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Cytotoxicity to *Artemia salina L.* of Marine Sponge Extracts from Surigao del Norte, Philippines

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ABSTRACT
The cytotoxic activities of the hexane, n-butanol, and water crude extracts of the marine sponges, Rhabdastrella globostellata, Callyspongia sp., Callyspongia aerizusa, Carteriospongia sp., and Cinachyrella sp. collected from the coast of Surigao del Norte, Philippines were determined using the brine shrimp lethality assay. Hexane and n-butanol crude extracts exhibited medium to high toxicity after 24 hours of exposure, while only the water crude extract of Callyspongia sp., manifested a high toxicity after 24 hours. CalspW has an LC_{50} (chronic, 24 hrs) value of 2.51 ppm, which is comparable to the positive control podophyllotoxin which has LC_{50} value of 2.4 ppm.
Keywords: *cytotoxicity, brine shrimp lethality assay, Philippine marine sponges, bioactivity, secondary metabolites.*

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INTRODUCTION

The study of secondary metabolites has historically proven to be of great benefit in drug discovery, providing an immense source of structurally novel bioactive molecules, many of which have become life-saving drugs or biomedical tools [1-3]. The marine environment is a rich source of both biological and chemical diversity of plants, animals, and microorganisms. Due to their adaptations to this unique habitat, marine organisms produce a wide variety of secondary metabolites unlike those found in terrestrial species. The kind of secondary metabolites they produce is related to the role of chemical defense played by these constituents [4]. Marine sponges are considered the richest sources of new marine natural products (NMNP), contributing to nearly 30% of all natural products discovered so far [5].

Brine Shrimp Lethality Assay (BSLA) is considered a useful method for the preliminary assessment of toxicity [6] of natural products. Numerous papers have been published reporting the effective role of BSLA as a tool for toxicity screening of marine sponges. It is not only rapid and convenient, but inexpensive as well. This method has led to isolation of important cytotoxic compounds that have potential pharmacological properties. The isolation of cytotoxic polyacetylenes from *Petrosia* sp. was guided by BSLA [7]. The crude extracts of marine sponges, *Pachastrella* sp. and *Jaspis* sp. collected from the south sea of Korea, exhibited significant brine shrimp cytotoxicity. Guided by this bioassay, fractionation and purification gave Pectenotoxin II and Psammaplin A, which were cytotoxic to human cancer cell lines [8]. In another study, BSLA was used as substitute for 9KB and 9PS cytotoxicities of two bioactive derivatives of avarol, diacetyl-avarol and 6'-hydroxy, 5'-acetyl-avarol [9].

This study was conducted to evaluate the potential cytotoxicity, using the Brine Shrimp Lethality Assay of the hexane, n-butanol, and water crude extracts of some marine sponges collected from the coast of Surigao del Norte, Philippines with the aim of discovering new bioactives that can be further studied for possible new drug leads.

MATERIALS AND METHODS

Collection of sponges

The marine sponges, *Rhabdastrella globostellata* (Rg), *Callyspongia* sp. (Calsp), *Callyspongia aerizusa* (Ca), *Carteriospongia* sp. (Carsp), and *Cinachyrella* sp. (Cisp), were collected off the coast of Barangay Lipata, Surigao City and the Municipality of General Luna, Siargao Islands, Surigao del Norte by hand scuba at a depth of 5-10 meters in April, 2014. The samples were immersed in seawater in separate plastic

containers and transported to the laboratory in a bucket of ice. Sand particles, trapped crustaceans, coral growth and other foreign materials were removed from the sponge samples. They were then chopped into small pieces and stored in the freezer. Prior to any further analysis or procedure, the marine sponge samples were freeze-dried for one to two days. Voucher specimens were prepared and taxonomically identified by Dr. Nicole J. de Voogd of the Naturalis Biodiversity Center, The Netherlands.

Solvent extraction and partitioning

Freeze-dried marine sponge samples were separately soaked in methanol-ethyl acetate (50:50) solvent mixture for at least 48 hours. The resulting crude extracts were concentrated *in vacuo* and then sequentially partitioned using hexane and 20% aqueous methanol to obtain the hexane and the 20% aqueous methanol crude extracts. The latter was further partitioned using n-butanol and water (50:50) to obtain n-butanol and water crude extracts. The hexane crude extracts (RgH, CalspH, CaH, CarspH, and CispH), the n-butanol crude extracts (RgB, CalspB, CaB, and CispB), and the water crude extracts (RgW, CalspW, CaW, CarspW, and CispW) were concentrated *in vacuo* and were subjected to cytotoxic activities.

Brine shrimp lethality assay (BSLA)

Brine shrimp (*Artemia salina*) eggs/cysts were allowed to hatch in sterile seawater (after incubation for 24 hours under illumination) at room temperature [10]. Hatched nauplii were collected using Pasteur pipet. Ten nauplii were delivered to each 5-mL crude extract (dissolved in sterile seawater) at concentrations of 10, 100, 500, and 1000 ppm. The test for each concentration was done in triplicates. The number of the dead and alive nauplii was recorded after 6 and 24 hours. The positive standard used was podophyllotoxin. Nauplii were considered dead if no internal or external movement was observed for 30 seconds. The percent mortality of the brine shrimps and the LC_{50} (Median Lethal Concentration) values of each crude extract were then calculated. This was done by plotting the percent mortality against the logarithm of the concentration of the crude extract [10] followed by Probit regression analysis.

RESULTS

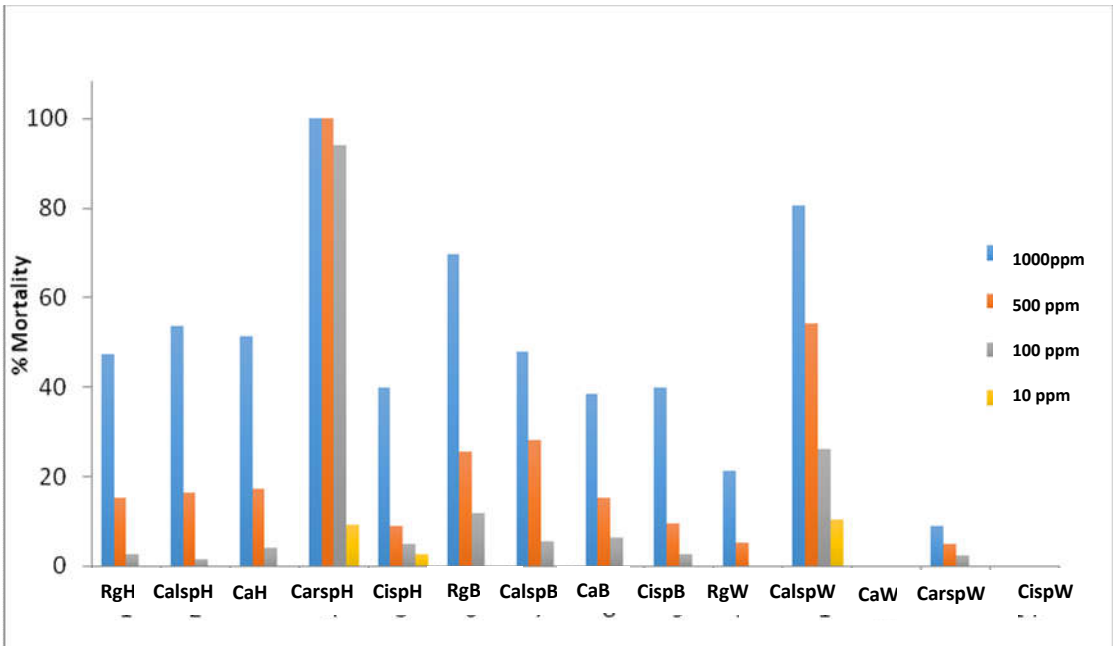

Figure 1: Percent mortality of the crude hexane, n-butanol, and water extracts of *Rhabdastrella globostellata* (Rg), *Callyspongia* sp.(Calsp), *Callyspongia aerizusa* (Ca), *Carteriospongia* sp. (Carsp), and *Cinachyrella* sp. (Cisp) after 6 hours

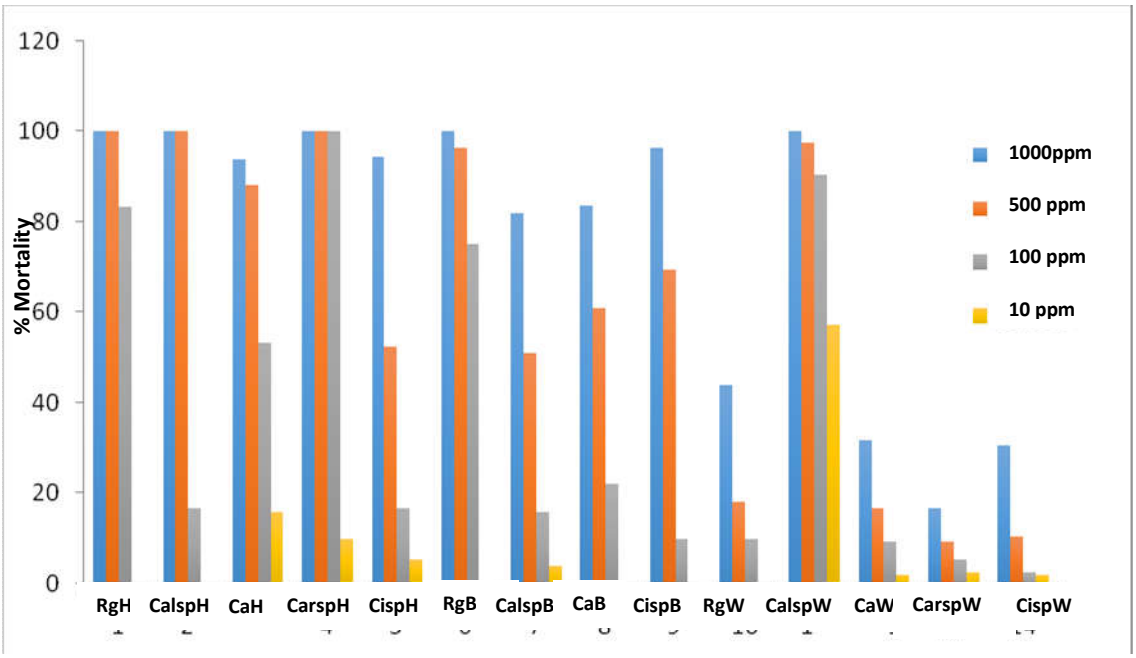

Figure 2: Percent mortality of the crude hexane, n-butanol, and water extracts of *Rhabdastrella globostellata* (Rg), *Callyspongia* sp.(Calsp), *Callyspongia aerizusa* (Ca), *Carteriospongia* sp. (Carsp), and *Cinachyrella* sp. (Cisp) after 24 hours

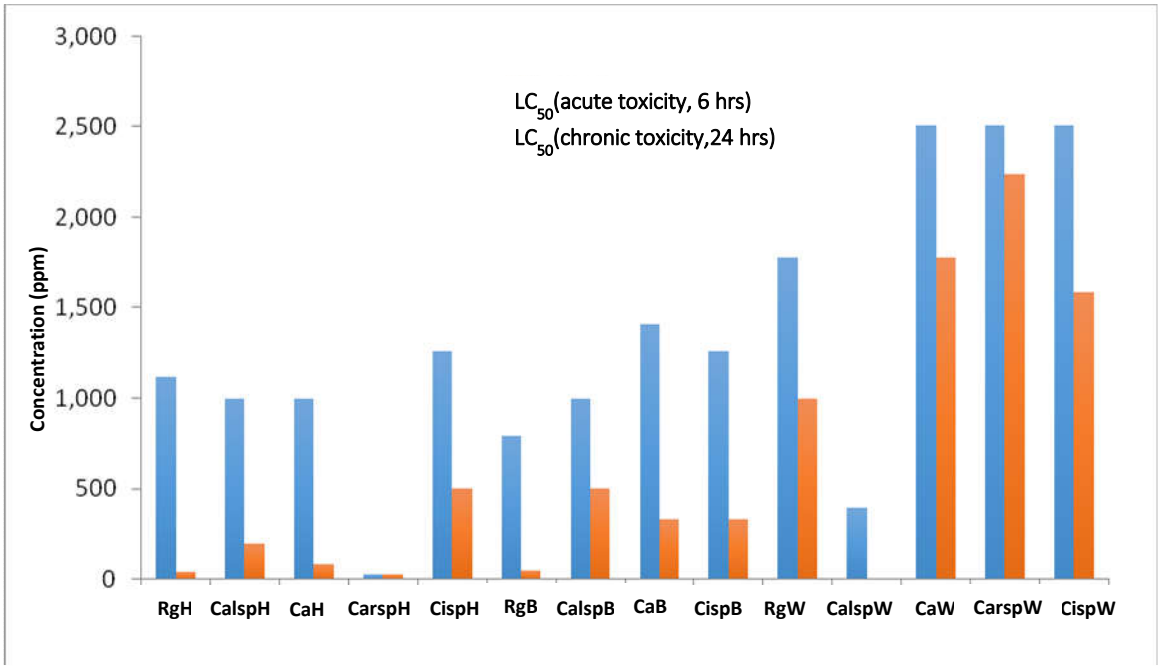

Figure 3: Toxicity of the crude hexane, n-butanol, and water extracts of *Rhabdastrella globostellata* (Rg), *Callyspongia* sp.(Calsp), *Callyspongia aerizusa* (Ca), *Carteriospongia* sp. (Carsp), and *Cinachyrella* sp. (Cisp) after 6 and 24 hours

Table 1: Toxicity summary of the crude extracts of the various Philippine marine sponges

Sponge extract	*Acute toxicity (ppm)*	*Chronic toxicity (ppm)*	*Toxicity description*
RgH	1,122.02	42.17	Nontoxic, acute Highly toxic, chronic
CalspH	1000	199.53	Low toxic ,acute Medium toxic, chronic
CaH	1000	84.14	Low toxic, acute Highly toxic, chronic
CarspH	28.18	26.61	Highly toxic
CispH	1,258.93	501.1872	Nontoxic,acute Low toxic, chronic
RgB	794.33	50.12	Low toxic, acute Highly toxic, chronic
CalspB	1000	501.19	Low toxic
CaB	1,412.54	334.97	Nontoxic, acute Medium toxic, chronic
CispB	1,258.93	334.97	Nontoxic, acute Medium toxic, chronic
RgW	1,778.28	1000	Nontoxic, acute Low toxic, chronic
CalspW	398.11	2.51	Medium toxic, acute Highly toxic, chronic
CaW	2,511.89	1,778.28	Nontoxic
CarspW	2,511.89	2,238.72	Nontoxic
CispW	2,511.89	1,584.89	Nontoxic

DISCUSSION

The results indicate that the crude extracts of the marine sponge samples were toxic to *A. salina* in dose-dependent manner. Of all the marine sponge crude extracts tested, only Cars pH at 500 and 1000 ppm concentrations exhibited 100% brine shrimp mortality after 6 hours (Figure 1). Other hexane extracts that gave a mortality of > 50% are CalspH (53.63%) and CaH (51.28%). Among the n-butanol crude extracts, only RgB (69.77%) gave a significant result at 1000 ppm. The 500 and 1000 ppm of CalspW, on the other hand, resulted to brine shrimp mortality of 54.24% and 80.65%, respectively. It can be clearly seen in Figure 2 that the % mortality of brine shrimp increased after 24 hours of exposure to the different crude extracts. CarspH still topped the list with 100% mortality in its 1000, 500, and 100 ppm hexane crude extract concentrations. Moreover, it is important to note that among the water crude extracts, only CalspW gave a mortality result of greater than 50%. Based on the brine shrimp percent mortality results, most of the bioactive components are in the hexane crude extracts of the marine sponge samples. Hence, most bioactive compounds from these marine sponges are non polar. Percent mortality results are given more significance when it is used to calculate the LC_{50} values to describe the degree of toxicity of the crude extracts. According to Meyer's toxicity index, LC_{50} < 1000 µg/mL (ppm) is toxic, while LC_{50} >1000 µg/mL is nontoxic. Moreover, a more detailed criterion is given by Clarkson as follows: LC_{50} >1000 µg/mL is nontoxic, LC_{50} 500-1000 µg/mL is low toxic, LC_{50} 100-500 µg/mL is medium toxic, and LC_{50} 0-100 µg/mL is highly toxic [11]. The acute and chronic toxicities of the marine sponge crude extracts are presented in Figure 3. After 6 hours (acute toxicity), most of the crude extracts are classified as nontoxic except CarspH (28.18 ppm), CalspW (398.11ppm), and RgB (794.33 ppm), which are considered as highly toxic, medium toxic, and low toxic, respectively. Most of the crude extracts, however, showed significant toxicities after 24 hours (chronic toxicity) ranging from low to highly toxic. Leading among them is CalspW (2.51 ppm), which is highly toxic and is comparable to podophyllotoxin which has LC_{50} value of 2.4 ppm [10]. From the summarized data in Table 1, a generalization can be drawn from this BSLA study. All hexane and n-butanol crude extracts are toxic after 24 hours of exposure and that the water extracts are nontoxic with a remarkable exception of CalspW which exhibited a medium to high toxicity activities. A study on the bioactivity of extracts of *Spongosorites halichondrioides* also concluded that its hexane and butanol extracts showed high toxicity [12]. In a study published in 2010, seven new cytotoxic cyclic peptides were isolated from the ethanol fraction of the marine sponge *Callyspongia aerizusa* collected from Indonesia [13]. Moreover, from the organic extract of *Callyspongia* species, new cytotoxic C_{22}-polyacetylenic alcohols were isolated [14].

CONCLUSION

The Brine Shrimp Lethality Assay of the hexane, n-butanol, and water crude extracts of the marine sponges, *Rhabdastrella globostellata*, *Callyspongia* sp., *Callyspongia aerizusa*, *Carteriospongia* sp., and *Cinachyrella* sp., from the Coast of Surigao del Norte, Philippines has produced a solid background with regards to the bioactivity of these marine sponges. The data obtained from this study were used as basis for a more detailed and thorough ongoing investigation of the bioactive components of these marine sponges.

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