Zoochemicals: secondary metabolites and toxicity potential of soft coral *Rumphella aggregata* (Nutting, 1910) from Tandubas, Tawi-Tawi, Philippines

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Abstract. Secondary metabolites are chemical compounds produced by majority of marine organisms and have several ecological functions. Identification of these bioactive compounds through zoochemical screening, and toxicity assessment are esssential for understanding their biological roles, safety and potential applications to human health, and drug development. Marine species exhibit different structural diversity, but only a few of them have been examined for pharmacological potential. *Rumphella aggregata* (Nutting, 1910) of Isididae family, is a species of soft coral that is abundantly found in the coastal areas of Tandubas, Tawi-Tawi, Philippines. It has served as an important nursery ground for juvenile fish and has been used to treat small cuts and wounds by the locals. However, scientific studies on the zoochemical composition and toxicity effect of *R. aggregata* are very limited. This study was conducted to evaluate the secondary metabolites and toxicity of the *R. aggregata* using the zoochemical analyses and brine shrimp lethality assay (BSLA) methods. The coral specimens, azooxanthellate, were extracted using methanol solvent, filtered, and then zoochemical screening was performed. The brine shrimp lethality assay was also employed to determine the toxicity of *R. aggregata* methanol extract. The results of the zoochemical screening revealed the presence of alkaloids, flavonoids, glycosides, phenols, tannins, saponins, triterpenoids, and steroids. The methanol extract exhibited an LC_{50} value of 1380 μg mL^{-1}, which is considered non-toxic. The findings suggest that *R. aggregata* extract may possess a potential source for pharmaceutical drugs and support its use in traditional medicine.
Key Words: alkaloids, corals, marine species, *Rumphella aggregata*, toxicity.

Introduction. Coral reefs are extremely valuable ecosystems that are essential to the dynamics of marine ecosystems by providing many animals' food and shelter (Xin et al 2016). Soft corals (Octocroallia: Alcyonacea) are found all over the world but mostly in tropical or subtropical waters (Frey 2022), and are the second most dominant group of large bottom-dwelling organisms on many tropical and temperate reefs, following reef-building scleractinian corals (Fabricius & Alderslade 2001). *Rumphella aggregata* (Nutting, 1910), a soft coral species belonging to family Isididae, is abundantly found in the coastal area of Tandubas, Tawi-Tawi. It is locally known as "Sahasah lamai-lamai" and is known as filter feeder and serves as an important nursery ground for juvenile fish (Baillon et al 2012). The locals believe that these corals served as protection grounds for the fish production and also have been used for medical purposes, acting as antibacterial for small cuts and wounds among fishermen.

R. aggregata has large, massive, bushy colonies that can reach a height of up to one metre with profuse regular bifurcate branching. The branching can be sparse or very abundant. The branches have bulbous or pointed tips and are spherical, smooth, and comparatively thick. The polyps are dispersed throughout the colonies and on both sides of the branches. The corals are azooxanthellate (Western Australian Museum Collections 2025). The black coral species, *R. aggregata* is also referred to as antipatharian coral. The dark, frequently black, skeletal structure of these corals, which is made up of a protein known as gorgonin, sets them apart. Together, the polyps, which are in charge of eating and reproduction, create the complex branching structure that is distinctive to black corals. From shallow waters to the depths of the abyss, *R. aggregata* can be found in a wide range of marine environments. On continental slopes, where currents provide them with food and shelter, they are very prevalent. These corals have a significant function in their ecosystem, providing habitat for a variety of marine life (Villar & Ylade 2014). This type of marine organism belongs to Isididae coral species. These organisms are modular colonies that are typically very branched. They have a rigid internal axis in the center of both the main stem and every branch, which is made up of either fused sclerites or sclerites made of calcite and gorgonin (Horvath 2019). Corals of the family Isididae are also used as a source of active natural substances (Han et al 2024).

Despite their apparent fragility, black corals are incredibly resilient and able to resist the severe conditions found in the deep water. They are essential to their environment because they give a variety of marine animals a place to live and food. However, they are sensitive to human activities because to their slow development rate and susceptibility to environmental changes, which emphasizes the significance of their protection (Olson 2023). Low light levels in this deep-sea habitat make it unsuitable for corals that depend on symbiotic algae for energy. However, black corals have adapted to survive in these environments and now rely on filter feeding (Bo 2014). Black corals are treasured by many cultures for medicinal purposes and to produce jewellery (Wagner et al 2012). Black corals create crucial habitats for a diverse array of marine life. Their complex branches provide shelter for fish, crustaceans, and other invertebrates. They also contribute to the overall structure of deep-sea reefs, providing a foundation for other organisms to colonize (Olson 2023; Yrea 2024).

Zoochemicals are chemical compounds found in animals, including secondary metabolites with natural bioactive properties, and are equivalent to phytochemicals in plants (Karnan et al 2023). These compounds play crucial roles in defense, communication, and ecological interactions within marine ecosystems. According to Liu et al (2022), secondary metabolites from corals have great diversity because these sessile organisms need exceptional chemical weapons for potential parasite predation and microbial colonization. Lay et al (2014) found that several secondary metabolites have anti-inflammatory qualities that can aid in lowering inflammation throughout the body and possibly easing the symptoms of inflammatory bowel disorders and arthritis. Therefore, it is crucial to comprehend the unique toxicity of these marine species in order to maximize their efficacy and reduce any negative effects on healthy cells. Although scientific studies on soft corals, particularly *R. aggregata* (Isididae), remain limited, there is a growing interest in their potential as sources of bioactive compounds. Research on the zoochemical composition and toxicity of *R. aggregata* is sparse, and the available data is insufficient to fully understand its medicinal properties. Therefore, this study aims to investigate the bioactive components and assess the toxicity potential of *R. aggregata* as a promising source for novel drug development.

Material and Method

Sample collection and preparation. The soft coral materials needed for this study were collected from the coastal waters of Tandubas Island, Tawi-Tawi. The map of the collection site is shown in Figure 1. The municipality of Tandubas is located in the province of Tawi-Tawi of the Bangsamoro Autonomous Region of Muslim Mindanao (BARMM), Mindanao, Philippines. The specimens of *R. aggregata* that were collected from the coastal water of Tandubas island were then later authenticated at the Herbarium of Mindanao State University - Tawi-Tawi College of Technology and Oceanography.

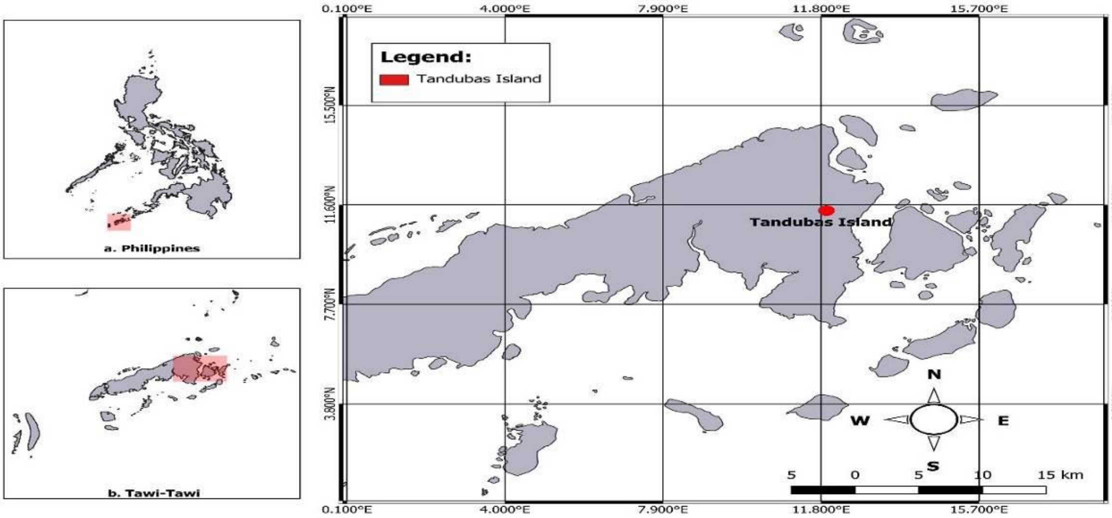

Figure 1. Collection site in the coastal water of Tandubas island, Tawi-Tawi, Philippines.

The Figure 2 shows the sample of *R. aggregata* when was freshly collected from the coastal water of Tandubas, before the methanol extraction taken by one of the researchers.

Figure 2. Sample of *Rumphella aggregata* when was freshly collected from the coastal water of Tandubas, Tawi-Tawi, Philippines.

Methanol extraction. The specimens were cleaned with tap water and rinsed carefully with distilled water, then air-dried at standard room temperature (25°C) before it was utilized for the methanol extraction and zoochemical screening. Prior to the various zoochemical component assays, the marine species was cut smaller and ground into a fine powder with an electric blender and steeped for approximately 48 hours in a methanol solution. Following this time frame, the solution was filtered using Whitman filter paper No. 1 and extracted in order to prepare the toxicity assay and zoochemical screening. The sample's filtrate was safely placed in a reagent bottle and kept in the refrigerator for later use.

Zoochemical analyses. All of the chemicals used in the study were obtained from the Integrated Science Laboratory at Mindanao State University - Tawi-Tawi College of Technology and Oceanography, located in Sanga-Sanga, Bongao, Tawi-Tawi, Philippines. To detect the presence of flavonoids, alkaloids, phenols, tannins, saponins, tripernoids, steroids, and cardiac glycosides, zoochemical screening was carried out using the procedures described by Goyal et al (2012), with a few minor adjustments. This involved steps such as material preparation, drying, methanol extraction, qualitative screening, and quantitative analysis. According to qualitative criteria, the findings of the zoochemical screening investigation were classified as either positive (+) or negative (-), signifying the presence or absence of bioactive components.

Figure 3 shows the specimens of the *R. aggregata* after it was air-dried and cut at standard room temperature prior to the zoochemical analyses.

Figure 3. Specimens of *Rumphella aggregata* after air-dried and cut.

Test for alkaloids. Two milliliters (2 mL) of hydrochloric acid (2N) were combined with a 2 mL of sample extract of *R. aggregata*, which was then filtered. The presence of alkaloids was indicated by the production of a brown or reddish-brown precipitate after a few drops of Wagner's reagent were applied to 2 mL of the filtrate.

Test for flavonoids. To determine the presence of flavonoids, Shihoda's test was used. Two milliliters (2 mL) of the extract were mixed with two to three drops of 10% sodium hydroxide. A yellow tint indicated the presence of flavonoids when a few drops of diluted hydrochloric acid were added.

Test for phenols and tannins. Ferric chloride assays were used to determine whether phenolic and tannins were present in the specimen. A volume of 2.5 mL of 5% FeCl was added to the 5.0 mL sample. A blue-black or greenish-black precipitate confirms the presence of tannins, whereas the creation of a reddish-brown precipitate indicates the presence of phenols.

Test for saponins. The olive oil test was used to detect the presence of saponins. Five milliliters (5 mL) of distilled water were mixed with 2.5 mL of the solution and agitated vigorously until a stable froth formed. After that, three drops of olive oil were added to the mixture and shook again. The presence of saponins is indicated by the formation of an emulsion.

Test for triterpenoids. To determine whether triterpenoids were present, the Salkowski test was used. Five milliliters (5 mL) of chloroform was added to 2.5 mL of the sample, and it was then filtered. Three to four drops of strong sulfuric acid were added to the filtrate after it had been filtered. After shaking the mixture, it was not disturbed. The appearance of a yellow tint in the lower layer would indicate the existence of triterpernoids.

Test for steroids. The Liebermann-Burchard test was used to detect the presence of steroids. Five milliliters (5 mL) of chloroform was added to the 2.5 mL sample, and it was then filtered. After filtering, the filtrate was mixed with 1 mL of concentrated sulphuric acid and 2 mL of acetic acid. The development of a blue-green ring indicates the presence of steroids.

Test for cardiac glycosides. The presence of cardiac glycosides was assessed using the Keller-Kiliani test. One milliliter (1 mL) of glacial acetic acid, one milliliter (1 mL) of 5% $FeCl_3$, and one milliliter (1 mL) of concentrated sulfuric acid were added to 2.5 mL of the sample. The formation of a green-blue hue indicates the presence of glycosides.

Brine shrimp lethality assay. This study employed the brine shrimp (*Artemia salina*) lethality assay (BSLA) for the toxicity test. Throughout the procedure, a serial dilution approach was used. Methanol extract was used to create five distinct concentrations: 500 ppm, 1000 ppm, 1500 ppm, 2000 ppm, and 2500 ppm. Each testing container was then filled with five milliliters (5 mL) of each concentration. A volume of 10 mL of saltwater were added to the sample's methanol extract in different quantities, and ten (10) nauplii were subsequently added. All test vials were exposed to continuous lighting for a whole day during this time. Following this time frame, the mortality rate was calculated by counting the number of surviving nauplii in each test concentration. Using the Finney (1971) approach, a Probit analysis was used to determine the LC_{50} values for the *R. aggregata*. Gupta et al (1996) defined a substance as hazardous if its LC_{50} value was less than 1000 μg mL^{-1}. Additionally, the proportion of nauplii mortality associated with each sample concentration was calculated.

Results and Discussion. The results on the zoochemical screening of *R. aggreggata* is summarized in Table 1.

Table 1

Zoochemical screening of *R. aggregata*

Bioactive compounds	*Test*	*Result*
Alkaloids	Wagner	+
Flavonoids	Shihodas	+
Phenols and tannins	Ferric chloride	+
Saponins	Modified	+
Triterpernoids	Salkowski	+
Steroids	Liebermann-Burchard	++
Cardiac glycosides	Keller-Kiliani	+

Legend: (+) and (++) signs indicate the moderate presence and strong presence, respectively, of the bioactive compound.

This study's findings revealed the existence of bioactive substances including alkaloids, flavonoids, phenols, tannins, saponins, triterpenoids, steroids, and cardiac glycosides.

The results of this study confirmed the existence of alkaloids. Alkaloids are nitrogenous substances having a range of biological functions (Souza et al 2020). Gao et al (2013) stated that corals produce numerous alkaloids, which are nitrogen-containing compounds with a wide range of biological activities. These alkaloids which are frequently extracted from corals or symbiotic fungi, have properties like antibacterial, antifungal and cytotoxic effects. Other studies also confirmed that soft corals are known to produce alkaloids alongside other secondary metabolites (Ong 2008; Putra et al 2016; Tanod et al 2019). Anti-inflammatory marine alkaloids are substances that have a lot of potential for use in pharmacology and medicine. Another study by Tangon et al (2021), which found that a variety of living things produce alkaloids, particularly higher organisms, suggested that these compounds may have therapeutic uses for cancer, viral infections, inflammation and immune modulation, neurological disorders and psychiatric conditions, and diabetes.

Flavonoids were also found in the *R. aggregata* specimens in this research. Soft corals contain flavonoids and other bioactive chemicals that have antioxidant and anti-cancer potential (Putra et al 2016; Tanod et al 2019). Flavonoids are a class of compounds which are highly present in nature, including in marine organisms, and have been found in a wide range of biological activities (Pereira et al 2024). According to Martins et al (2019), the scientific community has been interested in marine natural flavonoids because of their potential medicinal properties as well as their potential as anti-feedant and anti-fouling agents. The most commonly reported antibacterial and antifouling properties among these marine flavonoids are those with an uncommon mutation structure, which are not commonly found in nature or in terrestrial organisms. Further, Martins et al (2019) indicated that flavones and flavanols, which are mostly extracted from marine species, are the most prevalent groups of marine flavonoids. Bhagwat et al (2023) suggested in their study that eating a diet high in flavonoids may lower the risk of cardiovascular diseases, diabetes, and some types of cancer. On the other hand, there were also some studies on soft corals particularly *Nephthea* sp., *Sarcophyton* sp., *Sinularia* sp., *Aaptos* sp., and *Lobophytum* sp. that indicated no presence of flavonoids (Rozirwan et al 2020; Sahidin et al 2022), which needs further scientific investigations.

A blue-black or greenish-black precipitate confirms the presence of tannins, whereas the creation of a reddish-brown precipitate indicates the presence of phenols. This study indicated the presence of phenols and tannins in the specimens of *R. aggregata.* This result is parallel to that of a study by Hanafi et al (2020), which found that corals had a relatively high total phenol content and some phytochemical components, including tannins, phenols, and other bioactive substances. Tanod et al (2019) in their study also found polyphenols (tannins), saponins and other bioactive compounds in the chemical constituent analysis of soft corals extracts. According to Maie et al (2008), tannins are found in natural waters at a considerable concentration and are crucial to the ecology because they buffer the cycling of nitrogen in estuarine environments and preserve it.

There were also a presence of saponins as indicated by the formation of an emulsion. Soft corals, starfish, sea cucumbers, sponges, and small fish are the main sources of marine saponins (Kim et al 2021). Tanod et al (2019) and Sahidin et al (2022) confirmed in their studies the presence of saponins and other secondary metabolites in soft corals. Indeed, saponins have a wide range of pharmacological properties, such as immunomodulatory, antiviral, antibacterial, anti-inflammatory, antifungal, anticancer, and antioxidant actions (Xu & Yu 2021).

In this study, *R. aggregata* was also shown to contain triterpernoids. Accordingly, triterpenoids may be found in animals, ferns, fungi, marine organisms and higher plants and therefore are widely distributed in nature (Du et al 2014). Terpenoids are the most prevalent and diverse class of compound in coral, and novel skeletons of terpenoids are continually being discovered. Its pharmacological screening revealed strong biological activity (Liang & Guo 2013; Liu 2017). Due to their anti-inflammatory, analgesic, antipyretic, and tonic properties, triterpenoids are utilized medicinally in several Asian nations (Ovesná et al 2004). Superior biological actions against various malignancies, inflammations, and metabolic disorders have been demonstrated for both natural and synthesized triterpenoid drugs (Deng et al 2021). Furthermore, the study by Lin et al (2024) also validated this finding, indicating that corals have anticancer active chemicals and that it would be beneficial to investigate marine natural products for efficient therapeutic agents.

The results in this study also revealed the strong presence of steroids in the sample of *R. aggregata*. Ai et al (2006) confirmed steroids are abundant in corals. Steroids are used to reduce redness and swelling (inflammation). According to Ermolenko et al (2020), several secondary metabolites, such as glycosides and steroids, that have been extracted from marine invertebrates, such as soft corals, have been shown to have anticancer and other pharmacological properties. Liu (2017) also indicated that corals contain steroids, which exhibit amazing biological activity. Another study, conducted by Han et al (2024), revealed that many structurally active unique secondary metabolites, such as steroids, terpenoids, prostaglandins, and ceramides have been isolated from corals, and their significant pharmacological activities, such as cytotoxic and antiviral activities, have been widely noticed and studied by scientists. Furthermore, they concluded that steroid compounds also play important roles in antitumor, anticancer, and anti-inflammatory activities.

Finally, the formation of a green-blue hue indicates the presence of glycosides. This study revealed the presence of cardiac glycosides in the sample of *R. aggregata.* Cardiac glycosides in soft corals have been found to exhibit diverse bioactivities, including antihypertensive effects and potential anticancer properties (Ivanchina et al 2011; Yan et al 2021b). According to Lan et al (2003), soft corals produced a wide variety of glycosides, along with terpenes and steroids. In the study of Bernal et al (2011), they also showed that glycosides have a variety of significant functions in living things, such as anti-inflammatory, antihypertensive, antidiabetic, and antioxidant properties.

Collectively, the presence of these compounds has been reported to possess a wide range of pharmacological activities, including anticancer, antioxidant, and antimicrobial properties. The findings of this research on *R. aggregata* are also supported by the study of Cooper et al (2014), which highlighted the anti-inflammatory properties of certain coral species, particularly the soft coral and discussed the potential of coral extracts for bone repair and neurological benefits. Their study emphasized the need for further research to explore these applications. In a study conducted by Tanod et al (2019) on the analysis of the bioactive components of all soft corals crude extracts (particularly *Sinularia* sp., *Nephthea* sp., and *Sarcophyton* sp.) they revealed the presence of alkaloids, flavonoids, saponins, tannins, steroids, and triterpenoids. Further suggested that the potential of each extracts, could be developed as a potential anti-inflammatory agent. Also, in the study of Luyao et al (2019), soft corals are considered an extremely diverse group of marine organisms and have been shown to possess a rich variety of marine secondary metabolites. A study on soft coral species like *Sinularia* have been found to have a variety of phytochemical components. According to Yan et al (2021a), secondary metabolites from marine organisms represent a plentiful source of structurally diverse and bioactive products. Furthermore, more than 700 secondary metabolites have been reported to date, including terpenoids, norterpenoids, steroids/steroidal glycosides, and other types (Yan et al 2021b). They showed a broad range of potent biological activities. Nowak et al (2009) also investigated the inorganic components of black coral skeletons, revealing the presence of calcium, iodine, potassium, and iron, among others. These elements are distributed in a specific pattern within the coral structure, suggesting distinct biological and mechanical functions. Another study conducted by Ong (2008) on the phytochemical and microbiological screening of samples of soft corals from Matuod, Batangas, Philippines showed that the presence of the chemical constituents namely alkaloids, saponins, cardenolides, flavonoids, tannins and antraquinones. Our present study suggests that all samples significantly showed varying degrees of antimicrobial activity. According to Putra et al (2016), alkaloids, steroids, triterpenoids, flavonoids, saponins, terpenoids, and phenols were found in all fractions of soft corals, particularly those on the genus *Lobophytum*, that showed a high potential for biological activities like anti-inflammatory, antimicrobial, and antiviral activity. Moreover, the use of corals species for traditional medicine by the local people is documented in the study of Han et al (2024). It demonstrates how coral is frequently applied individually as an alternative to orthopedic materials in the treatment of conditions including bone hyperplasia and abnormalities. Corals are also frequently used to treat neurological conditions such primary headache, migraine, cerebral infarction, epilepsy, hypertension, and other conditions affecting the heart and brain. Ayllon et al (2019) suggested that several soft coral species from the Talisayan shallow seas in the Mindanao region might be good sources of bioactive chemicals for drug discovery. Furthermore, according to Karthikeyan et al (2022), the application of marine-derived bioactive compounds obtained from marine sources has become more significant due to their potential as treatments for a variety of illnesses and were also found that antibiotic, antiviral, neurodegenerative, anticancer, and anti-inflammatory bioactivities are only a few of the pharmaceutically significant bioactivities that marine natural compounds exhibit.

Therefore this study findings suggest that soft corals, particularly *R. aggregata*, produce a variety of secondary metabolites, most commonly including alkaloids, flavonoids, phenols, tannins, saponins, triterpernoids, steroids and cardiac glycosides which serve as defense mechanisms against predators and competitors. These compounds are often studied for potential pharmaceutical applications due to their diverse biological activities like anti-cancer, anti-inflammatory, and antimicrobial properties.

Brine shrimp lethality assay of R. aggregata. In this study, the BSLA was employed to evaluate the toxicity of *R. aggregata* extract. BSLA is a simple, high throughput toxicity test of bioactive chemicals (Harwig & Scott 1971). The BSLA is a widely used and cost-effective method for preliminary assessment of the toxicity of natural products. The lethal concentration (LC_{50}) of a test material was ascertained using the brine shrimp *A. salina* as a model organism. The LC_{50} represents the concentration of the substance that causes 50% in the test for marine organisms using the brine shrimp after a specific period of exposure. Biostat 2009 was used to assist in the Probit analysis method of data analysis. Probit analysis is a survival analysis technique that entails converting concentrations into logarithms and percentage mortalities into probits.

Table 2 demonstrates that the degree of mortality was inversely proportional with the extract's concentration. All of the shrimps in the solution survived before the 8-hour observation period. Despite this, the 500 μg mL^{-1} concentration revealed nearly the highest mortality, while the concentration of 2500 μg mL^{-1} showed the lowest mortality. The toxicity of *R. aggregata* using the brine shrimp lethality showed that the extract exhibited no significant toxicity to brine shrimp, as the calculated LC_{50} value of 1,380 μg mL^{-1} is considered non-toxic. It was observed that at higher concentrations of treatment extracts, the shrimps began to die after 8 hours, and after 48 hours, all of the shrimps died in the solution.

Table 2

Brine shrimp lethality assay of *R. aggregata*

Concentration (ppm)	*log10 (concentration μg mL^{-1})*	*Mortality (%)*	*LC_{50} (μg mL^{-1})*	*Category*
500	2.698970004	60%	1380	Non-toxic
1000	3	60%	1380	Non-toxic
1500	3.176091259	50%	1380	Non-toxic
2000	3.301029996	30%	1380	Non-toxic
2500	3.397940009	20%	1380	Non-toxic

This finding suggests that *R. aggregata* extract may possess a relatively low toxicity profile in the coastal area of Tandubas, Tawi-Tawi. In connection with this finding, Coll et al (1982) discovered that soft corals from the middle Great Barrier Reef region experience toxicity much less frequently than those from the northern region; the vast range of toxicity displayed by species of several highly common genera of soft corals indicates that toxicity is one of the factors contributing to the group's widespread distribution on the reef. The toxicity of coral extracts to brine shrimps seems to be more related to the species and possibly the degree of fish predation on soft corals than it is to the site's exposure to waves (Ayllon et al 2019). In another study, fraction of the soft coral *Lobophytum* sp. extract was confirmed that it is not toxic and also contains phenolic compounds (Sahidin et al 2022). Therefore, these soft corals have a significant impact on human health; yet, the health risks that come with such corals are often unrecognized or underestimated due to the low volume of cases documented (Dhakal & Gupta 2023).

Conclusions. The present study demonstrated the presence of a variety of secondary metabolites in soft coral *R. aggregata.* The diverse zoochemicals presence in this type of soft coral holds promise for discovering new bioactive compounds with potential medicinal applications, and for the development of new drugs and natural therapies for various diseases, based from what this study revealed. The non-toxic results obtained from the BSLA suggest that *R. aggregata* extract may possess a relatively low toxicity profile. These findings imply that this marine species may be a promising source of natural anticancer drugs. This is particularly important for traditional medicine, which uses this species to treat a variety of illnesses. However, further research is necessary to fully understand the potential applications of these natural compounds and to examine the therapeutic potential of *R. aggregata* and to develop safe and effective pharmaceutical medicines.

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

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