 1212-1215. DOI: 10.1016/j.jiac.2022.05.006.
Khalid Abbas R, Elsharbasy FS. 2019. Antibacterial activity of *Moringa oleifera* against pathogenic bacteria in Sudan. Intl J Curr Res 11 (01): 27-30. DOI: 10.24941/ijcr.33664.01.2019.
Kumar S, Pandey AK. 2013. Chemistry and biological activities of flavonoids: An overview. Sci World J 2013: 162750. DOI: 10.1155/2013/162750.
Lampropoulos PK, Gkentzi D, Tzifas D. Dimitriou G. 2021. Neonatal sepsis due to *Bacillus subtilis*. Cureus 13 (9): e17692. DOI: 10.7759/cureus.17692.
Lee CH, Kaang BK, Lee YD. 2014. Spawning behavior and egg development of *Aplysia kurodai* inhabiting the coastal waters of Jeju Island, Korea. Dev Reprod 18 (1): 25-31. DOI: 10.12717/DR.2014.18.1.025.
Love-Chezem T, Aggio JF, Derby CD. 2013. Defense through sensory inactivation: Sea hare ink reduces sensory and motor responses of spiny lobsters to food odors. J Exp Biol 216 (8): 1364-1372. DOI: 10.1242/jeb.081828.
Macedo MWFS, da Cunha NB, Carneiro JA, da Costa RA, de Alencar SA, Cardoso MH, Franco OL, Dias SC. 2021. Marine organisms as a rich source of biologically active peptides. Front Mar Sci 8: 667764. DOI: 10.3389/fmars.2021.667764.
Magalhães AP, Jorge P, Pereira MO. 2019. *Pseudomonas aeruginosa* and *Staphylococcus aureus* communication in biofilm infections: insights through network and database construction. Crit Rev Microbiol 45 (5-6): 712-728. DOI: 10.1080/1040841X.2019.1700209.
Marques DN, de Almeida AS, de Oliveira Sousa AR, Pereira R, Andrade AL, Chaves RP, Carneiro RF, de Vasconcelos MA, do Nascimento-Neto LG, Pinheiro U, Videira PA, Teixeira EH, Nagano CS, Sampaio AH. 2017. Antibacterial activity of a new lectin isolated from the marine sponge *Chondrilla caribensis*. Intl J Biol Macromol 109: 1292-1301. DOI: 10.1016/j.ijbiomac.2017.11.140.
Martins A, Vieira H, Gaspar H, Santos S. 2014. Marketed marine natural products in the pharmaceutical and cosmeceutical industries: Tips for success. Mar Drugs 12 (2): 1066-1101. DOI: 10.3390/md12021066.
Mashjoor S, Yousefzadi M. 2017. Holothurians antifungal and antibacterial activity to human pathogens in the Persian Gulf. J Mycol Med 27 (1): 46-56. DOI: 10.1016/j.mycmed.2016.08.008.
Motohashi S, Jimbo M, Naito T, Suzuki T, Sakai R, Kamiya H. 2017. Isolation, amino acid sequences, and plausible functions of the galacturonic acid-binding egg lectin of the sea hare *Aplysia kurodai*. Mar Drugs 15 (6): 161. DOI: 10.3390/md15060161.
Mustaev A, Malik M, Zhao X, Kurepina N, Luan G, Oppegard LM, Hiasa H, Marks KR, Kerns RJ, Berger JM, Drlica K. 2014. Fluoroquinolone-gyrase-DNA complexes two modes of drug binding. J Biol Chem 289 (18): 12300-12312. DOI: 10.1074/jbc.M113.529164.
Pepito AR, Delan GG, Asakawa M, Ami LJ, Yap EES, Olympia MS, Yasui K, Maningo AG, Rica RLV, Lamayo MHA. 2015. Nutritional quality of the egg mass locally known as "Lukot' of the wedge seahare *Dolabella auricularia* (Lightfoot, 1786). Trop Technol J 19: 1-6. DOI: 10.7603/s40934-015-0007-z.
Petersen L-E, Kellermann MY, Schupp PJ. 2020. Secondary metabolites of marine microbes: From natural products chemistry to chemical ecology. YOUMARES 9: 159-180. DOI: 10.1007/978-3-030-20389-4_8.
Prince JS, Johnson PM. 2015. Ultrastructural comparison of processing of protein and pigment in the ink gland of four species of sea hares. J Mar Biol 2015: 10-14. DOI: 10.1155/2015/847961.
Qin S, Xiao W, Zhou C, Pu Q, Deng X, Lan L, Liang H, Song X, Wu M. 2022. *Pseudomonas aeruginosa*: pathogenesis, virulence factors, antibiotic resistance, interaction with host, technology advances and emerging therapeutics. Signal Transduction Targeted Ther 7 (1): 199. DOI: 10.1038/s41392-022-01056-1.
Ruaza JRFC. 2022. Lethality and antimicrobial screening of sea hare (*Dolabella auricularia*) ink against the fish pathogens. Uttar Pradesh J Zool 43 (21): 51-57. DOI: 10.56557/upjoz/2022/v43i213212.
Shakouri A, Shoushizadeh MR, Nematpour F. 2017. Antimicrobial activity of sea cucumber (*Stichopus variegatus*) body wall extract in Chabahar Bay, Oman Sea. Jundishapur J Nat Pharm Prod 12 (1): 1-5. DOI: 10.5812/jjnpp.32422.
Shameem N, Kamili AN, Ahmad M, Masoodi FA, Parray JA. 2017. Antimicrobial activity of crude fractions and morel compounds from wild edible mushrooms of North western Himalaya. Microb Pathog 105: 356-360. DOI: 10.1016/j.micpath.2017.03.005.
Shariati A, Arshadi M, Khosrojerdi MA, Abedinzadeh M, Ganjalishahi M, Maleki A, Heidary M, Khoshnood S. 2022. The resistance mechanisms of bacteria against ciprofloxacin and new approaches for enhancing the efficacy of this antibiotic. Front Public Health 10: 1025633. DOI: 10.3389/fpubh.2022.1025633.
Sharma D, Patel RP, Zaidi STR, Sarker MMR, Lean QY, Ming LC. 2017. Interplay of the quality of ciprofloxacin and antibiotic resistance in developing countries. Front Pharmacol 8: 1-7. DOI: 10.3389/fphar.2017.00546.
Singh SB. 2022. Discovery and development of dolastatin 10-derived antibody drug conjugate anticancer. Drugs. J Nat Prod 85 (3): 666-687. DOI: 10.1021/acs.jnatprod.1c01135.
Srikacha N, Ratananikom K. 2020. Antibacterial activity of plant extracts in different solvents against pathogenic bacteria: An in vitro experiment. J Acute Dis 9 (5): 223-226. DOI: 10.4103/2221-6189.291288.
Srilekha V, Krishna G, Seshasrinivas V, Charya MA. 2017. Antibacterial and anti-inflammatory activities of marine *Brevibacterium* sp. Res Pharm Sci 12 (4): 283-289. DOI: 10.4103/1735-5362.212045.
Sukmiwati M, Ilza M, Putri AE, Sidauruk SW. 2020. Antibacterial activity of sea cucumber *(Holothuria atra*) against *Pseudomonas aeruginosa*. IOP Conf Ser: Earth Environ Sci 404: 012047. DOI: 10.1088/1755-1315/404/1/012047.
Swarna RR, Asaduzzaman AKM, Kabir SR, Arfin N, Kawsar SMA, Rajia S, Fujii Y, Ogawa Y, Hirashima K, Kobayashi N, Yamada M, Ozeki Y, Hasan I. 2021. Antiproliferative and antimicrobial potentials of a lectin from *Aplysia kurodai* (sea hare) eggs. Mar Drugs 19 (7): 1-14. DOI: 10.3390/md19070394.
Tam CC, Nguyen K, Nguyen D, Hamada S, Kwon O, Kuang I, Gong S, Escobar S, Liu M, Kim J, Hou T, Tam J, Cheng LW, Kim JH, Land KM, Friedman M. 2021. Antimicrobial properties of tomato leaves, stems, and fruit and their relationship to chemical composition. BMC Complement Med Ther 21 (1): 1-9. DOI: 10.1186/s12906-021-03391-2.
Tan Z, Deng J, Ye Q, Zhang Z. 2022. The antibacterial activity of natural-derived flavonoids. Curr Top Med Chem 22 (12): 1009-1019. DOI: 10.2174/1568026622666220221110506.
Tayone JC. 2017. Tracing metal concentrations in egg strings of sea hare, *Dolabella auricularia* from Pujada Bay. Intl J Adv Appl Sci 4 (2): 134-138. DOI: 10.21833/ijaas.2017.02.022.

Tayone JC. 2020. Investigation of chemical composition and antibacterial activity of ink from sea hare *Dolabella auricularia*. Walailak J Sci Technol 17 (6): 600-607. DOI: 10.48048/wjst.2020.3075.

Tayone JC, Del Rosario RM, Canencia OP, Tayone WC. 2021. Egg strings of *Dolabella auricularia*: Its in vitro antioxidants and antibacterial activities. Asian J Biol Life Sci 10 (2): 340-345. DOI: 10.5530/ajbls.2021.10.46.

Tokano M, Tarumoto N, Imai K, Sakai J, Maeda T, Kawamura T, Seo K, Takahashi K, Yamamoto T, Maesaki S. 2023. Bacterial meningitis caused by *Bacillus subtilis* var. natto. Intl Med 62: 1989-1993. DOI: 10.2169/internalmedicine.0768-22

Vasconcelos MA, Arruda FVS, Carneiro VA, Silva HC, Nascimento KS, Sampaio AH, Cavada B, Teixeira EH, Henriques M, Pereira MO. 2014. Effect of algae and plant lectins on planktonic growth and biofilm formation in clinically relevant bacteria and yeasts. Biomed Res Intl 2014: 1-9. DOI: 10.1155/2014/365272.

Xie Y, Yang W, Tang F, Chen X, Ren L. 2014. Antibacterial activities of flavonoids: Structure-activity relationship and mechanism. Curr Med Chem 22 (1): 132-149. DOI: 10.2174/09298673216661409161134443.

Yeo YL, Chia YY, Lee CH, Sow HS, Yap WS. 2014. Effectiveness of maceration periods with different extraction solvents on in-vitro antimicrobial activity from fruit of *Momordica charantia* L. J Appl Pharm Sci 4 (10): 16-23. DOI: 10.7324/JAPS.2014.40104.

Zaky AA, Simal-Gandara J, Eun J-B, Shim J-H, Abd El-Aty AM. 2022. Bioactivities, applications, safety, and health benefits of bioactive peptides from food and by-products: A review. Front Nutr 8: 1-18. DOI: 10.3389/fnut.2021.815640.

Zarrinmehr MJ, Daneshvar E, Nigam S, Gopinath KP, Biswas JK, Kwon EE, Wang H, Farhadian O, Bhatnagr A. 2022. The effect of solvent polarity and extraction conditions on the macroalgal lipids yield, fatty acids profile, and biodiesel properties. Bioresour Technol 344: 126303. DOI: 10.1016.j.biortech.2021.126303.

Zhou H, Chen L, Ouyang K, Zhang Q, Wang W. 2023. Antibacterial activity and mechanism of flavonoids from *Chimonanthus salicifolius* S.Y. Hu. and its transcriptome analysis against *Staphylococcus aureus*. Front Microbiol 13: 1103476. DOI: 10.3389/fmicb.2022.1103476.