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TOTAL FLAVONOIDS CONTENT, TOTAL PHENOLICS CONTENT, AND ANTIOXIDANT ACTIVITIES OF *Acanthaster planci* AND *Linckia laevigata* COLLECTED FROM CARMEN, AGUSAN DEL NORTE, PHILIPPINES

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Abstract

Marine invertebrates are known to contain metabolites with unprecedented diversity in terms of their molecular structures and bioactivities. The majority of the invertebrate phyla have been accounted for but only several studies on antioxidant activities have been made on Philippine sea stars. The main aim of this research is to determine the total flavonoids content, total phenolics content, and antioxidant activities of ethyl acetate and methanolic extract of *Linckia laevigata* and *Acanthaster planci*. Standard methods in plants were utilized to determine TFC and TPC while TEAC, DPPH, and FRAP assays were used to evaluate antioxidant activities. No flavonoids were detected in both species for the two solvents utilized while total phenolics were detected in both species and solvents utilized. Total phenolics were noted to be higher in methanol compared to ethyl acetate extract. *A. planci* also consistently recorded higher TPC for both solvents compared to *L. laevigata*. Consistently in DPPH and TEAC, *A. planci* had higher antioxidant capacity compared to *L. laevigata* for both solvents utilized. Overall, lower antioxidant capacities were observed for all three assays which could be due to the method of sample preparation, especially in the drying of samples which could have affected the natural antioxidants present in the body parts of the sea stars. It is recommended that conservation measures be implemented to protect these species which are a potential source of novel antioxidant compounds.

INTRODUCTION

Marine ecosystems provide a home to highly diverse living organisms, compared to terrestrial ecosystems, which provide numerous resources for human nutrition (food, nutraceuticals) and health (Hill & Fenical William, 2010). In like manner, marine invertebrates are considered useful in medical research due to their wide diversity in form and physiological competence (Suleria, Osborne, Masci, & Gobe, 2015). These properties of marine invertebrates exploited for medicines are brought about by the secondary metabolites they produce. Secondary metabolites are generally used by organisms to control ecological relationships especially those involved in defense against predation, competition for space and food, and interspecies communication (Skropeta & Wei, 2014).

Sea stars are excellent producers of secondary metabolites which are derivatives of lipid compounds. These substances include cholesterol derivatives (steroids), which are often glycosylated, and fatty acids, which are often in the form of amides of sphingosines (Slattery, 2010). The vast majority (>80%) of secondary metabolites discovered in sea stars are steroids while the minor component (14%) is comprised of sphingolipids. Furthermore, steroidal glycoside compounds in sea stars are predominantly responsible for their general toxicity (McClintock, Amsler, & Baker, 2013). In addition to the more than 800 metabolites, 24 miscellaneous compounds complete the

current extent of what is known as sea star secondary metabolites. Other secondary metabolites which including anthraquinones, alkaloids, phospholipids, peptides, fatty acids were reported to have been extracted from sea stars (Dong et al., 2011; McClintock et al., 2013). Although a relatively large number of secondary metabolites have been isolated, structurally elucidated, and identified in the body components of sea stars, our knowledge on their ecological significance is yet at an infancy stage (Mayer, Rodríguez, Berlinck, & Hamann, 2009).

In the Philippines, sea stars have only been investigated in terms of cytotoxicity against brine shrimp and certain strains of bacteria (Layson, Rodil, Mojica, & Deocaris, 2014), proximate biochemical composition (Walag & Del Rosario, 2018), and zoochemical screening (Walag, Del Rosario, & Canencia, 2019), although in terms of their biology, ecology, biogeography, and conservation, extensive studies have already been made (Bos, Gumanao, Alipoyo, & Cardona, 2008; Llacuna, Walag, & Villaluz, 2016; Medrano, 2015; Schoppe, 2000; Walag, Layaog, & Garcia, 2018). No literature yet is available on the total flavonoids content, total phenolics content, and antioxidant activities of Philippine sea stars. Understanding their potential benefits to humans allows for better protection and conservation of these species, especially that the water quality of Mindanao is declining (Walag, Canencia, & Fiedler, 2018). Thus, the main aim of this study is to determine the total flavonoids content, total phenolics content, and antioxidant activities of ethyl acetate and methanolic extract in selected sea stars from Goso-on and Vinapor, Carmen, Agusan del Norte, Philippines.

Figure 1. Collection site (red dot) in Barangay Gosoon, Carmen, Agusan del Norte with respect to Mindanao Island, Philippines.

MATERIALS AND METHODS

Study Area

The animal materials required for this study were 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 the 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 to their 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 and were brought to the laboratory. Photographs of specimens were also sent to the Institute of Environmental and Marine Science, Silliman University for the confirmation of the identification. The sea stars collected were *L. laevigata* and *A. planci*. A collection of aquatic samples using a non-destructive method was utilized. Gratuitous Permit (GP-2019-01) was also acquired from the Bureau of Fisheries and Aquatic Resources – Caraga Region before sampling and reconnaissance.

Fresh samples were collected during the lowest low tide 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 (>5inches 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 a smaller size using a clean pruning shear. Samples were subjected to drying using the oven method blanketed with nitrogen gas to minimize oxidation (Walag et al., 2019). Samples were homogenized separately using a mechanized grinder available at the Northern Mindanao Food Innovation Center. The sample materials were weighed and transferred to pre-weighed Ziploc bags. Each sample bag was labelled with its scientific name and a specific sample number.

Determination of Total Flavonoids and Total Phenolics

Extraction for total flavonoids and total phenolics

About 50 g of powdered samples were soaked in 95% methanol (1g of sample: 4 mL of solvent) for 48 hours. After, the crude extract was filtered using Whatman filter paper. Then another 100 mL of methanol was used for the second and third soaking (Walag, Cepeda, Galenzoga, & Sambaan, 2017). After an hour, the sample with methanol was subjected to another second and third filtration round. The samples were then concentrated under reduced pressure at 40°C using a rotary evaporator and were stored at -20°C. A similar procedure was also employed in the extraction using ethyl acetate as a solvent. There were two extracts, ethyl acetate extract (EAE) and methanolic extract (MeOHE)

Analysis of total flavonoids

The total flavonoids content in the methanolic and ethyl acetate extracts was determined using aluminum chloride colorimetric assay (Ardekani et al., 2011). An aliquot (1 mL) of each extract (1000 ppm) was added to a 10 mL volumetric flask with 4 mL of distilled water. Sequentially, 0.3 mL of 5% $NaNO_2$ was added and allowed to stand for 5 minutes and was followed by the addition of 0.3 mL of 10% $AlCl_3$. After the 5 minutes, 2 mL of 1M NaOH was added and diluted to 2.4 mL distilled water to make a 10 mL solution. The solution was then vigorously shaken, and the absorbance was read and recorded at 520 nm using a Jenway 6051 colorimeter. The same wavelength was used for the construction of a standard calibration curve using known solutions of quercetin in ethanol at 50, 100, 150, 200, and 300 ppm concentrations. The total flavonoids in the sample was derived from the calibration plot and expressed as mg quercetin/g of sample.

Analysis of total phenolics

The total phenolics content in the crude methanolic and ethyl acetate extracts of sea star was determined using the Folin-Ciocalteu method (Singleton & Rossi, 1965). An aliquot (1 mL) of each extract was added to a 25 mL volumetric flask containing 9 mL of distilled deionized water. A reagent blank was also run using only distilled deionized water. 1 mL of Folin-Ciocalteu phenol reagent was also added to the mixture and was shaken. After about 5 minutes, 10 mL of 7% Na_2CO_3 solution was added to the mixture and the solution was diluted up to 25 mL with deionized water. The mixture was then incubated for 90 minutes at room temperature, while the absorbance was read at 750nm using a UV-Vis spectrophotometer against the prepared reagent blank. The concentration of total phenolics was determined using a standard curve prepared using a standard solution of gallic acid with concentrations of 20, 40, 60, 80, and 100 g/mL and was expressed as mg Gallic acid/g sample.

Determination of Antioxidant Activities

The in vitro antioxidant activities of the methanolic and ethyl acetate extracts of selected sea stars was determined using DPPH antioxidant assay (Manzocco, Anese, & Nicoli, 1998), FRAP antioxidant assay (Benzie & Strain, 1996), and Trolox equivalent antioxidant capacity/ABTS radical cation decolorization assay (Seeram et al., 2006).

DPPH antioxidant assay

The DPPH solution was freshly prepared by dissolving 6 mg of DPPG to 50 mL of methanol, resulting in a 0.006% concentration. The freshly prepared DPPH was taken in the test tubes where extracts were previously added and were serially diluted (100-1000 µg) to attain a final volume of 2 mL. The mixture was then incubated for 30 minutes in the dark and the decrease in absorbance was measured at 517 nm using a UV-Vis spectrophotometer. Ascorbic acid was used as the standard for this study.

FRAP antioxidant assay

The extracts (at 200 µL) were each added to 3 mL of FRAP reagent. The FRAP reagent was prepared by mixing 5 mL of 10 mM of 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) in 40 mmol/L of HCl, 5 mL of 20 mM $FeCl_3$ and 50 mL of acetate buffer (0.3 mol/L, pH 3.6). The final reaction mixture was incubated in a water bat at 37°C for 10 minutes. The absorbance was then read at 593 nm using a UV-Vis spectrophotometer. A standard calibration curve was prepared using $FeSO_4 \cdot 7H_2O$ at 125, 250, 500, 750, 10 µmol/L concentrations. The ferric reducing antioxidant property was calculated from the calibration curve and was expressed as µmol/L of Fe^{+2}/g of dried sample.

Trolox equivalent antioxidant capacity/ABTS

The ABTS radical cation was prepared by adding 80 mg solid manganese dioxide to a 5 mM of aqueous stock

solution of ABTS prepared by 20 mL of 75 mM Na/K buffer at pH 7. The antioxidant standard that was utilized in this method is Trolox, a water-soluble analogue of Vit. E. A standard calibration curve was constructed using Trolox at 0, 50, 100, 150, 200, 250, 300, and 350 μM concentrations. Methanolic and ethyl acetate extracts of the samples were diluted appropriately according to the antioxidant activity in Na/K buffer at pH 7. The diluted samples were mixed with 200 μL of ABTS·+ radical cation solution and the absorbance was read at 750 nm using a UV-Vis spectrophotometer after 5 minutes has elapsed. The TEAC values were reported as mM Trolox equivalents based on the standard curve.

RESULTS AND DISCUSSION

Total Flavonoids Content

Flavonoids are naturally occurring compounds that exist in fruits, vegetables, and herbs that act as antioxidants from various kinds of free radicals (Baharara & Amini, 2015). No flavonoids were detected in both species of sea star and both solvents utilized as shown in Table 1. The results could be due to the limited detection capability of the instrument and method utilized. As mentioned in other studies, different classes of echinoderms possess flavonoids but of trace amounts. This means that there may be flavonoids in the samples as indicated in the qualitative testing done by Walag et al. (2019) but of very minute content. Trace amounts of total flavonoids were detected in aqueous extracts of a sea cucumber (*C. frondosa*) from 2.9 to 59.8 Rutin equivalents mg/g in various parts of the organism (Mamelona et al., 2007). Moreover, in the same study, it was noted that higher flavonoid contents were observed in water-rich and acetonitrile-rich fractions of the gonads where in this study, gonads were not considered part of the analysis due to quantity limitation and that methanol was used as the extracting solvent. Trace amounts of total flavonoids in the methanolic extract were also observed in brittle star *O. erinaceus* (1.26 ± 0.03 mg Q/g) which was believed to be part of the therapeutic capacity of brittle against oxidative damage-related diseases (Baharara & Amini, 2015).

Table 1. Total flavonoids and total phenolics contents of ethyl acetate and methanolic extracts

Species	Total Flavonoids Content (mg Q/g sample)		Total Phenolics Content (mg GA/g sample)	
	EAE	MeOHE	EAE	MeOHE
L. laevigata	nil	nil	0.020 ± 0.001	0.169 ± 0.008
A. planci	nil	nil	0.021 ± 0.002	0.402 ± 0.044
p-value	n/a	n/a	0.000*	0.000*

*significant at 0.05 level

In the ethyl acetate extracts, no flavonoids were also detected for both sea star species. As mentioned earlier, this could be due to the limited detection ability of the method and instrument used since in other classes of echinoderms, total flavonoids were found in trace amount in ethyl acetate extract like *E. mathaei* sea urchin, wherein trace total flavonoids were detected ranging from 0.47 to 4.81 mg Q/g of sample (Soleimani, Moein, Yousefzadi, & Amrollahi Bioki, 2016).

In a more general sense, the ability of organisms to produce total flavonoids can be predicted based on various ecological variables (Suguna, Bragadeeswaran, Natarajan, & Mohanraj, 2014). For example, the presence of predators exerts pressure on organisms to produce secondary metabolites that possess defense function as predicted by the Optimal Defense Theory (ODT) (Martins, Vieira, Gaspar, & Santos, 2014). In this study, large sizes of sea stars were believed to be a result of the absence of significant predators (i.e. sea turtles) and healthy ecosystems (neighboring intertidal zones are declared as Marine Protected Area). This means that, in the ODT sense, it would be a waste of energy for these sea stars to produce defense compounds when predators are absent in their ecosystem. Moreover, the elaborate chemical defense systems of living organisms is a result of a trade-off between the energetic cost of defense against growth and reproduction (Dong et al., 2011). As mentioned earlier, there seems to be no problem in terms of the growth of these organisms as reflected by their huge size compared to other studies which indicate that these organisms utilize their energy more for growth and not for defense.

Total Phenolics Content

Phenolic compounds are a collective term used to refer to metabolites possessing an aromatic ring with one or more hydroxyl substituents (Gomes, Dasari, Chandra, Kiss, & Kornienko, 2016). The total phenolics content of the methanolic extract of the two sea stars and are summarized in Table 1. As shown, total phenolics were detected in *A. planci* and *L. laevigata* methanolic extracts although the results seemed low compared to that in the literature. In one study on *A. planci*, 301.89 mg GAE /g sample was detected in ethanolic fraction (Lee, Hsieh, Hsieh, & Hwang, 2014).

The difference seemed to be high even if methanol is more polar than ethanol and that phenolic compounds are polar in nature. This could be attributed to the fact the samples in this study were subjected to conventional oven-drying with nitrogen blanket (to reduce oxidation) compared to the other study which was lyophilized. In the process of drying in this study, some phenols may have been exposed to oxidants thus the lower results.

Trace amounts (4.51 mg GAE /g sample) of total phenolics were also observed in brittle star methanolic extracts (Baharara & Amini, 2015). These phenolic compounds are believed to be highly correlated with the antioxidant potential in this species. Moreover, phenolics are also found in sea cucumbers (*H. leucospilota*, *H. scabra*, and *S. chlorontus*) ranging from 4.85 to 9.70 mg GAE/g sample in the aqueous extract and 1.53 to 2.90 mg GAE/g sample in the organic extract. This goes to show that phenolics are better detected in polar solvents (Althunibat et al., 2009). In another class of echinoderms, trace amounts (0.0044 to 0.3256 mg GAE/g) were recorded in the sea urchin *E. mathaei* (Soleimani et al., 2016). Moreover, similar to brittle stars, phenolic compounds in this sea urchin is believed to be responsible for the antioxidant properties of the extracts.

As shown, trace amount of phenolics was detected using this solvent and by mere visual inspection, there is no difference between total phenolics content in the two species in ethyl acetate extract. The low detection of total phenolics using ethyl acetate extract could be due to the slightly less polar nature of this solvent. In a similar study on phenolics content in *A. planci*, low levels were detected in both petroleum ether and ethyl acetate extracts which are non-polar and slightly polar in nature respectively (Lee et al., 2014). This further signifies that better phenol detection will be achieved using a strong polar solvent.

Differences in the TPC were also examined and tested using two-way ANOVA as summarized in Table 1. As shown, significant differences were observed for both species and solvent used and there was also an interaction between both species and solvent. The detection of more total phenolics in *A. planci* compared to *L. laevigata* could be of evolutionary importance since these compounds are known to possess defense functions in plants (Dai & Mumper, 2010). *A. planci* is characterized by a softer body wall compared to *L. laevigata* thus these compounds offer additional chemical defense against predators, parasites, and pathogens and thus could be beneficial for its survival. Moreover, phenolics are known to be responsible for the bitterness and astringency in plants and allows them to deter predators. *L. laevigata*, on the other hand, have a more rigid and elastic body wall compared to *A. planci* thus may need less additional chemical defense to deter potential predators. Besides, compared to *L. laevigata*, *A. planci* is more visible deeper parts of the marine ecosystem because of its bright color and thus may attract more potential predators thus the need to develop elaborate chemical defenses.

The low levels of TPC in both species could also be explained by the fact there were no known predators (like pufferfish, butterflyfish, damselfish, angelfish, etc.) observed in the sampling site. It is believed that the defensive nature of these compounds only exists as a response to selection pressures exerted by consumers as based upon the convergence of functions of many secondary metabolites across a broad section of organisms (Sumitha, Banu, & Deepa Parvathi, 2017). This supports the idea that phenolics may also function as chemical defenses in these organisms as shown in plants and other attached marine organisms.

The difference observed on TPC in the two solvents could be due to the polar nature of phenolic substances. Methanol is known to possess higher polarity compared to ethyl acetate as reflected in their polarity index. Methanol has a 5.1 polarity index while ethyl acetate has 4.4 (Gupta, Batra, Tyagi, & Sharma, 1997). This difference in the polarity could explain the difference in TPC results based on the solvent used.

Antioxidant Assay

The antioxidant capacities of the two species of sea stars in two different solvents were examined and compared to a standard water-soluble analog of Vitamin E, Trolox. The TEAC reflects the hydrogen donating and the chain-breaking capacity of the samples (Pérez-Jiménez et al., 2008). The TEAC for both sea star species in the methanolic extract is summarized in Table 2. As shown, lower levels of inhibition and Trolox equivalent content were shown for both species compared to Trolox equivalents noted in sea star *Echinaster sepositus* collected from Lecce, Italy (Stabili et al., 2018). This could be due to the levels of TPC on the same solvent as shown in Table 1.

Antioxidant capacity of different samples are highly correlated with the phenolics content (Althunibat et al., 2009; Soleimani et al., 2016; Toor & Savage, 2005). The percent inhibition when plotted against the TPC as GAE will show an $r = 0.99$, $p = 0.03$ for *L. laevigata* which indicates high correlation. This may confirm the standing notion that phenolics contribute to the antioxidant capacity and that the estimated phenolics content (Table 1) in the extract act as an antioxidant directly through the mechanism of the reduction of oxidized intermediate in the chain reaction. Moreover, no extraction from body walls were done in *E. sepositus* since the pure coelomic fluids were used in their study which could be the reason for the higher antioxidant capacity.

ABTS radical scavenging activity was also determined in *A. planci* in various extracts and was found to be highest in ethanol relative to other solvents used (Lee et al., 2014). It was suggested that the higher antioxidant activity in polar solvents was due to the high possibility of extracting polar compounds that are known to be antioxidants. This suggests that the antioxidant capacity observed in this study could be due to the polar antioxidant

compounds extracted using methanol (a polar compound) as a solvent.

Antioxidant capacity of the two sea star species were also examined in ethyl acetate extract and are summarized in Table 2. Low levels of antioxidant capacities were observed for both species under this extract which is similar to the result for *A. planci* where ethyl acetate showed the least radical scavenging activity (Lee et al., 2014). This could be because the contributing compound for antioxidant capacity is believed to be polar in nature. Although ethyl acetate extract is only slightly polar, there was still an antioxidant activity that was detected which suggests that the compounds contributing to this activity could range from slightly polar to polar.

The DPPH antioxidant capacities of the two species of sea stars in two different solvents were examined and compared to a standard water-soluble analog of Vitamin E, Trolox. The DPPH assay reflects the capacity of the extracts in transferring electrons or hydrogen atoms (Zhu, Lian, Guo, Peng, & Zhou, 2011). The DPPH antioxidant assay for the two sea star species methanolic extracts are summarized in Table 2. Low levels of antioxidant activity are observed for both species. This means that the samples were not effective in countering the DPPH oxidant.

Table 2. Antioxidant activities of ethyl acetate and methanolic extracts

Species	TEAC (mg Trolox/g sample)		DPPH (mg Trolox/g sample)		FRAP (mg Fe2+/ g sample)	
	EAE	MeOHE	EAE	MeOHE	EAE	MeOHE
L. laevigata	0.0049 ± 0.00006	0.0774 ± 0.0007	0.005 ± 0.000	0.0774 ± 0.00005	nil	0.00
A. planci	0.0079 ± 0.00006	0.0833 ± 0.0003	0.008 ± 0.000	0.0824 ± 0.00015	nil	0.02 ± 0.000
p-value	0.000*	0.000*	0.000*	0.000*		

*significant at 0.05 level

In a similar study, the aqueous extract of sea urchins (*Heliocidaris erythrogramma*) did not react with DPPH and showed no antioxidant activity for both dichloromethane and methanol extracts (Sheean et al., 2007). The same authors also found out that the extract only scavenged ROS and H_2O_2 which suggests that different antioxidants in extracts may behave differently in different antioxidant assays. This could explain why the antioxidant activity was observed in TEAC and not in DPPH. Also, antioxidant activity was observed in the sea star *A. pectinifera* (Kim et al., 2016) which could be due to the solvent (water) used which is highly polar compared to the solvent used in this study (methanol). Moreover, low levels of antioxidant capacities could be due to the method of drying utilized in the study were some of the antioxidants could have been exposed to further oxidation. The antioxidant capacity of the methanolic extract could also be due to the presence of saponins for both species (Walag et al., 2019). As revealed by a study, brittle stars (*Ophicoma erinaceus*) possess antioxidant capacity which was due to their saponin content as confirmed in both ABTS and DPPH methods (Amini, Nabiuni, Baharara, Parivar, & Asili, 2015; Baharara & Amini, 2015). It was suggested that the saponin's ability to terminate the radical chain reaction by electron or hydrogen donation to DPPH and ABTS could be the reason behind its antioxidant activity (Amini et al., 2015).

Antioxidant activity was also determined in ethyl acetate extract for both species of sea stars. Lower Trolox equivalents were noted for ethyl acetate extracts of both species. This is not surprising since in a similar study on sea urchins (*H. erythrogramma*), all slightly polar and nonpolar solvents used in the study were reactive to DPPH radical except for water, a strong polar solvent (Sheean et al., 2007). This implies that the compounds responsible for the antioxidant properties of the extract for this study are generally polar.

Iron is an important material for oxygen transport, cellular respiration, and various enzymatic activities in the human body. However, iron also poses a threat because of its reactivity, and ability to induce oxidative damage in the living tissues and cells (Choi, Jeong, & Lee, 2007). The reducing power analysis utilized in this study is anchored on the reduction of Fe^{3+} to Fe^{2+} which could be expressed as an antioxidant activity (Lee et al., 2014). The ferric reducing antioxidant potential of methanolic extract for both sea star species is summarized in Table 2. As shown, no Fe^{2+} was detected in *L. laevigata* while minimal Fe^{2+} was found in *A. planci*.

The ferric reducing ability of *A. planci* methanolic extract is expected since in a recent study, semi-polar and polar solvents were noted to extract antioxidant compounds responsible for chelating iron from this species (Lee et al., 2014). This implies that antioxidants from *A. planci* easily dissolve in semi-polar solvents and that they are polar. Moreover, the antioxidant capacity of the methanolic extract could be related to phenolic compounds, known antioxidants easily extracted in polar solvents. Consistently, the methanolic extract of *A. planci* had higher antioxidant capacity compared to *L. laevigata* for all antioxidant assays conducted, TEAC, DPPH, and FRAP. This implies that the antioxidant responsible for the antioxidant activity could range from slightly polar to polar. The absence of FRAP

antioxidant activity could be due to the polarity of ethyl acetate as the solvent used. It is known that most of the compounds responsible for the antioxidant properties of extracts are best extracted using a polar solvent. This also suggests and supports that the compounds responsible for the antioxidant activity of these organisms come from polar compounds.

CONCLUSION

No flavonoids were detected in both species for the two solvents utilized. This could be due to the limited detection capability of the instrument and method utilized since other classes of echinoderms possess flavonoids but of trace amounts. Meanwhile, total phenolics were detected in both sea stars and both solvents utilized. The differences in phenolic content was believed to be due to the polarity of solvent utilized since phenolics are polar. Moreover, the differences between species were ascribed to their difference in physical defense mechanism. Total phenolics were noted to be higher in methanol compared to ethyl acetate extract. *A. planci* also consistently recorded higher TPC for both solvents compared to *L. laevigata*.

Low levels of inhibition and Trolox equivalent content were observed for both species and solvent utilized. Higher antioxidant capacity was noted in methanol compared to ethyl acetate while *A. planci* consistently recorded higher antioxidant capacity in both solvents compared to *L. laevigata*. Low antioxidant capacity was noted for both species and solvent compared to the existing literature for DPPH assay. Relatively higher antioxidant capacity was noted in methanol extract for both species while *A. planci* consistently recorded higher antioxidant capacity for both solvents utilized. Lastly, low antioxidant capacity was noted for methanolic extract while no antioxidant capacity was detected in ethyl acetate extract for FRAP assay. For methanolic extract, *A. planci* recorded higher antioxidant capacity compared to *L. laevigata*. It is recommended that a similar study will be conducted in determining the antioxidant activity using various *in vitro* and *in vivo* methods to fully understand the potential of sea stars as a novel source of antioxidant compounds. Besides, the use of strong polar solvents like water and dimethyl sulfoxide (DMSO) to extract phenolic compounds is recommended in future studies.

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