Original Research Article

Zoochemical Composition of Selected Sea Stars Collected from the Coastal Waters of Carmen, Agusan Del Norte, Philippines

Angelo Mark P. Walag*, Romeo M. Del Rosario, Oliva P. Canencia

Department of Science Education, University of Science and Technology of Southern Philippines, Cagayan De Oro City, PHILIPPINES.

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ABSTRACT

Background: The marine ecosystem is home to 34 of 36 phyla in the animal kingdom. It is for this reason that substantial amount of marine-derived natural products with potential medicinal and therapeutic applications have been described, isolated and characterized. Majority of the invertebrate phyla have been accounted for, but few studies have been made on Philippine invertebrates. **Aim:** The goal of this research is to determine the zoochemical composition selected sea stars collected from the coastal waters of Carmen, Agusan del Norte, Philippines. **Methods:** Standard methods were employed to determine the zoochemical composition of *Linckia laevigata*, *Protoreaster nodosus* and *Acanthaster planci* ground samples. **Results:** All sea stars showed presence of alkaloids, flavonoids, saponins, triterpenoids and cardiac glycosides while no presence of coumarins and phenolics and tannins were detected. The presence of these zoochemicals in sea stars was confirmed by the available literature although differences were noted particularly on the absence of phenolics in this study. Based on this study, the selected stars appeared to be rich in saponins and steroids which are known to possess biological activity that could be used to develop drugs. **Recommendation:** The other metabolites can also be exploited in the developing marine-derived drugs. Further analyses can be done especially in isolating these compounds and structurally elucidating them. Moreover, the isolated metabolites can also be tested individually for its biological activities. It is also suggested that more studies be conducted on these organisms to further understand their biochemistry which can be utilized before they become endangered or more so, extinct.

Key words: Alkaloids, Phenolics, Saponins, Sea stars, Natural Products Chemistry.

Correspondence:
Prof. Angelo Mark P. Walag,
Department of Science Education, University of Science and Technology of Southern Philippines, C.M. Recto Ave, Lapasan, Cagayan De Oro City-9000, PHILIPPINES.
Phone no: +63 9177000485

Email: walag.angelo@gmail.com

INTRODUCTION

The oceans is the most diverse ecosystem with 34 of the 36 phyla represented.[1] It is with this reason that a substantial number of marine natural products with potential medicinal and therapeutic applications have been described, isolated, and characterized. Of the 34 phyla present in the oceans, marine invertebrates contain metabolites of unprecedented diversity of molecular structures and activities.[2] Various laborious efforts have been made to study the different metabolites present in these organisms that might soon lead to further drug development. These marine invertebrates that have been accounted for belong to a wide range phyla cutting across Porifera, Annelids, Coelenterates (Cnidaria and Ctenophora), Mollusks, Echinoderms and etc.[1,3,4] In the Philippines, efforts have been made to study secondary metabolites and bioactivities of these invertebrates like ascidians,[5] corals,[6] sponges,[7-11] sea stars, sea cucumbers, and brittle stars,[12] mollusks.[13] Although, several studies have been made on Philippine invertebrates, much of the efforts have been focused on sponges and other sessile organisms which are believed to possess diverse chemical defenses. Several phyla are yet to be studied that may possess lead compounds for future

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drug development studies. Moreover, various issues on water quality of both marine and freshwater have been noted that may affect abundance and diversity of the aquatic life.[14] Several studies were also conducted to evaluate the abundance and diversity of echinoderm species in Mindanao and have noted a decline in their population.[15-21]

Of the different phyla mentioned, echinoderms are spread worldwide and known to have more than 7000 species divided into five classes.[22] These echinoderms are also known as producers of bioactive metabolites.[23] They are also reported to have ability to produce chemical defenses against possible predators although recent studies are rare to find.[23] Although, this phylum has been found to possess various bioactive secondary metabolites, much of the studies conducted on echinoderms in the Philippines come from and are limited on traditional fields like ecology, biogeography, effects of pollution, and conservation of species. This gap in the literature, particularly in the study of secondary metabolites from echinoderms in the Philippines, further emphasizes the need to exert effort in unravelling the diverse chemical world of secondary metabolites before these animals become endangered due to the compounding effects of climate change, pollution, exploitation, and etc. The identification, structure elucidation, and characterization of secondary metabolites and their ecological role have led to the discovery of lead compounds with relevant pharmacological properties.[24]

Sea stars are invertebrates which belongs to class Asteroidea, phylum Echinodermata which are often found in marine environments although some are found in brackish waters.[25] These organisms produce unique natural products which has been found to be in demand in the fields of medicine.[26] In fact, under the phylum Echinodermata, three classes (Holothuria, Asteroidea, and Crinoidea) have been noted to produce elaborate chemical defenses against predation.[27] Furthermore, the secondary metabolites they produce are an integral part of their survival strategy to protect them against predators and parasites. These compounds sea stars produce like glycosides, sterols, cerebrosides, peptides, and gangliosides possess a wide range of bioactivities like being cytotoxic, antimicrobial, expectorant, analgesic, hemolytic, antioxidant and antitussive.[28-31] Sea stars in the Philippines has only been studied in terms of bioactivity against brine shrimp and certain strains of bacteria[12,32] and no literature yet available on the secondary metabolite screening. Thus, the main aim of this study is to determine the zoochemicals present in selected sea stars from Goso-on and Vinapor, Carmen, Agusan del Norte, Philippines.

MATERIALS AND METHODS

Study Area

The animal materials required for this study was collected from the intertidal zone up to shallow waters (0-5m) of Barangay Goso-on, Carmen, Agusan del Norte, Philippines. The map of the collection site is shown in Figure 1. The Municipality of Carmen is located in the province of Agusan del Norte of CARAGA Region or Region XIII. It is strategically located along the Western Agusan Corregidor, surrounded by the Butuan Bay in the north, Buenavista in the south, Nasipit in the east, and Misamis Oriental in the west.

Collection and identification of sea star specimens

A field reconnaissance was conducted to evaluate presence and abundance of marine sea stars selected for this study. Preliminary identification of specimens was conducted based on the field guide developed by Schoppe (2000) and the World Registry of Marine Species (WoRMS) using morphological characteristics. Specimens that were identified were photographed and then returned back to its natural habitat. One representative per specimen was collected and stored in polypropylene bags for further identification. Final verification and confirmation of species identification was through the use of collected and preserved specimens that were brought to the laboratory. Photographs of specimens were also sent to the Institute of Environmental and

Figure 1: Collection site in Barangay Gosoon, Carmen, Agusan del Norte.

Marine Science, Silliman University for the confirmation of the identification. The sea stars collected were *L. laevigata*, *P. nodosus*, and *A. planci*. A collection of aquatic samples using non-destructive method. Gratuitous Permit (GP-2019-01) was also acquired from the Bureau of Fisheries and Aquatic Resources – Caraga Region prior sampling and reconnaissance.

Fresh samples were collected during the lowest low tide of the month of April 2019 from the intertidal zone up to shallow (3-5meters deep) parts of the marine environment in Barangays Goso-on and Vinapor, Carmen, Agusan del Norte. Mature (>5 inches across) sea stars that were collected were washed with marine water to remove dirt and sand and were then placed in styrobox with ice and marine water for preservation. All collected samples were brought to Cagayan de Oro City for storage and further sample processing.

Sample Preparation

The specimens were sorted in polypropylene bags filled with marine water. One bag was used for each species to avoid contamination. Specimens were brought to the chemistry laboratory and reduced to smaller size using a clean pruning shear. Samples were subjected to drying using oven method blanketed with nitrogen gas to minimize oxidation. Samples were homogenized separately using a mechanized grinder available at the Northern Mindanao Food Innovation Center. The sample material were weighed and transferred to pre-weighed Ziploc bags. Each sample bag was labelled with its scientific name and a specific sample number.

Zoochemical Analyses

Test for Alkaloids. Presence of alkaloids was determined qualitatively using the Wagner's Test.

1. Wagner's Test. 50 mg (0.05 g) of the sample was stirred with 2 mL dilute HCl (2N) and filtered. The filtrate (2 mL) was added with few drops of Wagner's reagent (1.27 g iodine and 2 g potassium iodide dissolved in 5 mL of water and made up to 100 mL with distilled water). The samples were scored positive on the basis of turbidity or precipitation.

Tests for Coumarin. Presence of coumarins was determined using NaOH test and Ammonium hydroxide-UV light test.

1. NaOH Test. 0.05g of moistened dry powdered sample was placed in a test tube. The test tube was covered with filter paper soaked in dilute NaOH (10%). The mixture was kept in water bath and after some time, the filter paper was exposed to UV light. A yellowish-green fluorescence indicates presence of coumarin.[34]
2. Ammonium hydroxide-UV light test. A moistened dry powdered sample (0.05 g) was placed in a test tube and was covered with filter paper soaked in dilute ammonium hydroxide (10%) and was kept in water bath. After some time, the filter paper was exposed to UV light. An intense fluorescence indicates presence of coumarin.

Tests for Flavonoids. The presence of flavonoids was determined using Shinoda's test and ammonia test.

1. Shinoda's Test. 400 mg (0.4 g) sample was extracted with 10 mL ethanol was then filtered. 1 mL filtrate was added with magnesium ribbon and conc. HCl (5 drops) and allowed to react. A pink or red color indicated the presence of flavonoids.
2. Ammonia test. 30 ml of ethyl acetate was added to about 0.6 g of the powdered sample and was heated on a water bath for 3 minutes. The mixture was cooled, filtered and 4 mL of filtrate was shaken with 1 mL of dilute ammonia solution (10%). The layers are allowed to separate and the yellow color in the ammonia layer indicates the presence of flavonoids

Test for Phenolics and Tannins. The presence of phenolics and tannins was determined using ferric chloride test.

1. Ferric chloride test. 500 mg (0.5 g) sample was boiled in 30 mL distilled water and filtered. 2 mL filtrate was mixed with 1 mL 5% $FeCl_3$.. Formation of blue, green, or violet color indicated the presence of phenolic and tannin compounds.

Tests for Saponins. The presence of saponins was indicated using foam test and olive oil test.

1. Foam test. 1 g powdered sample was boiled in 10 mL distilled water for 15 min. After cooling, the extract was shaken vigorously to record froth formation. Presence of honeycomb froth above the surface after 30 mins indicates the presence of saponins.
2. Olive oil test. About 2 g of the powdered sample will boiled in 20 mL of water in a water bath and will then be filtered. 10 mL of the filtrate will be mixed with 5 mL distilled water and shaken vigorously to form a stable persistent froth. The froth will then be mixed with 3 drops of olive oil, shaken vigorously, and then observed for the formation of emulsions.

Test for Triterpenoids. The presence of triterpenoids was determined using Salkowski test.

1. Salkowski test. 0.6 g of the sample was extracted in 30 mL chloroform, and filtered. 2 mL of the

filtrate was treated with few (3-4) drops of concentrated sulfuric acid, shaken and allowed to stand. Presence of triterpenoids was indicated by the appearance of yellow color in the lower layer.

Tests for Steroids. The presence of steroids was indicated using the Liebermann-Burchard test and Salkowski test.

1. Liebermann-Burchard test. 0.6 g of the sample was extracted in 30 mL chloroform, and filtered. 2 mL of the filtrate was added with 2 mL acetic anhydride and 1 mL conc. sulfuric acid and allowed to react. A blue-green ring indicated the presence of steroids
2. Salkowski test. 0.6 g of the sample was extracted in 30 mL chloroform, and filtered. 2 mL of the filtrate was treated with few (3-4) drops of concentrated sulfuric acid, shaken and allowed to stand. Presence of steroids was indicated by the appearance of red color in the lower layer.

Test for Cardiac glycoside. The presence of cardiac glycosides was determined using the test for Keller-Kiliani Test.

1. Keller-Kiliani Test. 500 mg sample was boiled in 30 mL distilled water and filtered. 2 mL of the filtrate was added with 1 mL glacial acetic acid and 1 mL 5% $FeCl_3$ + 1 mL conc. sulfuric acid. A green-blue color indicated the presence of glycosides.

RESULTS

Zoochemical Composition

Alkaloids are commonly defined as any substance which contain one or more nitrogen atoms usually as part of a cyclic system.[35] In plants, alkaloids are known to function as protective agents against predators, growth regulators, and as waste products in metabolisms.[36] Alkaloids were found to be present in all three sea stars as indicated in the Wagner's Test result which was scored based on the presence of turbidity or precipitation as summarized in Table 1. Coumarin is the parent compound of a large class of naturally occurring phenolic compounds and have been found to be widely distributed to various plant families while only few were found in animals.[37,38] Coumarins were found to be absent in all sea stars investigated in this research. Flavonoids are known in plants as pigments, synthesized from phenylalanine, which exudes brilliant fluorescence when excited with UV light.[39] The production of flavonoids in living organisms has been believed to be a response against fungal parasites, predation, pathogens, and oxidative cell injury.[40] The presence of flavonoids in the selected sea star samples are presented in Table 1. All three sea stars possess flavonoids as indicated by Shinoda's Test and Ammonia Test although differing in terms of their intensity. All sea stars investigated were found to possess flavonoids in both tests conducted. Phenolics and tannins, on the other hand, were found to be absent in all sea stars. Saponins, triterpenoids, steroids, and cardiac glycosides were also found to be present in all sea stars ranging from trace presence to strong presence as summarized in Table 1.

DISCUSSION

Trace to moderate presence was observed in the three samples which coincide with the finding that echinoderms were the second least nitrogen-bearer compared to different marine invertebrates.[41] Furthermore, Baker reported that the three major producers of alkaloids, in proportion, in the marine ecosystem are organisms

Table 1: Zoochemicals present in selected sea stars using varied tests.

Zoochemical	Test	*L. laevigata*	*P. nodosus*	*A. planci*
Alkaloids	Wagner's Test	++	+	+
Coumarin	NaOH Test	-	-	-
	NH_4OH-UV Light Test	-	-	-
Flavonoids	Shinoda's Test	++	+	+
	Ammonia Test	+	++	++
Phenolics and Tannins	Ferric chloride Test	-	-	-
Saponins	Foam Test	++	++	++
	Olive Oil Test	++	++	++
Triterpenoids	Salkowski's Test	+	++	+
Steroids	Liebermann-Burchard Test	++	+++	++
	Salkowski's Test	+	++	++
Cardiac glycosides	Keller-Kiliani Test	++	+	+

Legend: +++ strong presence, ++ moderate presence, + trace presence and – absence.

under taxa of tunicates, worms, and bryozoan. Sponges were also reported to possess the most rich source of alkaloids in the marine ecosystem.[42] It was also noted that majority of the nitrogenous substances found in marine organisms are halogenated (25%), sulfur-bearing in one of several oxidation states (13%), polypeptides (8%), and possess N-O bond (7%).

Alkaloids were also isolated from sea stars *Lethasterias nanimensis chelifera,*[30] *Perknaster fuscus antarticus* (Antarctic sea star) which is considered to be the most ichthyotoxic echinoderm in the Antarctic,[43] and *Dermasterias imbricata* [44] Several alkaloidal substances were also isolated from *Pentaceraster chinensis,*[45] which possesses antimicrobial activity against Gram-postive and –negative bacteria, *Fromia monilis* and *Celerini heffernani,* which also possess cytotoxic activities.[46] These alkaloids, together with many other alkaloids, possess toxic effects by mimicking structurally and biosynthetically related neurotransmitters.[47] This further highlights that the production of alkaloids in sea stars may be attributed to its chemical defense nature as suggested by Waller and Nowacki (1978).

The moderate presence of alkaloids in *L. laevigata* suggests the need for further protection against possible predators due to the absence of thorns in its body walls. *P. nodosus* and *A. planci* possess a thick body wall and an elaborate system of thorns, respectively, which may be the reason of low alkaloid presence. Alkaloids are known to taste bitter in plants, which may also deter predators in marine ecosystems. Furthermore, it was also observed that both *P. nodosus* and *A. planci* specimens collected were relatively larger than *L. laevigata*. In marine organisms, tradeoff between growth and development and variation in defenses has been well observed in the Optimal Defense Theory first proposed by Rhoades (1979).

In marine ecosystems, two isocoumarins were found present in sponge-associated fungus *Aspergillus similanensi*[48] while one isocoumarin was isolated from the marine-derived fungus *Aspergillus flavus* (from a prawn species *Penaeus vannamei*).[49] Another species of Aspergillus, a marine-derived fungus, yielded a new dihydroxyisocoumarin from the ethyl acetate extract which showed inhibition of LPS-induced nitric oxide and prostaglandin E_2.[50] Another isocoumarin compound was isolated from marine-derived fungus associated with seagrass.[51,52] This shows that most coumarins in marine ecosystems are derived from associated fungus thus supports results of this study particularly in the absence of coumarins in the three sea star species.

The presence of flavonoids as coloring pigments in sea stars may be an elaborate defense mechanism to serve as warning against potential predators. This mechanism of possessing aposematic coloration has been well observed in small and obscure organisms.[53] Furthermore, organisms under the phylum Echinodermata, includes 6000 species possessing conspicuously colored parts like sea cucumbers, brittle stars, and sea stars. Moreover, asteroids (sea stars) are believed to have evolved with chemical defenses as an in part of their survival strategies.[26]

L. laevigata are sea stars characterized by uniformly blue color due to a blue, grey, pink, or purple pigment.[33] In the Philippines, *L. laevigata* species have been found to be in shades of blue[15,16] which is due to a pigment called linckiacyanin.[54] This linkicyanin is a compound with varied pigments that reflect their availability in terms of the animal's diet or metabolic activities. The role of this coloration in the skin of sea stars has been believed to be an adaptive camouflage rather than as light receptors. [54] This camouflage coloration in sea stars allows them to hide against possible predators. Similarly, *A. planci's* coloration varies from red to orange which also reflects its variability as an attribute to the animal's diet.[33] Red and orange are the most frequent colors among phylum Echinodermata, as exhibited by both *A. planci* and *P. nodosus*, which are due to varied combinations of carotenoids, carotenoproteins, flavins, melanins, quinones, and porphyrins.[55]

Phenolic compounds are naturally occurring antioxidants found in plants and other marine invertebrates particularly sea cucumbers and sea urchins.[31] Furthermore, sea cucumbers were found to possess high phenolic content in their digestive tract while low phenolic content in their gonads[56] *Holothuria scabra*, *Holothuria leucospilota*, and *Stichopus chloronatus* from Malaysia[57] *Strongylocentrotus droebachiensis,*[58] and *Curcumaria frondosa.* [56] These phenolic compounds collected from these sea stars also showed antioxidant activity.

Sea stars were also found to possess phenolic compounds. Reasonable amounts of phenolics were determined in three different extracts from *L. maculata* which can be a good source of antioxidants.[31] Pharmacological and chemical properties of phenolic compounds were also evaluated in two sea star species (*Astropecten irregularis* and *Luidia sarsii*) and one brittle star (*Ophiura albida*).[59] Phenolic compounds were also present in organic extract of brittle star *Ophiocoma erinaceus* which showed antioxidant anti-inflammatory properties. [60] Phenolics and tannins showed negative in all three star species although present in many studies on echinoderms, even in *A. planci* where phenolic compounds were identified.[61] and showed antioxidant and anticancer activities. The absence may be attributed to the fact that majority of the phenolics identified in the literature came from the pyloric caeca of these organisms and the material analyzed for this study is only the body wall.

Saponins are naturally occurring non-volatile surface-active glycosides mainly produced by plants but also lower marine animals and bacteria.[62,63] Saponins in sea stars generally play important roles in digestion, reproduction, and chemical signaling.[64] Saponins were moderately present in all sea star samples which is not surprising since a good amount of literature on saponin isolation in sea stars and other echinoderm species are available. To note, saponins were isolated in two different sea cucumbers *H. lesson*[65] and *H. moebii*[66] where the latter showed *in vitro* cytotoxicity against tumor growth in mice. Not only sea cucumber possess saponins but also sea stars, in fact, among echinoderms, sea stars and sea cucumbers possess saponins that is generally responsible for their toxicity.[67]

Glycosylated steroids produced by sea stars bear one to six sugar groups and are collectively called saponins because of their soap-like tendencies to form emulsions with water. Saponin groups commonly found in sea stars are often sulfonylated[25] which are referred to as asterosaponins. Several sea stars around the world have been a source of a handful of asterosaponins. Two new asterosaponins (luidiaquinoside and psilasteroside) were isolated from *Luidia quinaria* and *Psilaster cassiope* which showed marginal *in vitro* cytotoxicity against rat basophilic leukemia cells.[68] Sulfated steroidal glycosides together with three known asterosaponins were also isolated from *Culcita novaeguineae* which all showed moderate cytotoxicity against cancer cell lines.[69] Six new asterosaponins were also isolated from *Leptasterias ochotensis* which showed slight to moderate cytotoxicity against cancer cell lines.[70] Anti-inflammatory asterosaponins were also isolated from the ethanolic extract of an edible Vietnamese sea star *Astropecten monocanthus*.[71]

The saponins detected in this study can be confirmed by the different saponin isolation and structural elucidation available in the literature. Five saponin compounds were also isolated from *L. laevigata*, where four of which were known (thornasteroside, marthasteroside, ophidianoside, and maculatoside) and the other one was named as laevigatoside, where all of which were found to be steroidal glycoside sulfates.[72] A novel asterosaponin nodoside were also isolated from the sea stars *A. planci* and *L. laevigata*.[73] Another steroidal glycoside sulfate compound was isolated from the sea star *P. nodosus* and was further structurally elucidated.[74] Moreover, in a more recent study, polyhydroxylated sterols isolated from *P. nodosus* showed anti-inflammatory activities.[75] This abundant literature on saponin presence in sea stars goes to show that asteroids are good source of novel saponin compounds. The toxic properties of sea stars are believed to be associated with compounds similar to plant saponins.[61,67] Furthermore, it is because of the toxic nature of saponins that it is probable that saponins act as chemical defense against infectious fungi, protists, parasites, and predators.[76] This could be the reason for toxic nature of the spines of *A. planci* which showed positive presence of saponins. In addition, the butanolic fraction extract from *A. planci* contained cytotoxic asterosaponins.[61]

Triterpenoids are considered to be a ubiquitous non-steroidal secondary metabolite in both marine and terrestrial fauna.[77] Triterpene glycosides have been initially believed to be present and abundant only in sea cucumbers[78] in the marine fauna but later were found to be present in marine sponges.[79] In a more recent review, it was found out that triterpenoids are the most abundant secondary metabolite present in marine organisms (sponges, sea cucumbers, marine algae, marine-derived fungi).[80] In addition, four triterpenoids were also described and isolated from a marine sponge *Jaspis stellifera* in a more recent study.[81] In sea cucumbers, the triterpene glycosides are synthesized in the skin and are ejected when the animal is disturbed. This behavior is believed to be a defense mechanism holothuroid species possess.[82]

The abundance of triterpenoids in the marine life can be confirmed by the presence of this compound in all three sea star samples as indicated in the Salkoswki's Test. Although present in the specimens tested, it was suggested that the saponins present in sea cucumbers are triterpenoid glycosides are while in sea stars are steroidal glycosides.[67] Furthermore, it was also pointed out agree that sea cucumbers are producers of triterpenoid glycosides while sea stars are steroidal glycosides.[76] This could have been the reason for the non-availability of literature on the sea star triterpenoid compounds. Moreover, the presence of oligoglycosides in both asteroids and holothuroids suggests the phylogenetic closeness of both taxa.

Steroids are compounds which possess a fundamental structure of four fused carbon rings commonly known as steroidal nucleus[83] Sea stars are known to be excellent producers of secondary metabolites particularly steroids.[25] Moreover, the vast majority (>80%) of the secondary metabolites discovered in sea stars are steroids.[31] Unlike other echinoderms, sea stars are known to produce a wide variety of oxygenated steroids compounds like polyhydroxysteroids, polyhydroxysteroid mono- and biosides, and toxic steroid oligoglycosides.

[84] Several sea stars have been found to possess steroidal compounds where majority of which exhibits antineoplastic, antiviral, anti-inflammatory, and icthyotoxic activities.[85] Three sulfated steroidal pentaglycosides have been isolated from the sea star *C. novaeguineae* which all possessed marginal cytotoxic activity.[86]

As shown in Table 1, all three sea stars samples showed moderate to strong presence of steroids. This confirms the idea that sea stars are considered to be richest source of polyhydroxy steroids in the marine ecosystem.[87] Furthermore, steroids were identified in *P. nodosus* and were found to possess the most common steroids in sea stars.[88] Three more steroids were further isolated from the same sea star which was also found to be common in other sea stars.[73] Cytotoxic steroids were also isolated first from the same sea star which was later called cytotoxic nodoside.[87] Four steroids were also isolated from *A. planci* which also confirms the presence of steroids of this same species in this study.[89] Several studies also reported presence of steroidal glycosides present in *A. planci*.[90,91]

Steroid glycosides were also isolated from *L. laevigata* which confirms the results of this study. Two new steroids were isolated from this sea star and were found to possess two monosaccharide units.[92] Three more novel steroids were also isolated from the same sea star were found to possess two monosaccharide units.[93] These steroids isolated were described to be members of neuritogenic compounds.

Cardiac glycosides or cardiotonic glycosides possess a steroidal structure with an unsaturated lactone ring.[94] This metabolite is present in a limited number of plant families and is clinically used to treat congestive heart failure.[95,96] In marine ecosystems, cardiac glycosides together with steroid saponins consists the toxin called holothurin. This toxin produced by sea cucumbers have been found to cause skin papular eruptions and eye irritation.[97] Ingestion of this holothurin containing potent cardiac glycoside has also been noted to cause death.[98] This toxin produced by sea cucumbers might be related to cardiac glycosides detected in this study. These cardiac glycosides detected might serve as defense mechanism of sea stars against predators and parasites.

CONCLUSION

The presence of alkaloids in the three sea stars species coincides with the finding that echinoderms were the second least nitrogen-bearer compared to different marine invertebrates. The results also suggest that the presence of alkaloids in sea stars serves the role as protective agent against predators, as growth regulators, and as waste products of metabolism. This also suggests that differences in alkaloid presence in sea stars can be attributed to presence of potential predators. The absence of coumarins in sea stars was not surprising since majority of the coumarins isolated from marine ecosystems come from fungi associated with sponges, prawns, and sea grasses. This further suggests that sea stars do not produce coumarins as part of their defense mechanism. Flavonoids in sea stars serve as their coloring pigments which is part of their elaborate defense mechanism, serving as an advanced warning against predators. Phylum Echinodermata is known to possess conspicuously colored parts to deter potential predators. The differences of flavonoid presence can be attributed to the color of this marine organism. The more colorful, the more flavonoid compounds can be expected. The absence of phenolics in this study may be attributed to the fact that majority of the phenolics identified in the literature came from the pyloric caeca of these organisms and the material analyzed for this study is only the body wall. Saponins are generally present in sea stars which serve as chemical defense against infectious fungi, protists, and parasites, and predators. The presence of saponins in sea stars also supports the idea this metabolite possess toxic properties similar to saponin counterparts in plants. Triterpenoids are generally absent in sea stars since they are known to be producers of steroidal glycosides while the major producers of echinoderm triterpenoids are sea cucumbers. The triterpene glycosides preset in sea stars in this study is believed to be part a defense mechanism of these organisms similar to those of holothuroid species. Sea stars are considered to be richest source of polyhydroxy steroids in the marine ecosystem. These saponins collected from these organisms are believe to be part of their defense mechanism as saponins from other sea star showed potent cytotoxic activity against broad range of test subjects. Cardiac glycosides are known to be produced by sea cucumbers as part of the toxin they produce. The cardiac glycosides detected in this study are believed to be part of the elaborate defense mechanism of these organisms against predators and parasites especially in the toxic spines of *A. planci*.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUMMARY

All sea stars showed presence of alkaloids, flavonoids, saponins, triterpenoids and cardiac glycosides while no presence of coumarins and phenolics and tannins were detected. Moreover, the selected stars appeared to be rich in saponins and steroids which are known to possess biological activity that could be used to develop drugs.

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