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Toxicity of crude extracts from soft corals (Anthozoa, Alcyonacea) collected at varying wave exposure sites in Talisayan, Northern Mindanao, Philippines

Miguel E. Ayllon[1], Homero Earl S. Elpa[1], Ephrime B. Metillo[1*], Mylene M. Uy[2]

[1]Department of Marine Sciences, [2]Department of Chemistry, College of Science and Mathematics, Mindanao State University-Iligan Institute of Technology, Andres Bonifacio Ave., Iligan City 9200, The Philippines

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Abstract

Octocorallian soft corals are known for their ecological and pharmacological importance, but the bioactivity of crude extracts from Philippine species is less studied. This study determined the toxicity of crude extracts from species collected at wave-exposed and less wave-exposed nearshore sites at Talisayan, Southern Philippines. SCUBA diving was used to collect soft coral specimens at 3-5 m depth. *In situ* colony image and sclerites from each specimen were used to identify soft corals to the species level. The brine shrimp lethality assay was carried out to determine the toxicity of methanol extracts at 10, 100 and 1000 ppm concentrations. This study recorded three species (*Sarcophyton crassocaule*, *Isis hippuris*, *Cespitularia stolonifera*) from the less wave-exposed site, and eight from the wave-exposed area (*Sinularia* sp., *Si. flexibilis, Lobophytum crassum,* and *L. durum*, *Si. polydactyla*, *S. ehrenbergi*, *S. glaucum*, and *Dendronephthya hemprichii*). Toxicity of extracts against brine shrimps appears not to be due to the wave exposure of site, but more associated with the species and perhaps intensity of fish predation on soft corals. Extracts from *S. crassocaule*, *L. durum*, *Isis hippuris*, *L. crassum*, *S. ehrenbergi*, *C. stolonifera*, and *D. hemprichii* were highly toxic with LC_{50} values (in ppm) of 0.40, 3.45, 3.67, 4.25, 4.25, 26.92, and 28.18, respectively. *Si. flexibilis* was considered toxic with an LC_{50} value of 144.54 ppm. The unidentified species *Sinularia* sp. was found to be non-toxic. This study reveals that some soft coral taxa from Talisayan neritic waters are potentially good sources of bioactive compounds for drug discovery.

* **Corresponding Author:** Ephrime B. Metillo ✉ ephrime.metillo@g.msuiit.edu.ph

Introduction

Along with scleractinian or hard corals, soft corals are known for its spectacular biodiversity. Soft corals are indispensable component of coral reefs that provides local livelihood, natural seawalls that protect people from tidal waves and storm surge, and nursery areas of many marine species (Johnson and Marshall, 2007; Nakamura *et al.*, 2011). However, coral reefs worldwide are degraded and overexploited partly because the socio-economic value of coral reef biodiversity is less understood and underappreciated (Reaka-Kudla, 1997). Unraveling the pharmacological value of different species is a key to the sustainable conservation of coral reefs (Goh *et al.*, 2009).

Aside from their ecological importance, soft corals can also be a potential source of drugs. For instance, the soft coral *Lobophytum* sp. from Mindoro, Philippines were found to produce bioactive lobane diterpenes (Edrada *et al.*, 1998). Soft corals produce a variety of secondary metabolites such as diterpenoids, sesquiterpenoids, steroids and cembranoid derivatives (Limna Mol *et al.*, 2010; Duh *et al.*, 2012; Sung *et al.*, 2013; Mehdinia *et al.*, 2014; Phan and Vairappan, 2015). Additionally, they are also known to have antioxidant (Shahbudin *et al.*, 2011), antifouling (Limna Mol *et al.*, 2010), anti-cancer (Chou *et al.*, 2008) and anti-inflammatory (Duh *et al.*, 2010; Putra and Murniasih, 2015) properties that make them very ideal for drug development.

Soft corals are known to have cytotoxic properties that have sparked interest of many researches in biochemical science and drug industries (Goh *et al.*, 2009; Putra *et al.*, 2016). Preliminary toxicity testing is commonly done to test if an organism contains bioactive compounds that can be useful in future studies. Brine shrimp lethality assay (BSLA) is widely used by many researchers to predict cytotoxic activity of many compounds (Luyao *et al.*, 2019).

The brine shrimp, *Artemia* sp., is an aquatic, filter-feeding crustacean which is commonly used as model organism in many toxicity studies due to its low cost and ease of use (Murcia *et al.*, 2019).

Soft coral species are known to synthesize bioactive compounds or secondary metabolites in response to competitors, predators, fouling organisms and parasites, but toxicity of species vary geographically (Coll *et al.*, 1983). For example, toxicity of sponges can vary with habitats with different strength of water movement (Wilkinson and Evans 1989). Hence, the objectives of this study are to collect and identify species of soft corals from wave-exposed and less wave-exposed nearshore areas of Talisayan, Northern Mindanao, Philippines, and to conduct brine shrimp toxicity bioassay using the methanolic crude extracts of these soft coral species.

Materials and methods

Sampling and collection of specimens

Field sampling of the two sites was conducted on 26 to 29 October 2017 in the fringing reef of Talisayan, a Municipality in the Province of Misamis Oriental, located in the Northeastern coast of Mindanao Island, Southern Philippines. The wave-exposed site (outer) was located in Eastern section of Sipaca Point while the less wave-exposed site (inner) is on the western sheltered area of the Point (Fig. 1). Location of each site was known using a hand-held Garmin Global Positioning System.

Prior to detailed sampling in the area, initial visual survey and spot-check SCUBA dives were done to identify the locations of soft coral assemblages. The initial survey was also used to determine the depth of the collection sites. Specimens were collected from the sampling area from a depth of three to five meters also by SCUBA diving. Each sample was rinsed with seawater and immediately kept in ice. The samples were then brought to the laboratory within 7 hours and kept at 4°C in a fridge.

Measured each in triplicate readings, baseline physico-chemical parameters were determined during sampling. Water temperature and dissolved oxygen was measured using a YSI 556 DO meter. Salinity was determined using an Atago refractometer. A portable pH meter (brand) was used to measure pH level. The amount of total suspended solids from a one-liter

sample was analyzed using gravitational filtration method.

Sclerite extraction and species identification

Sclerite extraction was done as described by Janes (2008). The soft coral samples were soaked in a Petri dish with pure sodium hypochlorite.

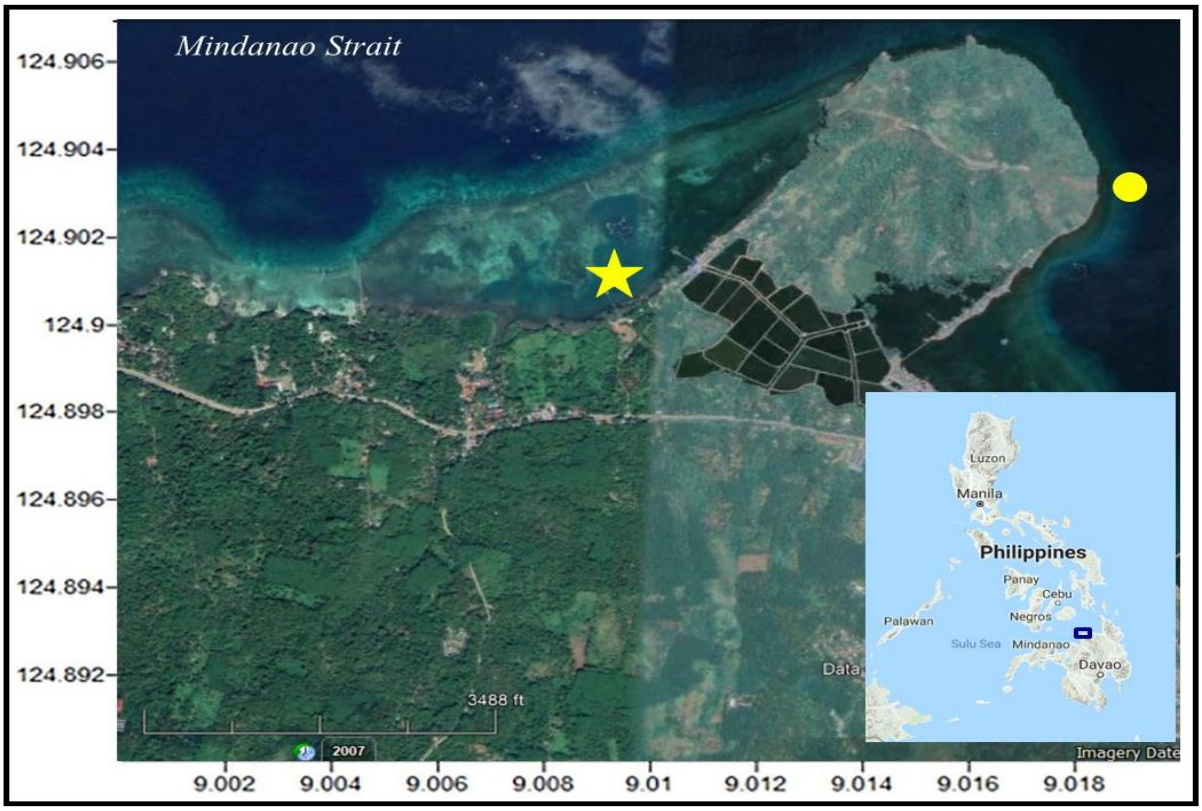

Fig. 1. Google Earth map showing the sampling sites (star-less wave exposed area; solid circle-wave exposed area) in Talisayan, Northern Mindanao, Philippines. Inset: map of the Republic of the Philippines with Talisayan enclosed in a square.

The samples were left for 8 hours with occasional swirling until tissues were completely dissolved and bubbles stopped forming at the surface. Sodium hypochlorite was siphoned using a pipette leaving the sclerites in the bottom of the test tubes undisturbed. Sclerites were rinsed by adding 10 mL of pure ethanol to the tube with gentle stirring, which was repeated five times to ensure no sodium hypochlorite will remain on the sclerites.

The sclerites were then stored in a vial containing pure grade ethanol with the vial sealed using parafilm and electrical tape. Isolated sclerites were mounted on a glass slide with a drop of Eukit and viewed under a stereomicroscope for identification. The book by Fabricius and Alderslade (2001) was used as reference for the soft coral identification.

Extract preparation

The methanol extraction of soft corals was done by following the methods of Phan and Vairappan (2015) with slight modification. Samples from the freezer were thawed, individually chopped and soaked in pure grade methanol at room temperature for seven days. The individual crude mixture was filtered using Whatman filter paper (no. 47 mm).

The filtrates were collected in an acid-washed round-bottom flask and were loaded into the rotary evaporator. The solvent was removed by rotary evaporation under reduced pressure (350 mbar) and at temperature below 40°C for 50-100 rpm. The residue collected was air-dried for seven days, weighed and stored in cool dry place.

The Brine Shrimp Lethality Assay (BSLA)

Following the BSLA of Meyer *et al.* (1982), the LC_{50} was determined from the 24-hour counts of *Artemia* mortality using probit analysis in SPSS software version 24 (SPSS, 2016). In case where data is insufficient for this technique, the dose-response data were transformed into a straight line by means of logit transformation. To validate the results, Kruskall-Wallis *H* test was done using the same software. The brine shrimp toxicity assay is as outlined below:

Decapsulation of brine shrimp:About one gram of *Artemia franciscana* (heretofore referred to as *Artemia*) was strongly aerated in a 1-liter beaker containing nano-filtered seawater. After one hour, the cysts were filtered using a 200 µm sieve screen, washed with clean water. The cysts were suspended in hypochlorite solution with continuous stirring for 7-15 minutes depending on color change from brown to light orange. The cysts were filtered and washed until the HCl smell was completely removed. The *Artemia* cysts were placed in a 1000mL beaker filled with filtered seawater and was properly aerated for 3 days which by then nauplii have already emerged from the cysts.

Dilution series: Using sterilized 10 mL test tubes, each extract was diluted to 10 µg/mL, 100 µg/mL and 1000 µg/mL. 50mg of extracts was dissolved in five mL methanol (solution A). 0.5 mL of solution A was pipetted to the tube with 10mL methanol (solution B). Appropriate amounts of solution (100µl B, 50µl A and 500ul A for 10 µg/mL, 100 µg/mL and 1000µg/mL) were transferred and placed in three different vials. Control vials were prepared using sea water, as well as methanol, and six replicates were prepared for each dose level.

Brine shrimp assay: Sterilized clean vials were used for the bioassay. Each vial was labeled with control (sterile filtered seawater only), methanol, 10 µg/mL, 100 µg/mL, and 1000µg/mL respectively. Ten actively swimming nauplii were pipetted and transferred to each sample test vial containing the designated treatment in five mL sterile filtered seawater. Each treatment was replicated six times.

The number of mortality after 24 hours was recorded. All test vials were incubated at room temperature and under proper illumination. The percentage mortality rate of brine shrimp nauplii was determined from the number of dead nauplii, and the LC_{50} was calculated for each sample. Toxicity grouping was based on Meyer *et al.* (1982), in which a substance is considered highly toxic if LC_{50} value < 30µg/mL (ppm), toxic if LC_{50} ranges from 30-1000µg/mL (ppm), and not toxic if LC_{50} is >1000µg/mL (ppm).

Statistical analysis

The LC_{50} was determined from the 24h counts using probit analysis in Microsoft Excel 2017. A one-way ANOVA was conducted on the two sampling points, comparing the means of mortality percentage from different treatments, followed by Tukey's multiple comparison test. A 95% confidence interval was used to compare toxicities of two population samples. All statistical analyses were performed using SPSS (SPSS, 2016).

Results

Physico-chemical conditions at the sampling sites

The inner less wave-exposed site was more turbid by an order of magnitude (56.0 ± 26.0 mg/L) compared to the outer wave exposed site (4.9 ± 2.6 mg/L) (Table 1). Salinity was lower at the inner site (28.7 ± 1.5) than at the outer (33.4 ± 0.5) site, due perhaps to the brackish water effluent from semi-intensive fish ponds near the inner site. Similar water temperature (in °C) was recorded at inner (30.0 ± 0.8) and outer (29.5 ± 0.2) sites. Dissolved oxygen at the outer site was lower (4.7 ± 0.1) than those at the inner site (6.0 ± 0.7), but similar slightly alkaline pH at inner (9.4 ± 0.3) and outer (9.2 ± 0.0) sites is attributed to the groundwater discharge which is very common at both sites.

Species identification

An overall of 11 species, 6 genera and 4 Families (Xeniidae, Isisidae, Alcyoniidae, Nephtheida) of soft corals were identified from the two sampling

locations (Fig. 2-5). The soft coral species with sclerites and *in situ* pictures of colonies found in the sampling areas were *Sarcophyton crassocaule* (Fig. 2A), *Isis hippuris* (Fig. 2B), *Cespitularia stolonifera* (Fig. 2C) from the less wave-exposed (inner) site, and *Sinularia polydactyla* (Fig. 3A), *Sinularia* sp. (Fig. 3B), *Si. flexibilis* (Fig. 3C), *Lobophytum crassum* (Fig. 4A), and *L. durum* (Fig. 4B), *S. ehrenbergi* (Fig. 4C), *S. glaucum* (Fig. 5A), and *Dendronephthya hemprichii* (Fig. 5B) from the wave-exposed (outer) site.

Table 1. Values of physico-chemical parameters recorded in the soft corals sampling sites in Talisayan, Northern Mindanao, Philippines.

Sampling Area	Temperature (°C)	Salinity (ppt)	Dissolved Oxygen (mg/L)	Total Suspended Solids (mg/L)	pH
Inner	30.6	30.0	6.6	75.4	9.1
	30.2	29.0	5.3	66.2	9.4
	29.1	27.0	6.1	26.5	9.6
Average ± SD	30.0 ± 0.8	28.7 ± 1.5	6.0 ± 0.7	56.0 ± 26.0	9.4 ± 0.3
Outer	29.6	34.0	4.7	5.7	9.2
	29.6	33.2	4.8	2.0	9.2
	29.2	33.0	4.6	7.0	9.2
Average ± SD	29.5 ± 0.2	33.4 ± 0.5	4.7 ± 0.1	4.9 ± 2.6	9.2 ± 0.0

Brine shrimp lethality bioassay

The efficacy of crude extracts varied significantly over increasing concentration ($p < 0.01$ for all). According to the results of Tukey-Kramer multiple comparison. The comparisons on the mean of 10 ppm with both 100 ppm ($p < 0.01$) and 1000 ppm ($p < 0.001$) were significantly different, but not significant when mean of 100 ppm mean was compared with 1000 ppm ($p < 0.23$).

Table 2. Effect of crude extracts from soft coral species collected at the inner less wave-exposed site and outer wave-exposed site on *Artemia* nauplii following the Brine Shrimp Lethality Assay (BSLA).

Area	Genera	Mean % *Artemia* Mortality			LC_{50} (ppm)
		10 ppm	100 ppm	1000 ppm	
Inner	*Sarcophyton crassocaule*	81	90	98	0.40†††
	Isis hippuris	63	80	95	3.67†††
	Cespitularia stolonifera	35	70	92	26.92†††
Outer	*Sinularia polydactyla*	22	61	85	63.24††
	Sinularia sp.	5	10	42	2754.22^{nt}
	Sinularia flexibilis	15	27	87	144.54††
	Sarcophyton ehrenbergi	65	90	100	4.25†††
	Sarcophyton glaucum	28	70	85	39.54††
	Dendronephthya hemprichii	33	72	90	28.18†††
	Lobophytum crassum	65	90	100	4.25†††
	Lobophytum durum	68	90	100	3.45†††

††† = highly toxic; †† = toxic; nt = non-toxic (after Meyer *et al.* (1982) scheme).

Table 2 shows the toxicity grouping of the 11 soft coral species according to Meyer *et al.* (1982). All three species from the inner less wave-exposed site were highly toxic, while the rest of the eight species from the outer wave exposed site showed varied results with all three toxicity categories represented. All results from the sea water and methanol control treatments showed no *Artemia* mortality and were not included in Table 1 but zero values were part of the probit analysis. It can be noted that *Sarcophyton*

crassocaule has the highest toxicity at 0.40 ppm. Although the three inner species were highly toxic, we cannot conclude with absolute certainty that location is a major cause of such high toxicity. This is because another *Sarcophyton* (*S. ehrenbergi*) species from the wave-exposed site was also highly toxic, but with higher LC_{50} value of 4.25 ppm than that of *S. crassocaule*.

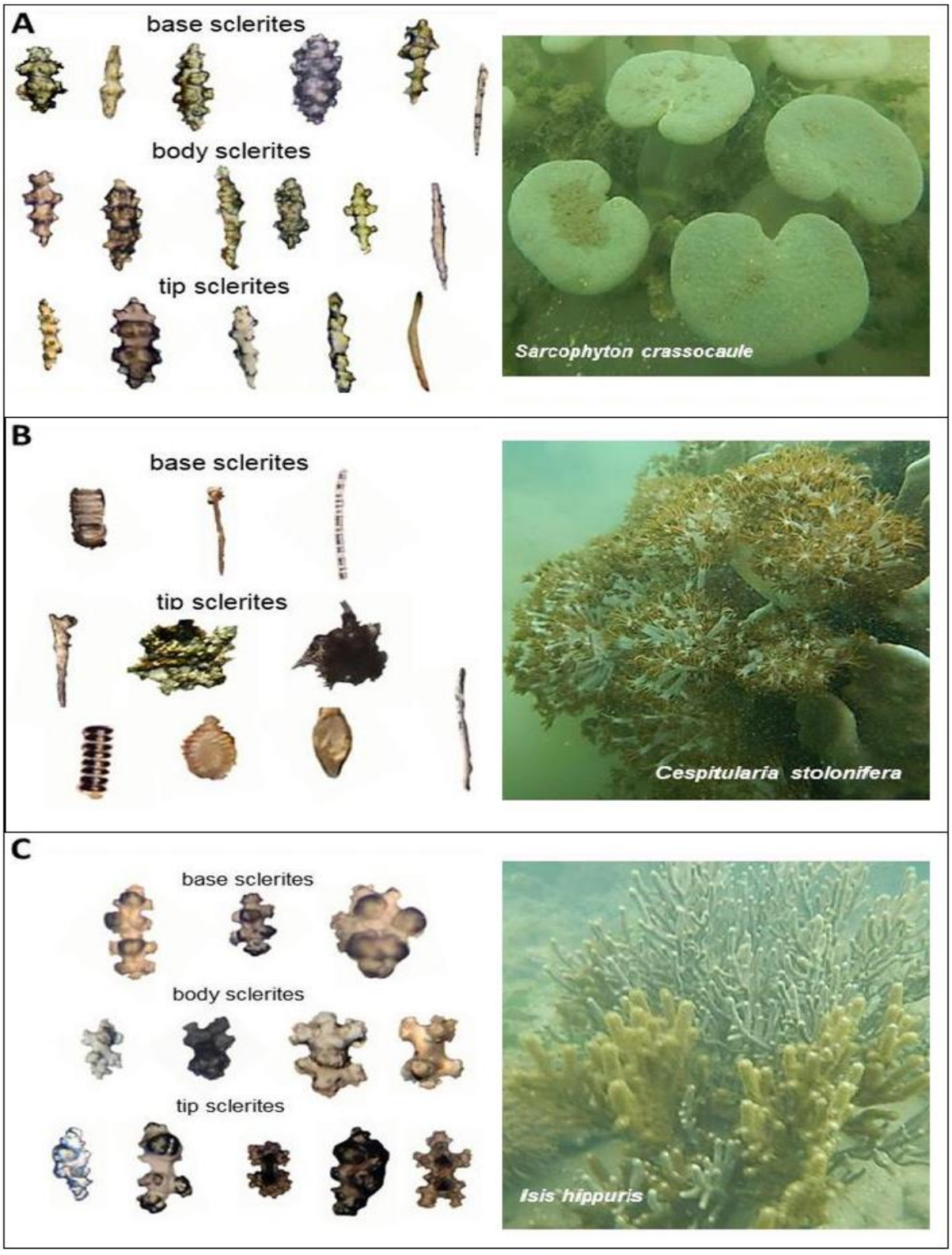

Fig. 2. Sclerites and *in situ* colony photographs of 11 soft coral species collected in this study. A. *Sarcophyton crassocaule* B. *Cespitularia stolonifera* C. *Isis hippuris*.

Discussion Results from the brine shrimp toxicity assay in this study indicated the presence of bioactive compounds in all 11 species of soft corals. Nearly all species of Alcyonacean soft corals collected appear to possess toxic compounds which triggered abnormal behavior in the test *Artemia* specimen.

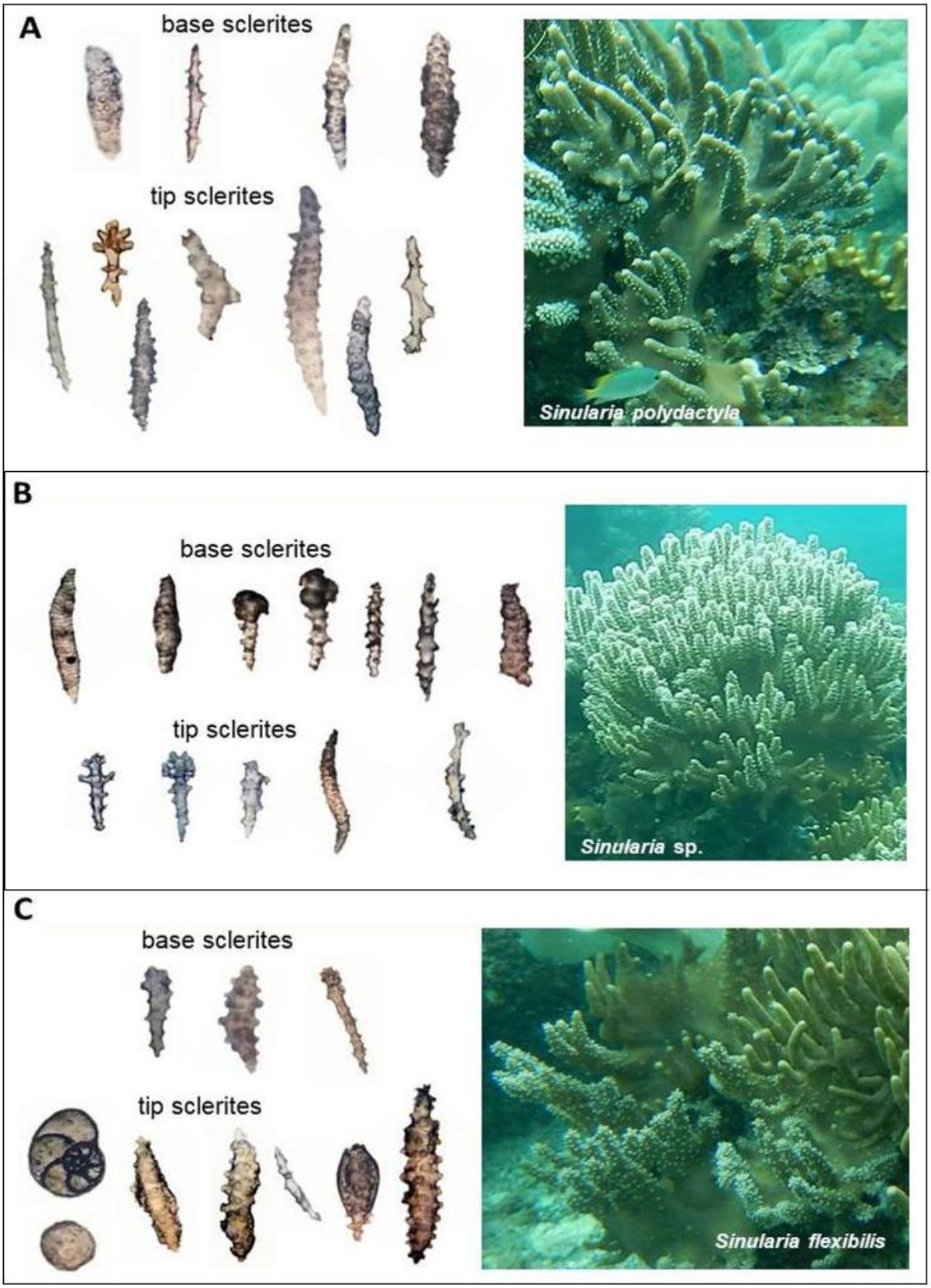

Fig. 3. (continuation) A. *Sinularia polydactyla* B. *Sinularia* sp. C. *Sinularia flexibilis*.

It is possible that these sub-lethal effects may be due either to the pharmacological properties of these compounds or to the relative concentrations of compounds present in the soft coral tissue. Our dilution studies demonstrated that even within the most toxic group of species, toxicity varied and members of the group could be ranked accordingly. Of the soft coral species tested *Sarcophyton crassocaule*, *Lobophytum durum*, *Isis hippuris*, *L. crassum*, *S. ehrenbergi*, *Cespitularia stolonifera*, and *Dendronephthya hemprichii* were highly toxic; *Sinularia flexibilis*, *Si. polydactyla*, and *S. glaucum* were considered toxic; and the unidentified species *Sinularia* sp. was non-toxic.

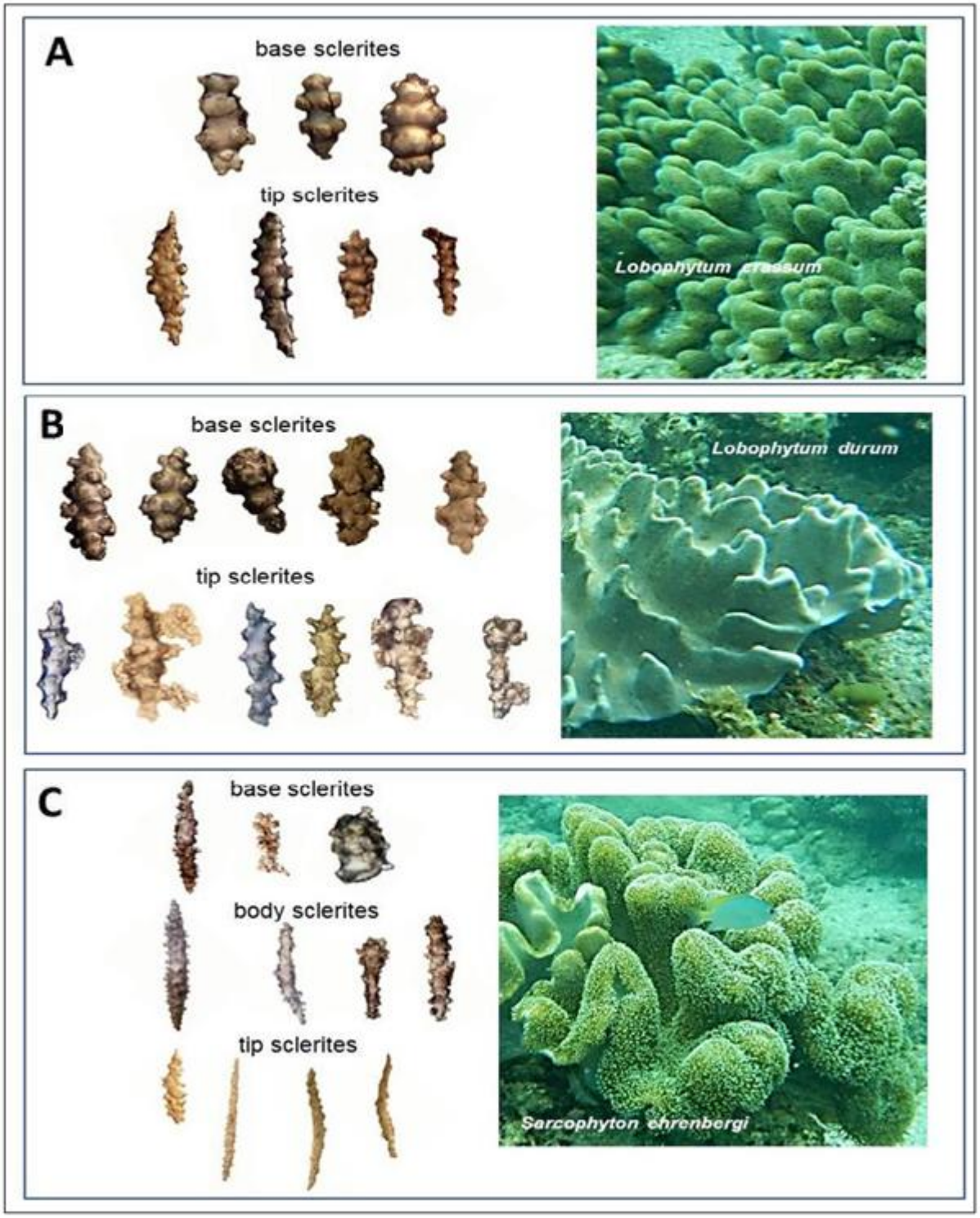

Fig. 4. (continuation) A. *Lobophytum crassum* B. *Lobophytum durum* C. *Sarcophyton ehrenbergi.*

A major finding that emerged is that the soft corals examined induced a wide range of responses in the test organism, as has also been found in other recent similar studies (Luyao *et al.*, 2019). Responses ranged from death within a very short period of time (24 hours) to intermediate effects. Many of the compounds from soft corals are known to play important ecological roles in the defense against predation (feeding deterrence and ichthyotoxicity) and competition for space via allelopathy (Sammarco and Coll, 1988). It has been found that the water around the soft corals *S. flexibilis*, can contain between 1 to 5 mgL^{-1} of flexibilide and dihydroflexibilide (Coll *et al.*, 1980). Such levels of toxins has been shown to induce mortality in several scleractinian corals enabling the soft coral to exert influence on neighboring organisms in competition for space or fouling interactions (Maida *et al.*, 1995; Maida *et al.*, 2001; Maida *et al.*, 2006).

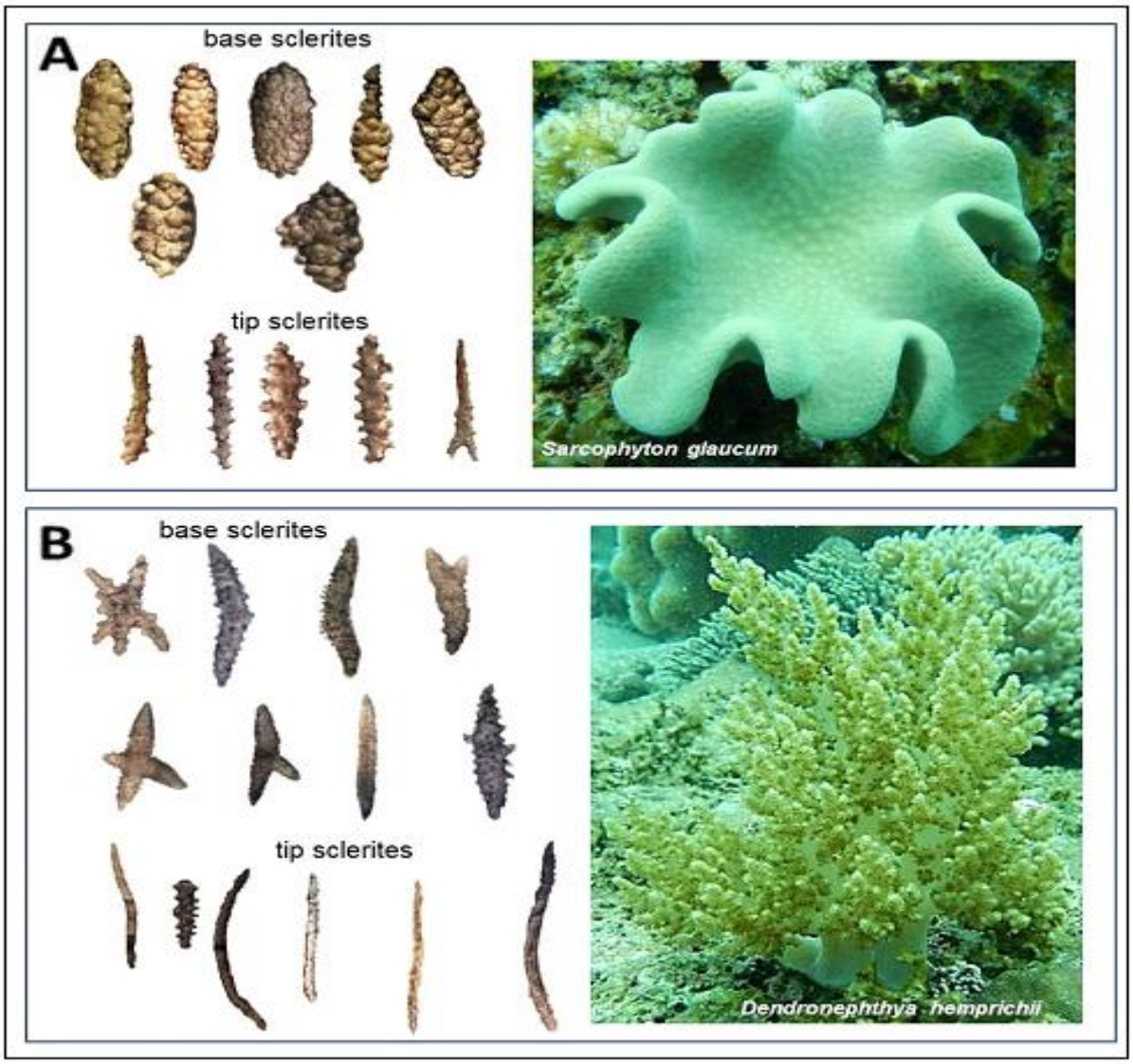

Fig. 5. (continuation) A. *Sarcophyton glaucum* B. *Dendronephthya hemprichii.*

Studies on several *Lobophytum* species proved that this genus contains bioactive compounds such as alkaloids, terpenoids, steroids, lobane, ceramide, flavonoids, saponins and phenols (Edrada *et al.*, 1998; Zhang *et al.*, 2006; Putra *et al.*, 2016). The result of this study agrees with Edrada *et al.* (1998) in which, the result on brine shrimp lethality bioassay in the two *Lobophytum* species was noted to be highly toxic with an LC_{50} of 4.25 ppm and 3.45 ppm. Similarly, *Sarcophyton crassocaule* in this study is

also considered highly toxic having an LC_{50} of 0.40 ppm. This species could be a strong candidate as source of bioactive compounds. The genus *Sarcophyton* is known for its wide array of bioactive components such as sarcophine, diterpene, cembranoids and steroids (Li *et al.*, 2005; Zhang *et al.*, 2005; Sheu *et al.*, 2011; Abdel-Wahhab *et al.*, 2012; Zhao *et al.*, 2013; Phan and Vairappan, 2015). *Isis hippuris* which has the highest LC_{50} value, belongs to the family Isiidae. It has been found to contain cytotoxic sesquiterpenes (suberosenol, suberosenol A acetate, suberosenol B acetate, subergorgic acid), which are metabolites known to exhibit potent cytotoxicity toward P-388, A549, and HT-29 cancer cell lines (Sheu *et al.*, 2000; Sayuti *et al.*, 2016). Also, *Cespitularia* sp. of the Xeniidae family like many other soft coral taxa, are remarkably rich in bioactive secondary metabolites (König *et al.* 1989, El-Gamal *et al.* 2005). On the other hand, the alcyoniid species of the outer area, *Sinularia polydactyla*, *Sarcophyton* sp. 2, and *S. ehrenbergi* were among the first marine taxa that were systematically screened for secondary metabolites (Tursch, 1976). Hirono *et al.* (2003) found in alcyoniids cembranoid diterpenes which function in chemical defense, in competition for space (allelopathy), against fouling, and inhibit reproduction of other organisms such as fishes and some genera of hard corals. *Dendronephthya* sp., which was one of the species of the outer area belongs to Nephtheidae that has been recognized as rich sources of novel and diverse chemical structures with interesting biological activities (Tomono *et al.* 1999; Li *et al.* 2005; Chao *et al.* 2008).

The broad distribution of toxicity both among and within genera was demonstrated as well as the trend from toxic to non-toxic is evident. It is known from many examples in the terrestrial and marine environments that toxicity often functions as a non-energy requiring defense mechanism in prey (Maiorana, 1979). This chemical defence has been favored by natural selection among terrestrial plants and animals such as the monarch butterfly *Danaus plexippus* and the crown-of-thorns starfish *Acanthaster planci* (Coll *et al.*, 1983). It has been suggested that toxicity in non-cryptic sedentary or sessile marine invertebrates may have evolved via natural selection due to high intensities of fish predation (Bakus, 1981; Sammarco *et al.* 1987).

The setting in the inner area might explain the occurrence of highly toxic soft corals. Floating milkfish pens are present very near our sampling sites, and it is very likely food from these fish cages lures several species of reef fishes into that area, and the increased number of grazing fish in that area may increase the chance of encounter with soft corals.

Therefore, the species of soft corals may be induced to mass-produce these bioactive substances as deterrents (Wylie and Paul, 1989; Van Alstyne *et al.*, 1994; Kelman *et al.*, 1999). On the other hand, the outer area species of soft corals would still be subject to predation which explains their toxicity levels.

Conclusion

This study dealt with the toxicity of 11 soft coral species from Talisayan, Northern Mindanao, Philippines.

The brine shrimp toxicity bioassay has indicated the presence of bioactive compounds from soft coral methanolic crude extracts, which showed positive toxic results. Samples from the inner less wave-exposed site produced highly toxic extract, particularly *Sarcophyton crassocaule* which had an LC_{50} of 0.40 ppm. Similarly, crude extracts from soft corals collected at the wave-exposed area also exhibited varied toxicity from non-toxic to highly toxic. These results suggest a good potential in drug discovery in the 11 soft coral species, and we recommend further elucidation of the bioactive compounds from the extracts of these species.

Acknowledgement

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References

Abdel-Wahhab MA, El-Nekeety AA, Hassan NS, El-Hefnawy, Kotb MM, El-Mekkawy, Khalil NA, Hanna AG. 2012. Hepatoprotective effect of sarcophine isolated from soft coral (*Sarcophyton glaucum*) in rats. Global Veterinaria **8(3),** 244–253.

Bakus GJ. 1981. Chemical defense mechanisms on the Great Barrier Reef, Australia. Science **211(4481),** 497–499.

Chou TZ, Liang CH, Wang GH, Liaw CC, Lee MF, Wang SH, Cheng DL. 2008. Extracts from *Cladiella australis*, *Clavularia viridis* and *Klyxum simplex* (soft corals) are capable of inhibiting the growth of human oral squamous cell carcinoma cells. Marine Drugs **6,** 595–606. http://dx.doi.org/10.3390/md6040595

Chao CH, Wen ZH, Chen IM, Su JH, Huang HC, Chiang MY, Sheu JH. 2008. Anti-inflammatory steroids from the octocoral *Dendronephthya griffin*. Tetrahedron **64,** 3554–60. http://dx.doi.org/10.1016/j.tet.2008.01.109

Coll JC, Bowden BF, Mitchell SJ. 1980. Marine natural products chemistry at the James Cook University of North Queensland. Chemistry Australia **47,** 259–263.

Coll JC, La Barrel S, Sammarco PW, Williams WT, Bakus GJ. 1983. Chemical defences in soft corals (Coelenterata: Octocorallia) of the Great Barrier Reef: a study of comparative toxicities. Marine Ecology Progress Series **8,** 271–278.

Duh CY, Puu SY, Wang SK. 2012. New 19-oxygenated steroids from the soft coral *Nephthea chabrolii*. Marine Drugs **10,** 1288–1296. http://dx.doi.org/10.3390/md10061288

Edrada RU, Proksch P, Wray V, Witte L, van Ofwegen L. 1998. Four new bioactive lobane diterpenes of the soft coral *Lobophytum pauciflorum* from Mindoro, Philippines. Journal of Natural Products **61,** 358–361. http://dx.doi.org/10.1021/np970276t

El-Gamal AAH, Chiang CY, Huang SH, Wang SK, Duh CY. 2005. *Xenia* diterpenoids from the formosan soft coral *Xenia blumi*. Journal of Natural Products **68,** 1336–1340. http://dx.doi.org/10.1021/np058047r

Fabricius K, Alderslade P. 2001. Soft corals and sea fans: A comprehensive guide to the tropical shallow water genera of the central-west Pacific, the Indian Ocean and the Red Sea. Australian Institute of Marine Science, and the Museum and Art Gallery of the Northern Territory.

Fabricius K, Déath G. 2001. Biodiversity on the Great Barrier Reef: Large-scale patterns and turbidity related local loss of soft coral taxa. Oceanographic Processes of Coral Reefs. Retrieved October 19, 2017 from epubs.aims.gov

Goh BL, Tan GE, Tan LT. 2009. Diversity, distribution and biological activity of soft corals (Octocorallia, Alcyonacea) in Singapore. Journal of Coastal Development **12(2),** 89–98.

Hirono M, Ojika M, Mimura H, Nakanishi Y, Maeshima M. 2003. Acylspermidine derivatives isolated from a soft coral, *Sinularia* sp., inhibit plant vacuolar H+-Pyrophosphatase. Journal of Biochemistry **133,** 811–816. http://dx.doi.org/10.1093/jb/mvg103

Janes MP. 2008. Advances in coral husbandry in public aquariums. Public Aquarium Husbandry Series **2,** 413–426.

Johnson JE, Marshall PA. 2007. Climate Change and the Great Barrier Reef. Great Barrier Marine Park Authority and Australian Greenhouse Office, Australia.

Kelman D, Benayahu Y, Kashman Y. 1999.

Chemical defence of the soft coral *Parerythropodium fulvum* (Forskål) in the Red Sea against generalist reef fish. Journal of Experimental Marine Biology and Ecology **238**, 127–137.
http://dx.doi.org/10.1016/S0022-0981(99)00016-7

König GM, Coll JC, Bowden BF, Gulbis JM, MacKay MF, La Barre SC, Laurent D. 1989. The structure determination of a xenicane diterpene from *Xenia garciae*. Journal of Natural Products **52,** 294–299.
http://dx.doi.org/10.1021/np50062a013

Li G, Deng Z, Guan H, van Ofwegen L, Proksch P, Lin W. 2005. Steroids from the soft coral *Dendronephthya* sp.. Steroids **70(1)**, 13–8.
http://dx.doi.org/10.1016/j.steroids.2004.09.003

Limna Mol VP, Raveendran TV, Parameswaran PS, Kunnath RJ, Sathyan N. 2010. Antifouling sesquiterpene from Indian soft coral, *Sinularia karavattiensis* Aldersalde and Prita. Indian Journal of Marine Sciences **39(2),** 270–273.

Luyao HN, Metillo EB, Uy MM. 2019. 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*. International Journal of Biosciences **14(2)**, 439–445.
http://dx.doi.org/10.12692/ijb/14.2.439-445

Maida M, Sammarco PW, Coll JC. 1995. Effects of soft corals on scleractinian coral recruitment. 1. Directional allelopathy and inhibition of settlement. Marine Ecology Progress Series **121,** 191–202.
http://dx.doi.org/10.3354/meps121191

Maida M, Sammarco PW, Coll JC. 2001. Effects of soft corals on scleractinian coral recruitment. II: allelopathy, spat survivorship and reef community structure. Marine Ecology **22(4),** 397–414.
https://doi.org/10.1046/j.1439-0485.2001.01709.x

Maida M, Sammarco PW, Coll JC. 2006. A diffusion chamber for assessing efficacy of natural anti-fouling defenses in marine organisms. Journal of Experimental Marine Biology and Ecology **337(1)**, 59–64.
http://dx.doi.org/10.1016/j.jembe.2006.06.008

Maiorana VC. 1979. Nontoxic toxins: the energetics of coevolution. Biological Journal of the Linnean Society **11(4)**, 387–396.

Mehdinia A, Fumani S, Rezaei H. 2014. Essential oils of a soft coral (*Sinularia* sp.) from Chabahar Bay of Iran. Journal of the Persian Gulf **5(15),** 51–58.

Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. 1982. Brine shrimp: A convenient general bioassay for active plant constituents. Journal of Medicinal Plant Research **45,** 31–34.
http://dx.doi.org/10.1055/s-2007-971236

Murcia SRS, Metillo EB, Uy MM. 2019. The brine shrimp toxicity of the philippine marine sponges *Axinyssa* sp., *Plocamionida* sp., *Forcepia* sp., *Pachymatisma* sp., and *Placospongia* sp.. International Journal of Biosciences **14(3),** 30–34.
http://dx.doi.org/10.12692/ijb/14.3.30-34

Nakamura M, Ohki S, Suzuki A, Sakai K. 2011. Coral larvae under ocean acidification: survival, metabolism, and metamorphosis. PLoS ONE, **6,** e14521.
http://dx.doi.org/10.1371/journal.pone.0014521

Phan CS, Vairappan CS. 2015. Isopropyl (ene)-type cembrane diterpene an important chemotaxonomical marker in Bornean soft coral genus *Sarcophyton*. Journal of Tropical Biology and Conservation **12**, 137–143.

Putra MY, Murniasih T. 2016. Marine soft corals as source of lead compounds for anti-inflammatories. Journal of Coastal Life Medicine **4(1),** 73–77.

http://dx.doi.org/10.12980/jclm.4.2016j5-226.

Reaka-Kudla M. 1997. The global biodiversity of coral reefs: a comparison with rain forests. In Biodiversity II: Understanding and Protecting our Biological Resources. The National Academy of Sciences, USA.

Sammarco PW, La Barre S, Coll JC. 1987. Defensive strategies of soft corals (Coelenterata: Octocorallia) of the Great Barrier Reef III. The relationship between ichthyotoxicity and morphology. Oecologia **74,** 93–101.
http://dx.doi.org/10.1007/BF00377351

Sammarco PW, Coll JC. 1988. The chemical ecology of alcyonarian corals (Coelenterata: Octocorallia). In: Scheuer, P.J. (Ed.), Bioorganic marine chemistry, Springer-Verlag, Berlin p, 87–116.

Sayuti M, Putri WDR, Yunianta. 2016. Phytochemicals screening and antioxidant activity test of *Isis hippuris* methanol extract. International Journal of ChemTech Research **9(7),** 427–434.

Shahbudin S, Deny, Zakirun AMT, Haziyamin TAH, Akbar JB, Taher M. 2011. Antioxidant properties of soft coral *Dendronephthya* sp. International Journal of Pharmacology **7(2)**, 263–267.
http://dx.doi.org/10.3923/ijp.2011.263.267

Sheu JH, Hung KC, Wang GH, Duh CY. 2000. New cytotoxic sesquiterpenes from the gorgonian *Isis hippuris*. Journal Natural Products **63(12),** 1603–1607.
http://dx.doi.org/10.1021/np000271n

Sheu Jh, Lin WY, Lu Y, Su JH, Wen ZH, Dai CF, Kuo YF. 2011. Bioactive cembranoids from the Dongsha Atoll soft coral *Sarcophyton crassocaule*. Marine Drugs **9,** 994–1006.
http://dx.doi.org/10.3390/md9060994

SPSS. 2016. IBM SPSS Statistics 24. IBM Corporation, NY, USA.

Sung PJ, Chen KH, Dai CF, Lu MC, Li JJ, Chen JJ, Chang YC, Su YD, Wang WH. 2013. Secondary metabolites from the soft coral *Sinularia arborea*. Marine Drugs **11,** 3372–3380.
http://dx.doi.org/10.3390/md11093372

Tomono Y, Hirota H, Imahara Y, Fusetani NJ. 1999. Four new steroids from two octocorals. Journal Natural Products **62,** 1538–41.
http://dx.doi.org/10.1021/np990246l.

Tursch B. 1976. Some recent development in the chemistry of Alcyonaceans. Pure and Applied Chemistry **48,** 1–6.
http://dx.doi.org/10.1351/pac197648010001

Van Alstyne KL, Wylie CR, Paul VJ. 1994. Antipredator defenses in tropical Pacific soft corals (Coelenterata: Alcyonacea). II. The relative importance of chemical andstructural defenses in three species of *Sinularia*. Journal of Experimental Marine Biology and Ecology **178(1),** 17–34.
http://dx.doi.org/10.1016/0022-0981(94)90222-4

Wilkinson CR, Evans E. 1989. Sponge distribution across Davies Reef, Great Barrier Reef, relative tolocation, depth, and water movement. Coral Reefs **8,** 1–7.
http://dx.doi.org/10.1007/BF00304685

Wylie CR, Paul VJ. 1989. Chemical defences in three species of *Sinularia* (Coelenterata, Alcyonacea): effects against generalist predators and the butterflyfish *Chaetodon unimaculatus* Bloch. Journal of Experimental Marine Biology and Ecology **129(2),** 141–160.
http://dx.doi.org/10.1016/0022-0981(89)90053-1

Zhang CX, Yan SJ, Zhang GW, Lu WG, Su JY, Zeng LM, Gu LQ, Yang XP, Lian YJ. 2005. Cytotoxic diterpenoids from the soft coral *Sinularia microclavata*. Journal of Natural Products **68,** 1087–1089.

http://dx.doi.org/10.1021/np058006v.

Zhang GW, Ma XQ, Su JY, Zhang K, Kurihara H, Yao XS, Zeng LM, 2006. Two new bioactive sesquiterpenes from the soft coral *Sinularia* sp. Natural Product Research **20(7),** 659–664.
http://dx.doi.org/10.1080/14786410500183233

Zhao HY, Shao CL, Li ZY, Han L, Cao F, Wang CY. 2013. Bioactive pregnane steroids from a South China Sea Gorgonian *Carijoa* sp. Molecule **18,** 3458–3466.
http://dx.doi.org/10.3390/molecules18033458