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Toxicity and antioxidant potential screening of extracts from five marine sponges collected off Zamboanga Peninsula, Philippines

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Abstract

Marine sponges contain unique secondary metabolites that are known to exhibit significant biological properties and can be used for various applications. In this study, five (5) marine sponges were collected off the coast of the Zamboanga Peninsula, Philippines and taxonomically identified as *Petrosia* sp., *Oceanapia ramsayi*, *Clathria* sp., *Ancorina cerebrum* and *Haliclona fascigera*. The marine sponges were sequentially extracted with ethyl acetate:methanol and ethanol:water to give the nonpolar and polar extracts, respectively. The sponge extracts were investigated for toxicity using the brine shrimp lethality assay and antioxidant properties through the 1,1-Diphenyl-2-picrylhydrazyl radical-scavenging assay and phosphomolybdenum method. Results show that almost all of the sponge extracts possess chronic toxicity against the brine shrimp *Artemia salina* Leach as LC_{50} values were observed to be less than 1000 ppm, with the nonpolar and polar extracts of *O. ramsayi* possessing the lowest LC_{50} values of 48.78 and 736.21 ppm, respectively. All sponge extracts exhibited low antioxidant properties with percent radical scavenging activity values from 0 to 22.74% and total antioxidant capacity value ranges of 17.79 - 341.04 Ascorbic Acid Equivalence (AAE) and 41.23 - 445.73 Butylated Hydroxytoluene Equivalence (BHTE).

Key words : Marine sponges, toxicity, total antioxidant, radical-scavenging, bioactivity

INTRODUCTION

Marine sponges thrive in unique physical and biological environments. In such conditions, they tend to produce compounds in response to several physiological processes like attracting prey, eluding predators and surviving competition. In addition, they are sessile and thus not possible for movement during external stress[1,2]. Therefore, they have evolved the ability to synthesize toxic compounds or to obtain them from marine microorganisms as their means of defense[3].

Sponges are considered as the chemical factory in marine environment because of their production of chemically diverse compounds. Other than the chemical diversity, the secondary metabolites also include the presence of covalently bound halogen atoms, mainly chlorine and bromine, presumably due to their availability in seawater[4]. In such, these compounds were reported to possess remarkable bioactivities[5]. Considering that the Philippines, specifically within the coast of Zamboanga in Mindanao is generally known for its rich marine environment, sampling sites for the search of marine sponges as much as the as the availability of the samples is feasible[6].

The brine shrimp lethality assay (BSLT) is considered a useful tool for preliminary assessment of toxicity. It has also been suggested as a test for natural products and as a general bio-assay for any pharmacological activity[7]. Antioxidant activity is an important and fundamental function in life systems. Many other biological functions such as the anti-mutagenic, anticarcinogenic, and anti-aging responses, originate from this property[8]. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) is a stable free radical. Upon accepting hydrogen from a corresponding donor, its solutions lose the characteristic deep purple (λ_{max} 515 - 517 nm) colour. DPPH is very popular for the study of natural antioxidants[9]. The phosphomolybdenum method is utilized for the spectrophotometric quantitation of total antioxidant capacity is based on the reduction of Mo(VI) to Mo(V) in presence of antioxidant compound and subsequent formation of a green phosphate/Mo(V) complex at acidic pH and at higher temperature[10].

The main purpose of this study was to screen the pharmacological potentials of extracts obtained from five (5) marine sponges collected from a coast off Zamboanga Peninsula, Philippines. Specifically, the nonpolar and polar extracts from the five (5) Philippine marine sponges namely *Petrosia* sp., *Oceanapia ramsayi*, *Clathria* sp., *Ancorina cerebrum* and *Haliclona fascigera* through sequential extraction with EtoAc:MeOH and EtOH:H_2O were prepared, respectively. The sponge extracts were subjected to bioactivity studied were in terms of potential cytotoxicity through brine shrimp lethality test (BSLT), antioxidant properties using 1,1- Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and total antioxidant activity by the phosphomolybdenum method.

MATERIALS AND METHODS

Sample Preparation

The marine sponges were collected by local divers within the depth of 7-10 feet from the shores of Brgy. Sinubong in Zamboanga City, Philippines with the geographical coordinates of 6°55'38.3"N 121°57'42.6"E on June 2015. The collected samples were properly labelled, placed in a plastic bag with ice in a cooler and sent immediately to the Bioorganic and Natural Products Chemistry laboratory in Mindanao State University-Iligan Institute of Technology, Iligan City, Philippines for storage in a freezer until work-up. Taxonomical identification of the collected marine sponges was done by Dr. Nicole J. de Voogd from the Biodiversity Naturalis Museum at Leiden, Netherlands and by Dr. Ephrime Metillo of the Biological Sciences Department, College of Science and Mathematics at MSU- IIT. The marine sponges were identified to be as *Petrosia* sp., *Oceanapia ramsayi*, *Clathria* sp., *Ancorina cerebrum* and *Haliclona fascigera*.

Each of the collected marine sponge samples was first washed with filtered and sterile seawater to remove any extraneous materials. After washing, each sponge material were cut in pieces, freeze- dried then soaked with sufficient amounts of 1:1 ratio of ethyl acetate:methanol solution (v/v) for three (3) days. The solutions formed were filtered, and the filtrates were concentrated *in vacuo* to give the nonpolar extract. The sponge residues were subsequently soaked in sufficient amounts of 1:1 ethanol:water (v/v) solution for three (3) days, with the resulting solutions filtered and concentrated *in vacuo* to afford the polar extract.

Brine Shrimp Lethality Test

The method for the cytotoxicity test was based from a study conducted by McLaughlin and Rogers[11] with slight modifications. In this test, 1000-, 500-, 100-, and 10-ppm solutions of the extracts were prepared in three replicates. The prepared test solutions were then subjected to toxicity test against the brine shrimp *A. salina*. The number of dead and alive nauplii were monitored, counted, and recorded after 6 and 24 hours. The results were then processed using of the Reed-Muench method and the acute and chronic lethal concentrations (LC_{50}) were determined[12].

The DPPH Radical Scavenging Method

The protocol of Lee and Shibamoto[13] was followed in this assay. For each of the sponge extracts, a 500-ppm concentration solution was prepared. Three hundred (300) µl of the prepared solutions was put into screw-capped test tubes and added with 3000 µl of methanolic solution of 0.1 mM DPPH (1,1-Diphenyl-2-picrylhydrazyl). The resulting solutions were thoroughly mixed and allowed to stand at room temperature for one hour. The absorbance for each mixture was measured at 517 nm against methanol as a blank. The percent of DPPH decoloration of the samples was calculated according to the formula:

$$\text{Antiradical Activity} = \quad \times 100$$

where A_{sample} is the absorbance of the sponge extract and $A_{control}$ is the absorbance of methanol. The DPPH radical scavenging activities of the extracts were compared with those of known antioxidants ascorbic acid and butylated hydroxytoluene (BHT).

Total Antioxidant Assay using Phosphomolybdenum Method

The protocol of Prieto et al[14] was employed for this assay. All sponge extracts were first prepared by dissolving in methanol to form a 200 µg/mL solution. An aliquot volume of 0.3 mL from the resulting samples were then added with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in test tubes. The tubes were covered, mixed vigorously and incubated at 95°C for 90 minutes. After cooling to room temperature, the absorbances of the solutions were measured at 695 nm. Triplicate analysis were performed for each sponge extract. Methanol was used as a blank. The total antioxidant capacity of the sponge extract was expressed as ascorbic acid equivalence (AAE) and butylated hydroxytoulene equivalence (BHTE) based on the linear calibration curves of the standards used.

RESULTS

Brine Shrimp Lethality Assay

The toxicities of the different sponge extracts towards *A. salina*, expressed as LC_{50} values, are presented in Table 1.

DPPH Radical Scavenging Activity

The results in the determination of the radical scavenging activity of the sponge extracts are summarized in Table 2.

Total Antioxidant Assay using Phosphomolybdenum Method

The total antioxidant capacities of the marine sponge extracts expressed as Ascorbic Acid Equivalence (AAE) and Butylated Hydroxytoluene Equivalence (BHTE) are shown in Table 3.

DISCUSSION

The results show that the nonpolar and polar extracts of the marine sponges *Petrosia* sp., *Clathria sp*., *A. cerebrum* and *H. fascigera* did not exhibit acute toxicity (after 6h- exposure) towards the brine shrimp *A. salina*. However, after exposure for 24 hours, almost all crude extracts exhibited chronic toxicity except that of the nonpolar extract of *A. cerebrum*. An LC_{50} value of ≤ 1000 µg/mL implies that an extract possesses toxicological properties[15]. The nonpolar extract of *O. ramsayi* possessed the lowest LC_{50} value among the nonpolar extracts studied after 6h- and 24h- exposure periods. Meanwhile, among the polar extracts, those of *O. ramsayi* and *Petrosia* sp. exhibited the lowest chronic and acute LC_{50} values. The sponge extract with the lowest LC_{50} values can be considered to have the most toxicological property among the extracts tested. In general, the *O. ramsayi* extracts (both nonpolar and polar) possess the most promising bioactivity in terms of potential antitumor properties.

Results as shown in Table 2 demonstrate the radical scavenging activities of the different crude sponge extracts using

Table 1: Toxicity of the marine sponge extracts against *A. salina.*

Sponge	LC_{50}, ppm			
	Nonpolar extract[a]		Polar extract[a]	
	Acute	Chronic	Acute	Chronic
Petrosia sp.	>1000.00	774.46	>1000.00	69.18
Oceanapia ramsayi	251.19	48.87	736.21	100.00
Clathria sp.	>1000.00	774.46	>1000.00	663.74
Ancorina cerebrum	>1000.00	>1000.00	>1000.00	359.75
Haliclona fascigera	>1000.00	226.99	>1000.00	903.65

[a] - Nonpolar = EtoAc:MeOH ; Polar = EtOH:H_2O

Table 2: DPPH radical scavenging activity of the marine sponge extracts.

Sponge Extract		Average Percent Inhibition, %				EC_{50}, ppm
		500 ppm	100 ppm	50 ppm	25 ppm	
Petrosia sp.	Nonpolar	0.31	0.55	0.52	0.00	>500
	Polar	3.84	0.48	0.00	0.00	>500
Oceanapia ramsayi	Nonpolar	22.74	4.80	3.93	2.07	>500
	Polar	20.30	2.78	2.78	0.00	>500
Clathria sp.	Nonpolar	3.62	3.00	2.04	3.86	>500
	Polar	0.41	0.00	0.00	0.00	>500
Ancorina cerebrum	Nonpolar	5.56	4.69	4.04	3.83	>500
	Polar	0.00	0.00	0.00	0.00	>500
Haliclona fascigera	Nonpolar	1.95	0.65	0.00	0.00	>500
	Polar	0.00	0.00	0.00	0.00	>500
BHT[b]		92.82	69.23	41.62	24.63	108.22
Ascorbic acid[b]		97.06	96.48	75.23	49.53	61.86

[a] - Nonpolar = EtoAc: MeOH, Polar = EtOH: H_2O

[b] - Reference standards used.

Table 3: The Total antioxidant capacity of the marine sponge extracts.

Sponge extract*		Total antioxidant capacity	
		BHTE	AAE
Petrosia sp.	Nonpolar	445.73	341.04
	Polar	41.23	17.79
Oceanapia ramsayi	Nonpolar	97.99	63.14
	Polar	91.23	57.74
Clathria sp.	Nonpolar	141.68	98.06
	Polar	79.07	48.02
Ancorina cerebrum	Nonpolar	181.32	129.74
	Polar	77.27	46.58
Haliclona fascigera	Nonpolar	328.17	247.09
	Polar	44.38	20.31

* - Nonpolar = EtoAc: MeOH, Polar = EtOH: H_2O

the DPPH method. In general, all of the sponge crude extracts bear little to no activity as all of the samples yielded percent activity values ranging from 0 - 22.74% within the gradient concentrations used, which was most likely due to the effect of the combination of several phytochemicals present in the extract. Nonetheless, it can also be noted that among the sponge extracts tested, the nonpolar extracts relatively possessed scavenging activity against DPPH. The polar extracts for *H. fascigera* meanwhile did not exhibit any effect at all within the concentration range used. It can be then hypothesized that a higher concentration (>500 ppm) is needed for the said sample to exhibit an activity. Though some exhibited radical scavenging activities, all of the extracts possessed EC_{50} values greater than 500 ppm.

Based on the results, the antioxidant capacity of the nonpolar extracts of all marine sponges were higher compared to those of the polar extracts. Furthermore, the total antioxidant capacity of the nonpolar extracts expressed as AAE and BHTE in increasing order is *O. ramsayi* < *Clathria* sp. < *A. cerebrum* < *H. fascigera* < *Petrosia* sp. while the order for the polar extracts is *Petrosia* sp. < *H. fascigera* < *A. cerebrum* < *Clathria* sp < *O. ramsayi*.

CONCLUSION

Results indicate that except for the nonpolar extract of *Ancorina cerebrum* , almost all of the marine sponge extracts contain toxicological properties at chronic exposure. Moreover, both the nonpolar and polar extracts of *O. ramsayi* should further be studied as they hold the most promising properties among the

samples tested in terms of toxicological property and antioxidant activities.

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