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Evaluation of potential cytotoxicity of the extracts from soft corals collected from the Philippine Sea of Mindanao: *Sarcophyton glaucum, Lobophytum pauciflorum, Sinularia flexibilis* and *Lobophytum crassum*

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Abstract

Soft corals have been shown to possess a rich variety of marine secondary metabolites and are considered an extremely diverse group of marine organisms. This study explored the potential cytotoxicity of soft corals collected from the Philippine Sea of Mindanao namely *Sarcophyton glaucum* (Sg), *Lobophytum pauciflorum* (Lp), *Sinularia flexibilis* (Sf) and *Lobophytum crassum* (Lc). Polar (P) and nonpolar (NP) extracts of the soft corals were prepared by sequential extraction of the freezed-dried samples with 50:50 ethanol-water and 50:50 ethylacetate-methanol, respectively. The resulting extracts were tested for their cytotoxic activity using the brine shrimp *Artemia salina* lethality test (BSLT). Results show that amongst the five soft corals, The polar extract of *S. glaucum* (SgP) exhibited interesting bioactivity with acute and chronic LC_{50} values of 324.00 ppm and 35.50 ppm, respectively. The results indicate that the soft coral *S. glaucum* may contain potential antitumor or pesticidal components and is thus worthy of further investigation.

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Introduction

The Philippine archipelago is located in the Pacific Ocean. It is composed of more than 7,100 islands with a coastline stretching over 36,289 km. The country has one the most populous and diverse aquatic ecosystems in the world. The marine waters of the Philippines contain significant marine habitats – coral reefs, seagrass beds, mangrove forests and the deep seas. These habitats are estimated to host at least 2,000 species of fish, 5,000 species of clams and other mollusks and crustaceans, 22 species of whales and dolphins, more than 900 species of seaweed, and more than 400 species of corals. Because of this rich diversity in marine life, some experts have acknowledged the Philippines as the global center of marine biodiversity (Ocean Defender, 2013).

In terms of coral species, the Philippines has the largest recorded number. The Philippine corals, consisting of at least 430 species in more than 70 genera, represent about 50% of the coral species in the world. In 2005, scientist using Global Information System (GIS) assessed the distribution of marine life in the Indo-Malay-Philippine Archipelago (the area of highest marine diversity) and discovered that the Philippines has higher concentration of marine species per unit area than anywhere in Indonesia (Sinha, 2012).

The biotechnological potential of soft corals has attracted the attention of researchers because of their ability to produce powerful toxins and venoms (Turk *et al.*, 2009). Soft corals (Octocorallia, Alcyonacea) are known to contain a rich variety of marine secondary metabolites and are considered an extremely diverse group of marine organisms. The biologically active substances from corals have not only great significance in chemical geology but also express various biological activities such as antitumor, antibacterial, antiviral, and antifungal (Coll, 1992).

This study investigated the potential cytotoxicity of the soft corals *Sarcophyton glaucum*, *Lobophytum pauciflorum*, *Sinularia flexibilis* and *Lobophytum crassum*collected from the Philippine Sea in Mindanao.

Materials and methods

Collection and identification of soft corals

Five species of marine soft corals (Fig. 1) were collected from Carmen, Agusan del Norte, Philippines by self-contained underwater breathing apparatus (SCUBA) diving as well as snorkeling last November 2014. The sampling site has the coordinates 9.086^0N and 125.219^0E in the Philippines. Two colonies from each species were sampled, one for the purpose of extraction and bioactivity testing, and the other for identification.

The samples were collected by means of scalpel or a pair of scissors and placed in zip lock plastic bags. The collected soft coral specimens for systematic study were then stored in sterile containers and transported to the Natural Products and Bioorganic Research Laboratory of the Department of Chemistry and the Department of Science and Technology-Philippine Council for Health Research and Development (DOST-PCHRD) Tuklas Lunas Development Center at Mindanao State University- Iligan Institute of Technology (MSU-IIT), Iligan City, Philippines.

The collected soft coral species were identified by one of the authors (EB Metillo). For every sample, a method adopted from Benayahu and van Ofwege (2011) was applied, where small squares of approximately 1 cm were cut from the colony with a scalpel and then mounted on a glass slide with 2 drops of bleach. Once the bubbles have ceased, the sclerites were spread out by stirring, and the specimen was examined under a microscope equipped with a camera and a stage micrometer. When the sclerites were very dark and difficult to distinguish from the tissue remains, clearing was carried out using a mixture of phenol-xylene.

Preparation of soft coral crude extracts

The samples were frozen immediately after collection. The frozen samples of soft corals were left to defrost, broken into small pieces and extracted at room

temperature. Polar (P) and nonpolar (NP) extracts of the soft corals were prepared by sequential extraction of the freeze-dried samples with 50:50 ethanol-water and 50:50 ethylacetate-methanol, respectively. The extracts were filtered through Whatman no. 1 filter paper and dried at 40 ^{0}C using a rotary evaporator. The resulting extracts were tested for their cytotoxicity.

Toxicity Assay: Brine shrimp lethality test (BSLT)

The cytotoxicity of the soft coral extracts was measured following the protocol of Meyer *et al.*, (1984). The extracts were subjected to BSLT using four concentrations: 1000-, 500-, 100-, and 10-ppm. In each concentration, solvent was allowed to evaporate and further dried under nitrogen gas. Dimethyl sulfoxide (DMSO) was added to the solution to enhance solubility of the extracts. Ten brine shrimps were placed to each test tube, and diluted with sterile, filtered seawater to make 5000 µl so as to obtain the required concentrations. The test tubes were kept under illumination. Control experiments using DMSO was performed for the four concentrations.

The test was done in triplicates. The number of alive A. *salina* was then counted and recorded for each replicate after 6 and 24 hours. As a measure of the extract's toxicity, the lethal concentration for 50% mortality after 6 and 24 hours of exposure corresponding to the acute and chronic LC_{50} respectively, and 95% confidence intervals were determined using the Reed-Muench, as the measure of toxicity of the extract. LC_{50} values greater than 1000 ppm for extracts were considered inactive (Meyer *et al.*, 1982; Costa *et al.*, 2012).

Results and discussion

The effect of various concentrations of the samples on brine shrimp larvae after 6 hours (acute LC_{50}) and 24 hours (chronic LC_{50}) are summarized in Figs 2-5 and Table 1.

Table 1. LC_{50} Values of the Soft Coral Extracts Against the Brine Shrimp *A. salina.*

Soft Coral	Extract	6 hours Exposure (Acute LC_{50})	24 hours Exposure (Chronic LC_{50})
Sarcophyton glaucum	SgNP	>1000.00 ppm	1000.00 ppm
	SgP	324.00 ppm	35.50 ppm
Lobophytumpauciflorum	LpNP	>1000.00 ppm	406.00 ppm
	LpP	501.00 ppm	158.00 ppm
Sinularia flexibilis	SfNP	>1000.00 ppm	631.00 ppm
	SfP	>1000.00 ppm	120.00 ppm
Lobophytum crassum	LcNP	>1000.00 ppm	>1000.00 ppm
	LcP	>1000.00 ppm	447.00 ppm

As shown by the mortality rates of the brine shrimp after 6-hour exposure to the soft coral extracts, (Figs 2 and 3), the soft coral extracts exhibited considerable activities specifically the polar extracts of *S. glaucum* and *L. pauciflorum* with brine shrimp mortalities of 98.1% and 90.7%, respectively, at 1000-ppm concentration.

The cytotoxic activity of the polar extracts at 500-ppm are also significant. However, the activities of the nonpolar soft coral extracts are not considerable.

After 24-hour exposure (Figs 4 and 5), the polar extracts showed commendable mortality effects on the brine shrimp with 100%for *S. glaucum, L. pauciflorum,* and *S. flexibilis*. Even at 500-ppm, the activity shown was still great. Meanwhile for the nonpolar extracts at 1000-ppm, LpNP indicated the highest mortality with 89.1% followed by SfNP (70.2%), SgNP (69.2%), and lastly the LcNP (33.3%).

At 100- and 10-ppm, almost all of the extracts were considered nontoxic against the brine shrimps.

Fig. 1. Photographs of the soft corals collected from the waters off Agusan del Norte, Philippines. 1: *Sarcophytonglaucum* (Sg). 2: *Lobophytumpauciflorum* (Lp). 3: *Sinulariaflexibilis* (Sf). 4: *Lobophytumcrassum* (Lc).

The brine shrimp mortality of the nonpolar extracts signified moderate cytotoxic activity when compared to that of the polar extracts.

According to Meyer *et al.*, (1982) and Costa *et al.*, (2012), LC_{50} values greater than 1000 ppm can be considered inactive. Therefore, as shown in Table 1, the nonpolar and polar extracts of SfNP, SfP, LcNP andLcP cannot be considered active for it took higher concentrations (>1000-ppm) for these extracts to kill 50% of the brine shrimps.Among the soft coral extracts, only two exhibited significant acute LC_{50} values; SgP (324.00 ppm) and LpP (501.00 ppm).Moreover, based on the chronic LC_{50} values, seven out of the eight extracts are considered active. In summary, the decreasing toxicity of the extracts to the brine shrimps after 6-hour exposure is, SgP (324.00 ppm) >LpP (501.00 ppm). For chronic toxicity, the order is, SgP (35.50 ppm) > SfP (120.00 ppm) > LpP (158.00 ppm) >LpNP (406.00 ppm) >LcP (447.00 ppm) > SfNP (631.00 ppm) >SgNP (1000.00 ppm).Polar extracts of *S. glaucum* and *L. pauciflorum* gave consistent appreciable values both in acute and chronic LC_{50}.According to Ullah *et al.* (2013),crude extract or fractions resulting in LC_{50} values less than 100 µg/mL (ppm) were considered significantly active and indicatedthe presence of potent bioactive compounds for further investigation.

Therefore, among the two notable marine samples, *S. glaucum* is considered to be the most active. Momtaz (2016) reported that polar extracts (ethanol, chloroform, and aqueous) of*S. glaucum* collected from the Egyptian Red Sea coast, Gulf of Suez,Al- Ain Elsokhna, indicated insecticidal activities against adults of *Sitophilus oryzae*a Rice weevils. Liang *et al.*

(2013) found that two hundred and five terpenes had been isolated from the genus *Sarcophyton* including eleven sesquiterpenes, 165 diterpenes, 29 biscembranoids, some of which had novel skeletons. They exhibited various biological features, such as anti-feedant, anti-inflammatory, antiviral, and antifouling activities. These and other researches confirm the potential of *S. glaucum* as a source of antitumor or pest control agents.

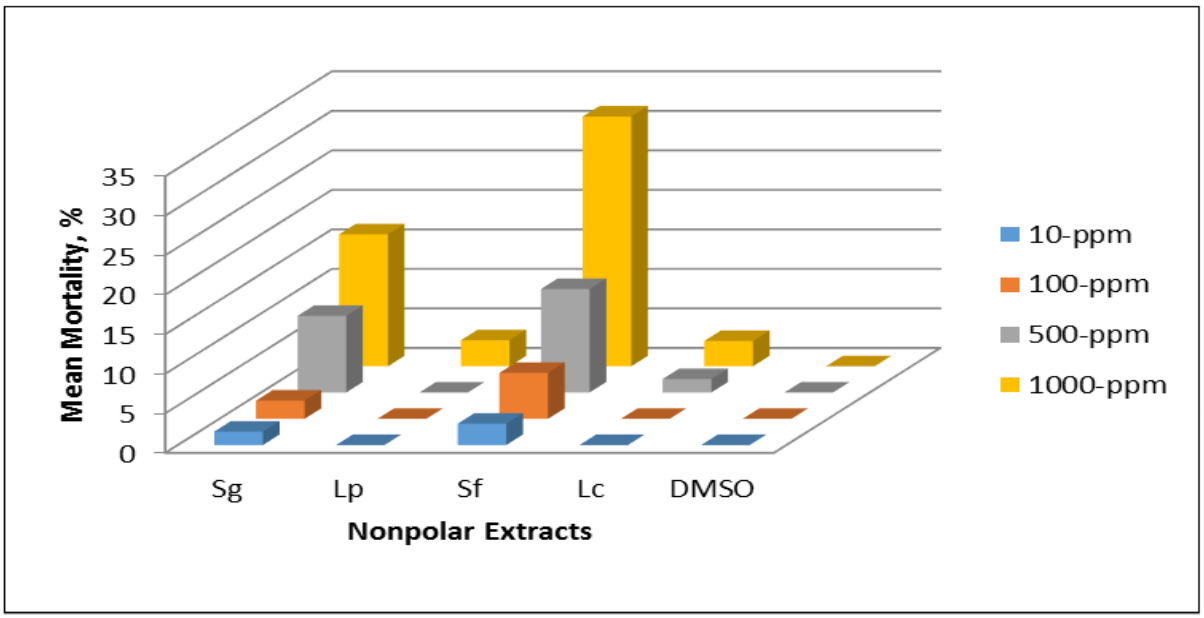

Fig. 2. Mortality of the Brine Shrimps after 6-hour Exposure to Various Concentrations of the Soft Coral Nonpolar Extracts.

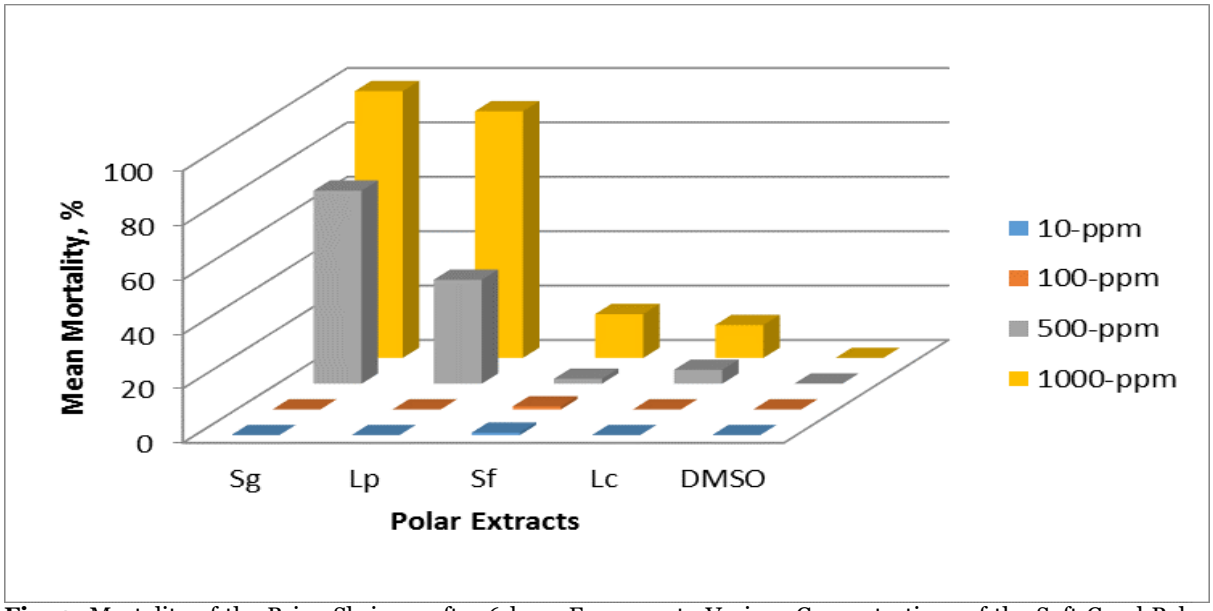

Fig. 3. Mortality of the Brine Shrimps after 6-hour Exposure to Various Concentrations of the Soft Coral Polar Extracts.

The LC_{50} of the nonpolar extracts of the marine samples signified moderate cytotoxic activity when compared to its polar extracts. The results of the study suggested that *L. crassum* is the least toxic; however, Peng *et al.* (2018) found that its nonpolar extract contained a distinctive chemical profile which is toxic to leukemia cell lines. The marine sample was collected in Taiwan. Somehow, specimens collected in

different geographical locations may have different secondary metabolite profiles due to factors such as salinity, temperature, light intensity, pollution levels, as well as predation pressures, and also the nature of bacterial symbionts may play a part in the occurrence and concentrations of specific secondary metabolites (Gallimore, 2017).

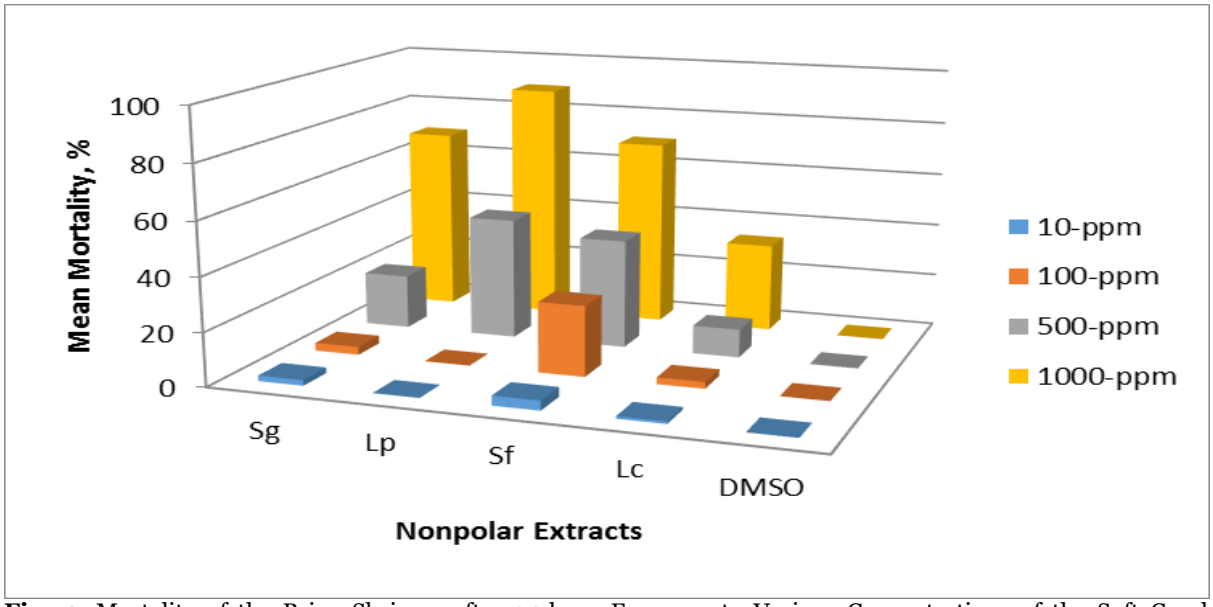

Fig. 4. Mortality of the Brine Shrimps after 24-hour Exposure to Various Concentrations of the Soft Coral Nonpolar Extracts.

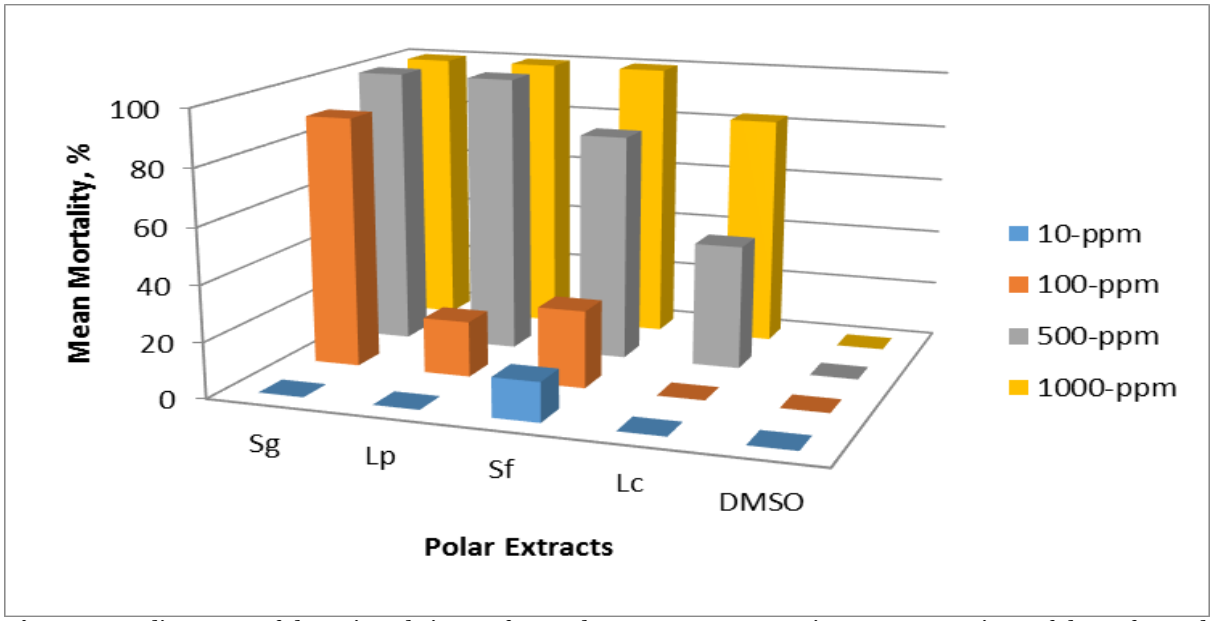

Fig. 5. Mortality Rates of the Brine Shrimps after 24-hour Exposure to Various Concentrations of the Soft Coral Crude Polar Extracts.

Conclusion

In conclusion, the study reports that among the four soft corals, the polar extract of *S. glaucum* gave a favorable activity in this assay with acute and chronic LC_{50} values of 324.00 ppm and 35.50 ppm, respectively, against the brine shrimps. Several studies have shown that the brine shrimp assay has been an excellent method for preliminary investigations of toxicity, which could also have positive correlation with antitumor, trypanocidal and

pesticidal activities. Therefore, the polar extract of *S. glaucum* is worthy for further investigation. In general, the nonpolar extracts of *S. glaucum*, *L. pauciflorum*, *S. flexibilis* and *L. Crassum* signified moderate cytotoxic activities against the brine shrimp *A. salina* when compared to those of the polar extracts. The correlation between concentration and exposure time was significant. The mortality of brine shrimps increased with increasing concentration level and time of exposure from 6 hours to 24 hours.

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