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ANTIOXIDANT AND ANTIMICROBIAL POTENTIALS OF THE EXTRACTS OBTAINED FROM THE FIVE MARINE SPONGES COLLECTED OFF THE COASTS OF AGUSAN DEL NORTE, PHILIPPINES: *AXINYSSA SP, PLOCAMIONIDA SP, FORCEPIA SP, PACHYMATISMA SP* AND *PLACOSPONGIA SP*

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ABSTRACT: Five marine sponges were collected off Carmen, Agusan del Norte, Philippines and taxonomically identified as *Axinyssa sp*., *Plocamionida sp*., *Forcepia sp*., *Pachymatisma sp* and *Placospongia sp*. The "non-polar" (EtOAc-MeOH) and "pola"r (EtOH-$_{H2O}$) extracts of the marine sponges were prepared and subjected to 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, a total antioxidant capacity assay using the phosphor molybdenum method and antimicrobial assay using the paper disc diffusion method. The marine sponge extracts exhibited low antioxidant activity as indicated by both the percent antiradical activity values obtained which ranged from 0.89% to 5.41% and the total antioxidant capacity values expressed as Ascorbic Acid Equivalence (AAE) ranging from 11.99 to 126.67 AAE and Butylated Hydroxytoluene Equivalence (BHTE) with a value range of 6.64 to 148.30 BHTE). The non-polar extracts from all the marine sponges as well as the polar extract from the marine sponge *Forcepia sp*. showed antibacterial activity against the two bacterial strains, *B. subtilis* and *E. coli.* However, the marine sponge extracts no significant antifungal activity against the two fungal strains, *A. niger* and *S. cerevisiae*. The results indicate the potential of the marine sponges as sources of antioxidant and antibacterial compounds.

INTRODUCTION: Drugs from marine resources offer an unparalleled opportunity for Pharma-cological investigation and hence have received significant attention.

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Recent years in natural product chemistry as a promising new area of study. Extracts from marine organisms, whether obtained from culture or directly from a collected sample, continue to be an essential cornucopia of novel natural products, varying widely in both chemical structures and biological activities.

There have been several recent reviews detailing the different classes of novel marine products or the various types of biological activities reported from these compounds.

Hence, the secondary metabolites produced in marine organisms can be bioactive compounds for drug discovery [1, 5]. Among all the marine organisms investigated, marine sponges are recognized as the most abundant sources of new marine natural products, with more than 5000 compounds as of 2019, contributing to nearly 30% of all marine natural products discovered so far [6]. This makes marine sponges champion producers in relation to the diversity of products that have been found from them. With this myriad of new marine natural products available, numerous studies have revealed bioactive compounds from marine sponges that are classified as anti-inflammatory, antitumor, immuno- or neuro-suppressive, antiviral, anti-malarial, antibiotic, or antifouling [4, 7]. Bioassays offer a special advantage in the standardization and quality control of heterogeneous natural products [8].

Extracts have been screened for biological activity, such as antioxidant, antimicrobial and cytotoxic activities, to achieve applied meaning and significance to the compounds isolated from these natural sources [9]. A simple method to determine antioxidant activity utilizes the stable 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical, which has a characteristic deep purple color that turns to yellow as the odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant [10, 12].

The phosphomolybdenum method is utilized for the determination of the total antioxidant activity based on the reduction of Mo(VI) to Mo(V) by the antioxidant compound and the subsequent formation of a blue-green phosphate/ Mo(V) complex at acidic pH [13, 14]. Determination of bacterial resistance to antimicrobials is an important part of the management of infections and diseases [15]. Antimicrobial activity can be evaluated based on the paper disc diffusion method, which determines the sensitivity or resistance of pathogenic aerobic and anaerobic bacteria to various antimicrobial samples [16, 18]. The main objective of this study was to determine the potential antioxidant and antimicrobial activity of the non-polar and polar extracts obtained from the five marine sponges *Axinyssa sp*., *Plocamionida sp*., *Forcepia sp*., *Pachymatisma sp* and *Placospongia sp*. collected off the coasts of Agusan del Norte, Philippines. The non-polar and polar extracts from the five (5) Philippine marine sponges, namely were obtained by solvent extraction with ethyl acetate-methanol (EtOAc-MeOH) and ethanol-water (EtOH-H_2O), respectively. The antioxidant potential of the sponge extracts was evaluated by DPPH free radical scavenging activity and total antioxidant capacity using the phosphomolybdenum method. The antimicrobial activity of the sponge extracts was evaluated using the paper disc diffusion method against the four respective test organisms – *Bacillus subtilis*, *Escherichia coli*, *Aspergillus niger* and *Saccharomyces cerevisiae*.

MATERIALS AND METHODS:

Collection and Taxonomic Identification of Marine Sponge Samples: Fresh samples of the five marine sponges were collected off the coasts of Brgy. Vinapor, Carmen, Agusan del Norte, Philippines (9005'13.6"N, 125013'12.4"E) on November 2015. The collected samples were properly labelled, stored in containers filled with ice and transferred to the Natural Products and Bioorganic Research Laboratory of the Department of Chemistry of Mindanao State University - Iligan Institute of Technology, Iligan City, Philippines. The collected sponges were then immediately cut and freeze-dried (Eyela FDU-2200 Freeze-dryer). Voucher specimens of the five marine sponges collected were prepared in accordance with a standard protocol [19,] and taxonomical identification was done by Dr. Ephrime B. Metillo of the Department of Biological Sciences, College of Science and Mathematics at MSU-IIT. The marine sponges were identified as *Axinyssa sp*., *Plocamionida sp*., *Forcepia sp*., *Pachymatisma sp* and *Placospongia sp*.

Solvent Extraction of Marine Sponge Samples: Each of the five marine sponge samples was soaked in adequate amount of 1:1 ethyl acetate-methanol mixture for 72 h. The resulting mixture was then filtered, concentrated in vacuo using rotary evaporator and weighed to give the corresponding ethyl acetate-methanol (non-polar) extract. The sponge residue from the first extraction was then soaked in 1:1 ethanol-water mixture for 72 h. The resulting mixture was then filtered, concentrated in vacuo, freeze-dried and weighed to give the corresponding ethanol-water (polar) extract.

DPPH Free Radical Scavenging Activity: DPPH Free Radical Scavenging assay was carried out to investigate the antioxidant potential of the marine sponge extracts, and the method was based on the protocol described by Lee and Shibamoto20. Four concentrations (500, 100, 50, and 25 ppm) were prepared from the extracts for the assay. Three hundred (300) µL of the prepared solutions were transferred into screw-capped test tubes, and 3000 µL of methanolic solution of 0.1 mM DPPH was added into the test tubes. The resulting mixtures were shaken thoroughly and allowed to stand at room temperature for one hour without exposure to light. The absorbance for each solution was measured at 517 nm using a UV-Vis Spectro-photometer (Double-beam LI-2800) against methanol as a blank. The percent of DPPH decoloration of the samples was calculated according to the formula:

$$\text{Antiradical Activity} = (A_{control} - A_{sample})/ \text{A-control} \times 100$$

Where a sample is the absorbance of the marine sponge extracts and a control is the absorbance of the methanol as the control. The evaluation of the DPPH free radical scavenging activity of the samples was also compared with a known antioxidant, ascorbic acid.

Total Antioxidant Capacity: The antioxidant activity of the compound is determined based on the total antioxidant capacity protocol described by Prieto *et al* [21]. For each sponge extract, A 200 - ppm solution was prepared at three replicates. Exactly 0.3 mL of each resulting solution was combined with freshly prepared 3 mL reagent solution (containing 0.6 Msulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The resulting reactions solutions were placed in screw-capped test tubes, incubated in a water bath at 950 °C for 90 min and were allowed to cool for 30 min after incubation. The absorbance of each solution was measured at 695 nm using a UV-Vis Spectrophotometer (Double-beam LI-2800). The antioxidant capacity of each solution was expressed as Ascorbic Acid and BHT equivalence using a linear equation (concentrations at 10, 100 and 200 ppm versus absorbance) established with ascorbic acid and butylated hydroxytoluene (BHT) as the standards and ethanol as the negative control measured at the same wavelength.

Antimicrobial Assay: Paper Disc Diffusion Method - The antibacterial and antifungal activity of the extracts was evaluated by the paper disc diffusion method described by Guevara *et al.* [22]. A 1000- ppm solution was prepared from each sponge extract to evaluate their bioactivity against four test organisms - *B. subtilis*, *E. coli*, *A. niger*, and *S. cerevisiae*. The solvent used in the preparation of the extracts was the negative control, and amoxicillin and nystatin were the positive control for the antibacterial and antifungal activity, respectively. Six (6) mm diameter discs were immersed into the marine sponge extracts.

The moistened filter disc was then laid gently on a previously prepared Petri dish containing an agar solution and swabbed with the test organism. The diameter of the inhibition zone around the discs was then measured after the incubation of the plates in an inverted fashion at 350 °C for bacteria in the dark for 24 h and 270 °C for mold and yeast also in the dark for 2-3 days.

TABLE 1: THE DPPH FREE RADICAL SCAVENGING ACTIVITIES OF THE MARINE SPONGE EXTRACTS AT VARIOUS CONCENTRATIONS

Marine Sponge	Extract	Antiradical activity, %				EC_{50}, ppm
		25 ppm	50 ppm	100 ppm	500 ppm	
Axinyssa sp.	Nonpolar	0.89	1.51	1.97	3.44	>500.00
	Polar	1.78	2.27	2.49	4.45	>500.00
Plocamionida sp.	Nonpolar	1.63	2.03	2.40	2.70	>500.00
	Polar	1.72	2.03	2.24	3.32	>500.00
Forcepia sp.	Nonpolar	1.84	2.15	2.46	3.66	>500.00
	Polar	1.35	1.87	2.33	3.23	>500.00
Pachymatisma sp.	Nonpolar	1.23	2.58	2.92	4.70	>500.00
	Polar	2.12	2.73	3.44	5.19	>500.00
Placospongia sp.	Nonpolar	1.94	2.89	3.32	4.67	>500.00
	Polar	2.80	3.07	3.66	5.41	>500.00
Ascorbic Acid (Standard)		17.27	46.61	91.61	96.47	56.46

RESULTS AND DISCUSSION: The scavenging activities against the DPPH radical of the marine sponge extracts and the effective concentration of the extracts required to scavenge the DPPH radical by 50% (EC50) are shown in **Table 1.**

The results indicate the polar extract of *Placospongia sp*., to possess the highest antiradical activity in all of the concentrations among all of the extracts tested. However, in comparison to the exhibited scavenging character of the reference standard antioxidant in the various concentrations, in this study, all of the marine sponge extracts do not possess significant and efficient capacity to scavenge the DPPH free radical. All of the extracts yielded low percent antiradical activity values ranging from 0.89-5.41% within the various concentrations tested. Correspondingly, all of the marine sponge extracts displayed median effective concentration (EC50) values greater than 500.00 ppm, which are not comparable to that of the standard antioxidant, ascorbic acid (56.46 ppm). These results can be correlated with the study done by Garcia-Davis on marine sponge Suberites aurantiacus, an abundant marine sponge in the Mexican Pacific, which has the same Order (Order Hadromerida) as the marine sponge Placospongiasp [23]. In the study, the ethanolic extract of S aurantiacus possessed a poor efficiency as a DPPH, 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and nitric oxide (NO) radical scavengers. The total antioxidant capacity of each of the marine sponge extracts expressed in terms of ascorbic acid equivalence (AAE) and butylated hydroxytoluene equivalence (BHTE) – which signify hydrophilic [24] and lipophilic [25] antioxidants are summarized in **Table 2.**

TABLE 2: TOTAL ANTIOXIDANT CAPACITY OF THE MARINE SPONGE EXTRACTS EXPRESSED AS ASCORBIC ACID EQUIVALENCE (AAE) AND BUTYLATED HYDROXYTOLUENE EQUIVALENCE (HBTE)

Marine Sponge	Type of Extract	AAE	BHTE
Axinyssa sp.	Nonpolar	126.67	148.30
	Polar	84.21	95.85
Plocamionida sp.	Nonpolar	110.88	128.79
	Polar	41.75	43.40
Forcepia sp.	Nonpolar	104.93	121.44
	Polar	17.31	13.21
Pachymatisma sp.	Nonpolar	50.64	54.38
	Polar	11.99	6.64
Placospongia sp.	Nonpolar	79.21	89.68
	Polar	36.12	36.44

Low to moderate total antioxidant capacity values were obtained for all of the crude marine sponge extracts as shown by the results. Among the extracts tested, the non-polar extract of *Axinyssa sp*. showed the highest total antioxidant activity both in terms of AAE and BHTE values. Most of the marine sponge extracts with high lipophilic antioxidant content consistently show relatively high hydrophilic antioxidant content as depicted by their BHTE and AAE values, respectively. However, most of the extracts have higher BHTE values compared to their AAE values signifying that they may possibly possess more lipophilic antioxidants than hydrophilic antioxidants. The family Halichondriidae where the genus *Axinyssa* belongs to is known to contain a wide range of bioactive metabolites such as steroids, nitrogenous sesquiterpenes, dimeric sesquiterpenoids, and fatty acids among others. These compounds are useful as potential therapeutics in which one, in particular, is due to their antioxidant activity [26]. The results for the antibacterial activity of the marine sponge extracts with considerable activities against *B. subtilis* and *E. coli* are summarized in **Tables 3** and **4.**

Most of the marine sponge extracts showed active to very active antibacterial activity against *B. subtilis* except for the polar extracts of *Plocamionida sp*., *Pachymatisma* sp, and *Placospongia sp*. The non-polar extract of *Forcepia sp*. exhibited the highest antibacterial activity against *B. subtilis* among all the extracts. Moreover, almost all of the marine sponge extracts also showed antibacterial activity against *E. coli* except the polar extract of *Axinyssa sp*. although some of them may be specified as inactive since they have ZOI less than 10.0 mm.

The high antibacterial activity exhibited by the non-polar extract of *Forcepia sp*. against *B. subtilis* can be related with the results obtained from the study of tetracyclic sesquiterpenes isolated from the Korean sponge C. Gombawuiensis and mcyaperoxides A and B isolated from the Thai sponge Mycale sp. which exhibited antibacterial activity against several strains of bacteria, including *B. Subtilis* [27, 28]. The marine sponges *Forcepia sp*., C. gombawuiensis, and Mycale sp. all belong to the Order Poecilosclerida.

TABLE 3: ZONE OF INHIBITION OF SELECTED MARINE SPONGE EXTRACTS AGAINST *B. SUBTILIS*

Sample	Type of extract	Ave. Zone of Inhibition (mm)[a]
Axinyssa sp.	Non-polar	14.8±1.1
	Polar	19.5±1.0
Plocamionida sp.	Non-polar	15.8±1.3
Forcepia sp.	Non-polar	39.6±0.9
	Polar	14.2±2.0
Pachymatisma sp.	Non-polar	14.7±2.1
Placospongia sp.	Non-polar	14.0±1.0
Amoxicillin[b]	-	42.3±2.4

a<10 mm inactive, 10-13 mm partially active, 14-19 mm active, >19 mm very active23; b Positive control

TABLE 4: ZONE OF INHIBITION OF SELECTED MARINE SPONGE EXTRACTS AGAINST *E. COLI*

Sample	Type of Extract	Ave. Zone of Inhibition (mm)[a]
Axinyssa sp.	Non-polar	11.3±1.5
Plocamionida sp.	Non-polar	11.0±1.2
	Polar	9.0±1.1
Forcepia sp.	Non-polar	13.0±1.4
	Polar	9.3±1.0
Pachymatisma sp.	Non-polar	14.0±1.5
	Polar	10.2±0.4
Placospongia sp.	Non-polar	15.0±0.0
	Polar	10.4±1.5
Amoxicillin[b]	-	38±2.6

a<10 mm inactive, 10-13 mm partially active, 14-19 mm active, >19 mm very active 23; b Positive control

All of the marine sponge extracts did not exhibit any antifungal activity ested against *A. niger* and *S. cerevisiae* since they did not inhibit the growth of any of the two fungi.

CONCLUSION: The results from the antioxidant assays as evaluated by the DPPH Free Radical Scavenging and Total Antioxidant Capacity methods, in general, indicate low antioxidant potential among all of the marine sponge extracts. Moreover, the DPPH Free Radical Scavenging assay results suggest that the extracts do not possess significant scavenging capacity, which correlates to the low to moderate total antioxidant capacity values seen in the Total Antioxidant Activity assay. The majority of the marine sponge extracts subjected to antimicrobial assay exhibited moderate to very active antibacterial activity against representative bacterial strains *B. subtilis* and *E. coli*, which indicate that the marine sponge species have potential as antibacterial agents.

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CONFLICTS OF INTEREST: Nil

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