RESEARCH ARTICLE

Assessing Antioxidant Activity and Phenolic Content of Marine Sponges from Mauritius Waters

Preeti Nitisha Oogarah[1], Avin Ramanjooloo[1*], Jay Rovisham Singh Doorga[1], Cindy Meyepa[1], Robertus Wilhelmus Maria van Soest[2], Daniel Edgard Pierre Marie[1]

[1]*Mauritius Oceanography Institute, Avenue des Anchois, Morcellement de Chazal, Albion*
[2]*Netherlands Centre for Biodiversity Naturalis, Leiden, The Netherlands*

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ABSTRACT

The ocean provides a plethora of structurally-diverse bioactive compounds that are potential candidates for drug development. Marine sponges have been studied over the past decades and have been found to be a rich source of these bioactive chemicals. This study is focused on the antioxidant properties of marine sponges collected from Mauritius waters. A total of 141 extracts derived from 47 marine sponges from Mauritius waters were tested for their antioxidant property, using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and ferric reducing antioxidant potential assay (FRAP). Additionally, their total phenolic content was determined by the Folin-Ciocalteu method. The methanol extract of *Axinella donnani* (ADM) displayed the highest DPPH activity (92.15 ± 0.09%), whilst the ethyl acetate extract of *Pseudosuberites* sp. showed the highest FRAP activity (10.57 ± 0.39 mm Fe^{2+}/g of extract). A significant correlation between antioxidant capacity and total phenolic content for methanol/butanol extracts were found.

Keywords: Antioxidant, DPPH, FRAP, Marine sponge, Mauritius, Total phenolic content.

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INTRODUCTION

Marine sponges are the most primitive multicellular invertebrate that is present in the marine ecosystem. As a defense mechanism against environmental stress factors, such as, predators and competition for space with other sessile organisms, marine sponges produce various classes of secondary metabolites, such as, alkaloids, steroids, glycosides, macrolides, and terpenoids.[1] These metabolites exhibit pharmacological properties, such as, anticancer, antibacterial, antifungal, antioxidant, antimalarial, anti-inflammatory, and other activities.[2,3] Among marine organisms seaweeds and sponges represent one of the richest sources of natural antioxidants and antimicrobials.

Antioxidants are compounds or systems that delay autoxidation by inhibiting the formation of free radicals and have widespread applications in medicine, cosmetics, and food industries.[4,5] The screening for potent antioxidants from natural sources as potential protective agents is of great relevance as they play a crucial role as reactive oxygen species (ROS) chelating agents.[6] Free radicals in the biological systems are mostly derivatives of oxygen known as "ROS"; however, other reactive species, such as, nitrogen derivatives "RNS" also exist. A balance between free radicals and antioxidants is necessary for the proper physiological function of the body. Antioxidants have a potential positive impact on human health due to their ability to attack reactive oxygen species (ROS) that are harmful to the body, thereby preventing damage to membrane lipids, proteins, and deoxyribonucleic acid (DNA).[7,8]

The antioxidant capacity of compounds has been related to the prevention of several diseases, including cancer, coronary heart diseases, inflammatory disorders, and neurological degeneration.[9] Free radicals and other reactive oxygen/nitrogen/chlorine species contribute to the development of several age-related diseases and to the aging process itself by causing oxidative stress and oxidative damage.[10,11] The implication of oxidative stress in the history of several acute and chronic clinical disorders, such as, cancer, atherosclerosis, and diabetes has led to the suggestion that anti-oxidants can be prophylactic agents against such diseases.[12-14] Anti-oxidant drugs protect tissue damage induced by free radicals by preventing their formation, scavenging them, or by promoting their decomposition.[15] There are relatively few investigations on the anti-oxidant activity of sponge extracts and the main objective of this study was to investigate this property for marine sponge extracts from Mauritius waters.

**Author for Correspondence: aramanjooloo@moi.intnet.mu*

MATERIALS AND METHODS

Study Area

Of volcanic origin, the main island of Mauritius (Figure 1) emerged from the South West tropical waters of the Indian Ocean some 8 million years ago. The island enjoys a tropical maritime climate throughout the two seasons of the year: summer and winter. About 150 km of fringing coral reefs shelter diverse marine biodiversity and enclose a lagoon of total area approximating to 243 km^2.[16] Another island, Saint Brandon, which forms part of the territory of Mauritius is of interest in the current study. Of volcanic origin, the island of Saint Brandon is characterized by its basaltic boulder shoreline and peripheral fringing reef.[17]

Chemicals

Hexane, ethyl acetate, butanol, and methanol were purchased from SFDC Ltd. Folin-Ciocalteu phenol reagent, ferrous sulfate heptahydrate, and ascorbic acid were purchased from Loba Chemie Ltd. 2,2-diphenyl-1-picrylhydrazyl, quercetin, and gallic acid were purchased from Sigma-Aldrich. 2,4,6-tri (2-pyridyl)-s-triazine was purchased from Fluka Ltd.

Sponge Collection

A subset of 46 sponge specimens was collected through scuba diving at depths varying from 5 to 40 meters around Mauritius. One specimen, *Phyllospongia papyracea*, was collected in the coastal waters of Saint Brandon. Samples were photographed *in situ* for better species characterization and identification. The species were identified by Dr. Rob Van Soest and a voucher sample of each species is kept at the Zoological Museum of University of Amsterdam, Netherlands.

Preparation of Sponge Extracts

Freshly collected marine sponges were set free of any debris, cut into small pieces, weighed, and freeze-dried. Two extraction protocols were used. The first batch of dried sponges (with mass ranging between 49 to 400 grams) was macerated with MeOH/CH_2Cl_2 1:1 for 24 to 48 hours. After maceration, the solution was filtered and evaporated to dryness on a rotatory vacuum evaporator set at a maximum temperature of 40°C to obtain the crude extract. This crude extract was dissolved in distilled H_2O and partitioned with hexane, ethyl acetate, and butanol to afford non-polar, semi-polar, and polar extracts, respectively. The second batch (with mass ranging between 0.2–136 grams) of dried/wet sponges was macerated and partitioned in the same way as described for the first batch except for the final water extract being evaporated and dissolved in MeOH to afford the polar extract (MeOH extract). Inorganic salts present in both BuOH and MeOH extracts were removed by re-dissolving the extract in MeOH, followed by filtration.

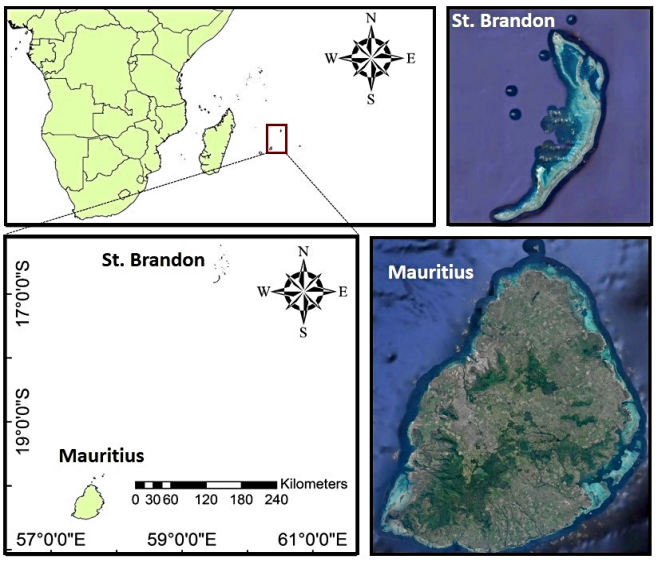


Figure 1: Study area of Mauritius (20.34°S, 57.55°E) and St. Brandon (16.58°S, 59.61°E) located in the southwest Indian Ocean

2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

A modified method described by Chang *et al.*[18] was used for performing the DPPH radical scavenging activity in a 96 well plate. DPPH (180 µL) of methanol solution (0.2 mM) was added to the sample solution (20 µL). The absorbance was measured at 517 nm in a microplate reader after incubating at 37°C for 30 minutes. DPPH radical scavenging ability was calculated using the following equation:

$$\text{DPPH radical scavenging ability (\%)} = \left\{\frac{(A_0 - A_1)}{A_0}\right\} \times 100$$

Where A_0 is the absorbance of the control (without sample) and A_1 is the absorbance in the presence of the sample.

Ferric Reducing Antioxidant Potential (FRAP) Assay

Briefly, FRAP reagent was prepared at 37°C from acetate buffer (300 mmol/L, pH 3.6), 2,4,6-tri[2-pyridyl]-s-triazine (TPTZ) (10 mmol/L) in HCl (40 mmol/L) and $FeCl_3$ (20 mmol/L) in the ratio of 1:1:10, according to the method first described by Benzie *et al.*[19] Ferrous sulfate heptahydrate ($FeSO_4.7H_2O$) was used as a standard and TPTZ working reagent used as a blank solution. Sample solution and the standards (15 µL) were mixed with FRAP reagent (135 µL). The mixture was then incubated at 37°C and the absorbance was measured at 593 nm spectrophotometrically after 4 minutes. The results were expressed as Fe^{2+}/g of extract and calculated as mean value ± SD (n = 3).

Determination of Total Phenol Content

Total phenolic content was estimated by the Folin-Ciocalteu method described by Singleton and Rossi.[20] Samples (20 µL) were added to 1:10 diluted Folin-Ciocalteu reagent (100 µL) in the 96 well plates. After 4 minutes, saturated sodium carbonate (80 µL, 75 g/L) was added. The samples were incubated for 2 hours at room temperature, after which the absorbance was measured at 765 nm. Gallic acid (0–200 mg/L) was used for the standard calibration curve. The results were expressed as gallic acid equivalent mg (GAE)/g of extract, and calculated as mean value ± SD (n = 3).

Statistical Analysis

The data were subjected to a series of statistical tests performed using the "statistics and machine learning toolbox" available on the MATLAB R2015a programming environment. Due to the small size of the dataset investigated, normality tests preluded parametric tests. Normality tests were performed using the Shapiro Wilk's test, while homogeneity of variances was checked using Levene's test. The non-normality and inequality of variances in the data revealed from both tests suggested the use of non-parametric inferential statistics. A Kruskal Wallis test was, therefore, employed as a non-parametric substitute for the one-way analysis of variance (ANOVA), followed by a Mann-Whitney U test as a non-parametric alternative to the unpaired t test. The correlation analysis among the assay methods was performed using Spearman's correlation test as a non-parametric alternative to Pearson's correlation test. Box-and-whisker plots were drawn to help with the descriptive statistics of the marine sponge datasets. Principal component analysis (PCA) was employed to classify marine sponges based on their radical scavenging activity levels. Detection of clusters was enabled through the dimensionality reduction technique by exploring the variability in the DPPH datasets for all extracts.

RESULTS AND DISCUSSION

DPPH, FRAP, and Total Phenolic Content (TPC) of Studied Sponges

Owing to the presence of different bioactive components with antioxidant potential in natural extracts, many methods have been used to investigate various samples in recent years. In the current study, the evaluation of free radical scavenging activity of 47 marine sponges (141 extracts classified as hexane, ethyl acetate, and butanol/methanol extracts) was performed by DPPH (1,1-diphenyl-2-picrylhydrazyl) method. This method is based on the reduction of DPPH, a stable purple color free radical that (after being reduced by an antioxidant) turns into a yellow product.

As shown in Figure 2, all sponge extracts tested at 1 mg/mL showed varying degrees of DPPH activity. The highest DPPH activities at the same concentration for hexane, ethyl acetate, and methanol/butanol were observed on *Acanthostylotella* sp. (84.24 ± 0.12%), *Hyrtios erectus* (68.72 ± 2.66%), and ADM (92.15 ± 0.09%), respectively. The lowest DPPH activities for the same order of extracts and at similar concentrations were reported for *Neopetrosia exigua* (1.31 ± 5.24%),

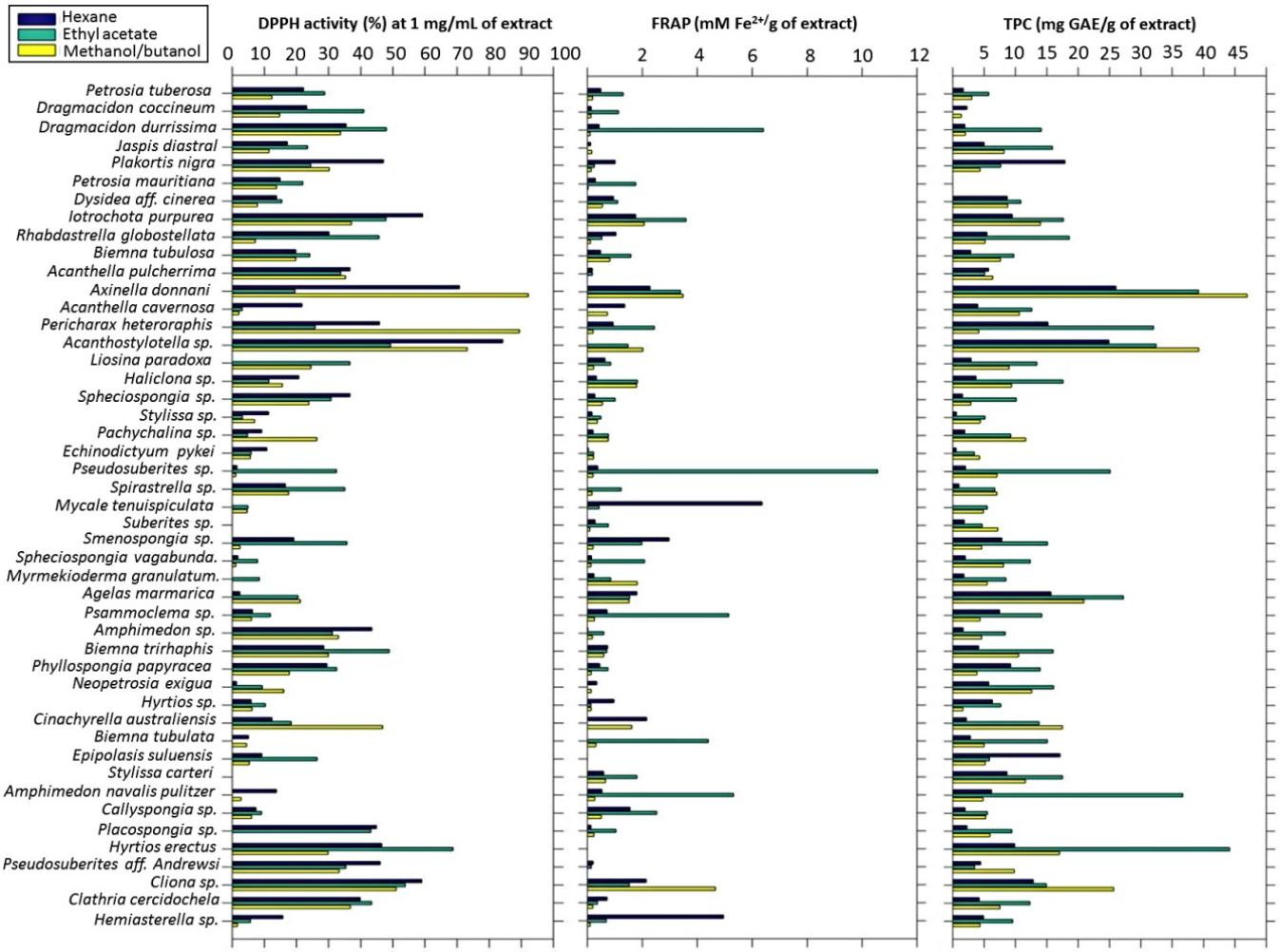


Figure 2: Percentage DPPH activity, FRAP, and TPC contents of hexane, ethyl acetate, and butanol/methanol extracts of sponge specimens (values represent means ± SD, n = 3)

Acanthella cavernosa (3.1 ± 0.77%), and *Spheciospongia vagabunda* (1.06 ± 0.38%), respectively. From Figure 3A, it can be inferred that on average, ethyl acetate extracts of the marine sponges displayed higher levels of DPPH radical scavenging activity in comparison with the same dose of their hexanolic and methanolic/butanolic extracts. However, for the three sponges (ADM, *Pericharax heteroraphis*, and *Acanthostylotella* sp.) containing exceptionally high antioxidant activities, hexanolic and methanolic/butanolic extracts were more active than the ethyl acetate extract at the same concentration (Figure 2).

The ferric reducing ability of plasma (FRAP) of the sponge extracts are also presented in Figure 2. This ability indicates that the antioxidant compounds are electron donors and could reduce the oxidized intermediate of lipid peroxidation processes, thus, acting as primary and secondary antioxidants.[21] The ferric-reducing ability of the extracts was found to vary from 0.01 to 10.57 mM Fe^{2+}/g of the extract with a mean value of 1.15 mm Fe^{2+}/g of extract. The trend for the ferric ion reducing activities of the 141 sponge extracts tested varied markedly from their DPPH scavenging activities. The FRAP contents of *Mycale tenuispiculata* (6.36 ± 0.31 mM Fe^{2+}/g of extract), *Pseudosuberites* sp. (10.57 ± 0.39 mM Fe^{2+}/g), and *Cliona* sp. (4.66 ± 0.05 mM Fe^{2+}/g) were found highest for hexane, ethyl acetate, and methanol/butanol, respectively. The lowest FRAP values for the same order of extracts were observed on *Acanthostylotella* sp. *(*0.01 ± 0.01 mM Fe^{2+}/g), *Hyrtios* sp. (0.13 ± 0.01 mM Fe^{2+}/g), and *Petrosia mauritiana* (0.03 ± 0.01 mM Fe^{2+}/g), respectively.

There was a wide range of phenolic concentrations in the sponge extracts analyzed, as shown in Figure 2. The total phenolic content on the other hand varied from a minimum of 0.55 mg GAE/g of extract to a maximum of 46.93 mg GAE/g of extract, with an average value of 10.02 mg GAE/g of extract over the sample set of collected marine sponges, as measured by the Folin-Ciocalteu method. Among the selected extracts, the hexane extracts of *Echinodictyum pykei* and *Stylissa* sp. possessed the lowest phenolic content. Similar to the results obtained for radical scavenging assay, the respective hexanolic and methanolic/butanolic extracts of ADM and *Acanthostylotella* sp. displayed the highest TPC activity (Figure 2).

DPPH activity of the extracts was found to vary from 1.06 to 92.15%, with an average of 24.87% over the entire dataset of collected marine sponges. The highest levels of DPPH radical scavenging activity is observed for methanolic/butanolic extracts, as highlighted by the outliers present in the boxplot of Figure 3. Additionally, ethyl acetate extracts of marine sponges exhibit the higher mean, median, and spread of the distributions in the FRAP and TPC assays, as observed in Figures 3B and C, respectively.

Statistical Hypothesis Testing

A Shapiro Wilk's test revealed that the datasets were non-normal (Table 1) at 0.05 level significance. Consequently, non-parametric inferential statistics were used. A Kruskal Wallis test (Table 2) revealed highly significant differences among the three extracts for the FRAP ($\chi^2 = 20.65$, $p < 0.001$) and TPC ($\chi^2 = 30.56$, $p <= -0.001$) assays. No significant difference was, however, observed for the hexane, ethyl acetate and methanol/butanol extracts corresponding to the DPPH ($\chi^2 = 3.42$, $p = 0.181$) assay. Pairwise comparison using the Mann-Whitney U test (two-tailed) showed that there is no statistical difference between DPPH of the hexane and ethyl acetate extracts ($U = 903$, $p = 0.623$); DPPH of the hexane and methanol/butanol extracts ($U = 903$, $p = 0.241$); DPPH of the ethyl acetate and methanol/butanol extracts ($U = 924.5$, $p = 0.063$); and FRAP of the hexane and methanol/butanol

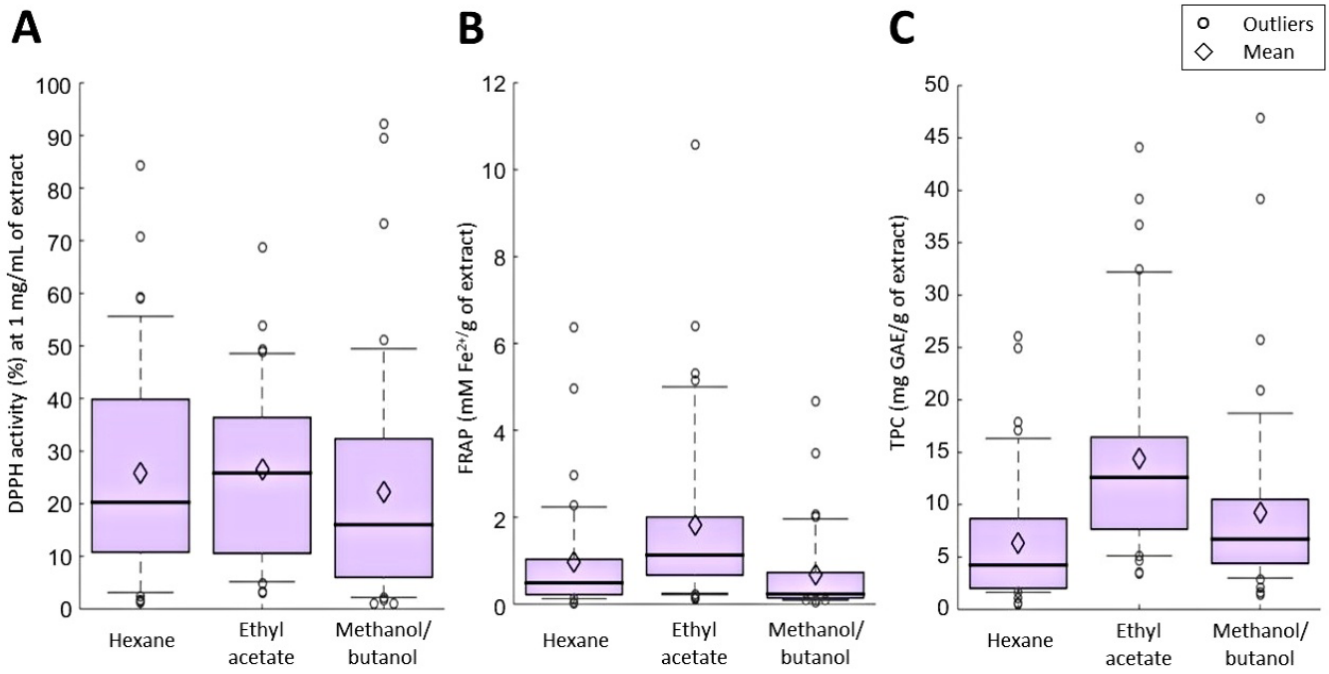


Figure 3: Box plots showing variation in **(A)** DPPH, **(B)** FRAP, and **(C)** TPC levels for hexane, ethyl acetate, and methanol/butanol extracts

Table 1: Summary of normality test conducted using the Shapiro Wilk's method; significance level: α = 0.05

		Shapiro Wilk's test		
	Assay methods	*Statistic*	*df*	*Significance*
Hexane	DPPH	0.919	41	0.008
	FRAP	0.662	42	< 0.001
	TPC	0.792	44	< 0.001
Ethyl acetate	DPPH	0.955	42	0.009
	FRAP	0.707	40	< 0.001
	TPC	0.844	44	< 0.001
Methanol/butanol	DPPH	0.813	42	< 0.001
	FRAP	0.634	41	< 0.001
	TPC	0.662	45	< 0.001

Table 2: ANOVA, non-parametric χ^2 *(*Kruskal Wallis) test for three extracts corresponding to each assay technique

Assay type	*Source*	*Sum of squares*	*df*	*Mean square*	χ^2	*Significance*
DPPH	Columns	4,704.3	2	2,352.13	3.42	0.181
	Error	170,045.7	125	1,360.37	-	-
	Total	174,750	127	-	-	-
FRAP	Columns	27,523.2	2	13,761.6	20.65	< 0.001
	Error	139,121.3	123	1,131.1	-	-
	Total	166,644.5	125	-	-	-
TPC	Columns	47,448.3	2	23,724.2	30.56	< 0.001
	Error	162,157.7	133	1,219.2	-	-
	Total	209,606	135	-	-	-

extracts (U = 903, p = 0.08). All other pairwise comparisons among each assay group subset were found to be statistically different as represented in the box plot (Figure 2). For each extract, a separate Kruskal Wallis test revealed highly significant differences for hexane (χ^2 = 85.4, p < 0.001), ethyl acetate (χ^2 = 82.05, p < 0.001), and methanol/butanol (χ^2 = 84.04, p < 0.001).

Correlation among Antioxidant Assay Methods

With the objective to rationalize the antioxidant potential of the marine sponge extracts, linear regression plots were produced and the corresponding Spearman correlation coefficients were derived. Strong positive linear correlations were observed between the ferric reducing capacity and total phenolic content of ethyl acetate (ρ = 0.65) and methanolic/butanolic (ρ = 0.63) extracts (Figure 4). This infers that in general, for the ethyl acetate and methanolic/butanolic extracts, marine sponges with the highest FRAP contents had the highest TPC levels, whilst those with the lowest FRAP contents were characterized by the lowest TPC levels. This is particularly true for ADM, which had the highest ferric reducing capacity (3.48 mM Fe^{2+}/g) and possessed the highest total phenolic content (46.93 GAE/g) in the methanolic/butanolic extract. Along the same lines, *Dragmacidon coccineum*, which was characterized by its lowest total phenolic content (1.37 mg GAE/g) among all sponges investigated, had one of the lowest FRAP levels (0.13 mM Fe^{2+}/g) in the methanolic/butanolic extract.

Poor correlations were observed between DPPH and FRAP for the hexane (ρ = 0.004) and ethyl acetate (ρ = 0.11)

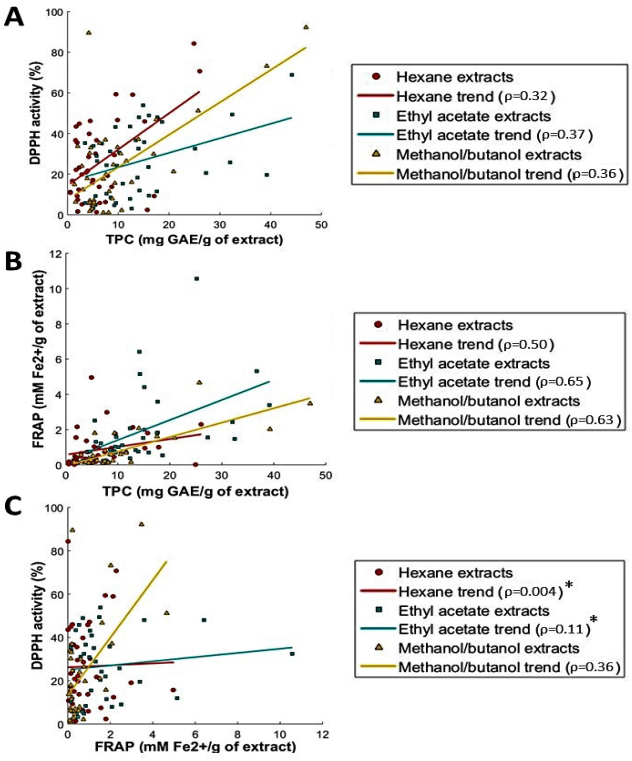
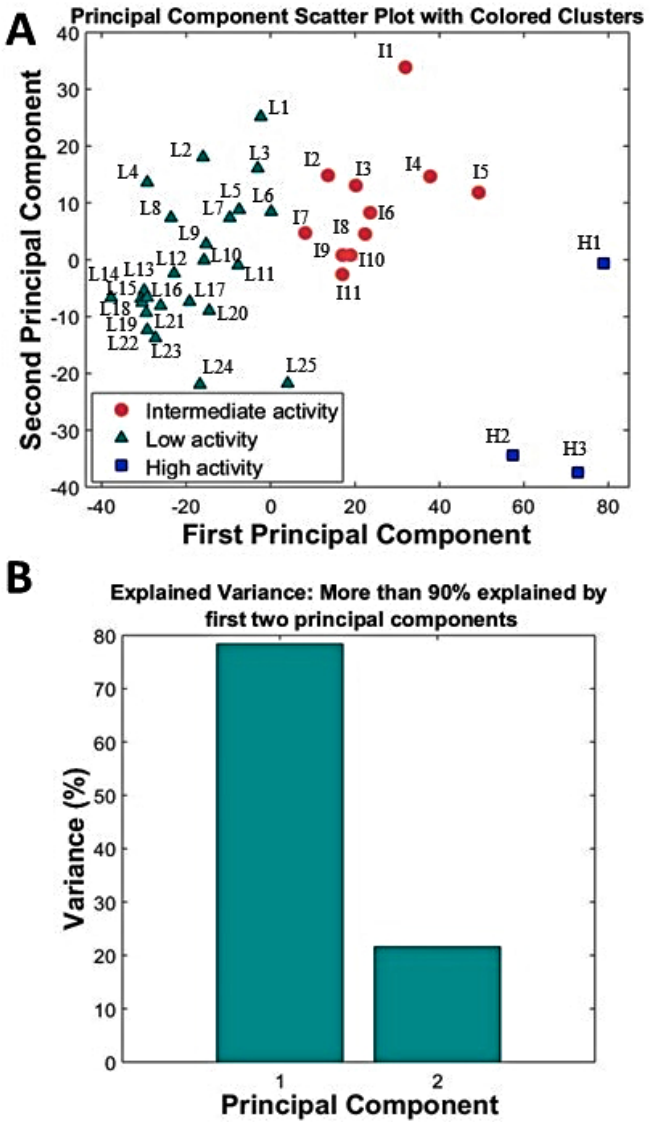


Figure 4: Linear regression plots and Spearman's correlation coefficients of **(A)** DPPH against TPC, **(B)** FRAP against TPC, and **(C)** DPPH against FRAP for hexane, ethyl acetate, and methanol/butanol extracts; all correlations were significant at the 0.05 level (two-tailed) except for values marked with an asterisk (*)

extracts (Figure 4C). The coefficient values were not significant ($p > 0.05$). At the exception of the hexanolic and ethyl acetate extracts, a moderate positive linear correlation was observed for methanol/butanol extract for DPPH against FRAP ($\rho = 0.36$). A moderate positive linear correlation was also noted for DPPH against TPC (hexane, $\rho = 0.32$; ethyl acetate, $\rho = 0.37$; methanol/butanol, $\rho = 0.36$), whilst a strong positive linear correlation was observed for FRAP against TPC (hexane, $\rho = 0.5$; ethyl acetate, $\rho = 0.65$; methanol/butanol, $\rho = 0.63$). This gives an indication that both phenolic compounds and ferric ion reducing activities may possibly be the main source of DPPH radical scavenging activity. The methanol/butanol extracts displayed significant correlations among the three assays.

Principal Component Analysis (PCA)

The PCA was performed to group marine sponges based on their radical scavenging activity levels. PCA reduces the dimensionality of the data while retaining most of the variation in the dataset.[22] Based on the dimensionality reduction technique, marine sponges sharing fairly similar characteristics may be grouped. Consequently, the dissociation and identification of the most active marine sponges are not based on arbitrary judgments of threshold values, but rather computed from the relative distances among the principal components of the marine sponge DPPH dataset. This is attributed to the variability in the datasets. A hierarchical cluster tree was generated from the sample data to determine the clusters of marine sponges sharing similar variability and gathering them into homogeneous categories. PCA was performed on the radical scavenging activity dataset of hexane, ethyl acetate, and methanol/butanol extracts for all 47 marine sponges investigated. Out of the list of marine sponges studied, eight sponges had missing values either for the hexane, ethyl acetate, or methanol/butanol extracts, corresponding to the



Low activity extracts

L1	*Rhabdastrella globostellata*
L2	*Smenospongia sp.*
L3	*Dragmacidon coccineum*
L4	*Pseudosuberites sp.*
L5	*Spirastrella sp.*
L6	*Phyllospongia papyracea*
L7	*Petrosia tuberosa*
L8	*Epipolasis suluensis*
L9	*Jaspis diastral*
L10	*Petrosia mauritiana*
L11	*Biemna tubulosa*
L12	*Dysidea aff. cinerea*
L13	*Acanthella cavernosa*
L14	*Spheciospongia vagabunda*
L15	*Hyrtios sp.*
L16	*Psammoclema sp.*
L17	*Agelas marmarica*
L18	*Echinodictyum pykei*
L19	*Callyspongia sp.*
L20	*Haliclona sp.*
L21	*Hemiasterella sp.*
L22	*Stylissa sp.*
L23	*Neopetrosia exigua*
L24	*Pachychalina sp.*
L25	*Cinachyrella australiensis*

Hexane: 14.46%
Ethyl acetate: 19.16%
Methanol/Butanol: 12.57%

Intermediate activity extracts

I1	*Hyrtios erectus*
I2	*Biemna trirhaphis*
I3	*Dragmacidon durrissima*
I4	*Iotrochota purpurea*
I5	*Cliona sp.*
I6	*Clathria cercidochela*
I7	*Spheciospongia sp.*
I8	*Pseudosuberites aff. Andrewsi*
I9	*Acanthella pulcherrima*
I10	*Amphimedon sp.*
I11	*Plakortis nigra*

Hexane: 43.50%
Ethyl acetate: 42.39%
Methanol/Butanol: 34.02%

High activity extracts

H1	*Acanthostylotella sp.*
H2	*Pericharax heteroraphis*
H3	*Axinella donnani*

Hexane: 66.94%
Ethyl acetate: 31.55 %
Methanol/Butanol: 84.92%

Figure 5: *(A)* Score plot from the PCA to classify 39 Mauritian marine sponges based on their radical scavenging activities corresponding to hexane, ethyl acetate, and methanol/butanol extracts; **(B)** Variance plot showing that the first two principal components account for more than 90% of variance in dataset

DPPH assay and were consequently not represented in the score plot from the PCA of Figure 5A.

Results of the analysis showed that the first two principal components explained all (100%) of the variation in the dataset. The first and second principal components accounted for 79 and 21% of the total variation, respectively (Figure 5B). The first principal component scores showed that the sponges with higher radical scavenging activities aggregated to the right (square blue labels), whilst those characterized as having inherently low activity levels were grouped to the left (triangular green labels) of the PCA score plot of Figure 5A. Sponges with intermediate levels of radical scavenging activities were found in the middle (circular red labels) of the score plot.

About 35% of sponges classified had intermediate to high levels of radical scavenging activities, with the highest levels observed for *Acanthostylotella* sp., *P. heteroraphis*, and ADM, representing average DPPH values of 66.94, 31.55, and 84.92% corresponding to hexane, ethyl acetate, and methanol/butanol extracts, respectively. The low activity extracts observed average DPPH values of 14.46, 19.16, and 12.57% corresponding to hexane, ethyl acetate, and methanol/butanol extracts, respectively.

Based on the results obtained by the PCA, the extracts hexane extract of *Axinella donnani* (ADH), ADM, methanol extract of *P. heteroraphis* (PHM), hexane and methanol extracts of *Acanthostylotella sp.* (ASSH), and ASSM were clustered in the high activity group. The IC_{50} of these extracts were hence, studied. The IC_{50} representing the sample concentration required for inhibiting 50% of the DPPH free radical for the group of highly active sponges identified in the previous stage is represented in Figure 5. The IC_{50} of ADH, ADM, PHM, ASSH, and ASSM, as presented in Figure 6, are 192.5, 61.71, 64.22, 32.31, and 30.55 µg/mL, respectively. Positive controls were ascorbic acid and quercetin, and their IC_{50} were 7.38 and 9.48 µg/mL, respectively.

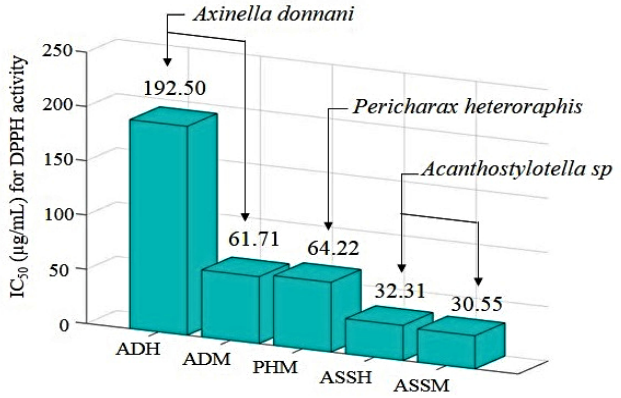


Figure 6: IC_{50} for DPPH activities of potent extracts (ADH, ADM, PHM, ASSH, and ASSM)

Nowadays, antioxidants have gained more importance on account of their positive effects as health promoters in the treatment of cardiovascular problems, cancers, and the aging process amongst others. The increased interest worldwide to identify antioxidants compounds from natural sources led us to determine the antioxidant activity of Mauritian sponge extracts. Of the 47 marine sponge species studied, the highest DPPH activities were recorded for extracts derived from *Axinella donnani* (ADH and ADM), *Acanthostylotella sp.* (ASSH and ASSM), and *P. heteroraphis* (PHM). The extracts obtained from ADM, *Acanthostylotella* sp., and *P. heteroraphis* (Figure 7) have also been studied for their cytotoxic activity,[23] acetylcholinesterase inhibitory activity,[24] and α-glucosidase inhibitory activity.[25] These studies showed that ADH and ADM were cytotoxic (≥ 70% at 50 µg/mL). ADM displayed significant α-glucosidase inhibitory activity (> 90% at 1 mg/mL) and moderate acetylcholinesterase inhibitory activity (≥ 50% at 0.1 mg/mL). PHM displayed weak activities (< 50%). ASSM displayed moderate acetylcholinesterase inhibitory activity (≥ 50% at 0.1 mg/mL), significant α-glucosidase inhibitory activity (> 90% at 1 mg/mL), and moderate cytotoxic activities. ASSH displayed weak cytotoxic and acetylcholinesterase inhibitory activities but the α-glucosidase inhibitory activity of 70% at 1 mg/mL. It has been reported by Lee *et al*. that the presence of phenolic compounds could be the main contributors to both α-glucosidase and antioxidant activities.[26] However, significant differences in TPC were noticed between the different sponge species tested and the solvents used for extraction (Figure 2). In general, ethyl acetate extracts showed higher amounts of total phenolic content, when compared to hexane and methanol/butanol extracts. However, certain species, such as, ADM and *Acanthostylotella* sp. contained higher amounts of TPC in methanol/butanol extracts than ethyl acetate extracts. This variation could be due to the type of solvent used.

Figure 7: Sponges identified as having high radical scavenging activity levels: **(A)** P. heteroraphis; **(B)** ADM; **(C)** Acanthostylotella sp.

It has been reported by Farvin *et al*. that the type of solvent influences the selectivity of phenolic compounds.[27] According to Waterman *et al*., phenolic compounds are generally more soluble in polar organic solvents.[28] However, in this study, the phenolic content in ethyl acetate extracts were found to be higher than that of their hexane and methanol/butanol counterparts. Moreover, the marked inter-species variability in TPC could be due to other external factors. Connan *et al*. reported that environmental factors, such as, light, depth, salinity, nutrient level, seasonality, as well as, intrinsic factors, such as, age and type of tissue influences the content of total phenolic compounds in brown algae.[29]

There are relatively few research publications on the antioxidative effect of sponge extracts. According to available literature, a number of metabolites derived from marine sponges, such as, indole derivatives, aromatic alkaloids, aromatic polyketides, and phenolic compounds have exhibited strong antioxidant potential compared to vitamin E and ascorbic acid.[30,31] A scientific study showed that alkaloids, phenols, steroids, terpenoids, tannins, and saponins were detected in the sponge extracts derived from *Plakortis nigra* from Mauritius waters and TPC varied from 2.28 ± 0.072 to 12.79 ± 0.236 mg gallic acid equivalents per gram extract. Likewise, the research findings in this study corroborate with the reported occurrence of phenols in Mauritian *Stylissa* sp. and *Biemna tubulosa* extracts of different polarities.[32]

In this connection, Shaaban *et al*. also investigated the DPPH activity of four marine sponges *Smenospongia*, *Callyspongia*, *Niphates*, and *Stylissa* collected from the Red Sea at Egyptian coasts.[33] At 1 mg/mL, the DPPH activity of the extracts was 30.6, 72.2, 40.2, and 38.5%, respectively. Additionally, the inhibitory activity of the DPPH demonstrated by the sponge extracts was mainly attributed to some terpenoidal analogs.[34] In another study by Berrué *et al*., steroidal glycosides isolated from the Caribbean sponge *Pandaros acanthifolium* exhibited high antioxidant and cytoprotective activities.[35] Sponge microbes (marine sponge derived fungus and yeast) have also been studied for their antioxidant capacity. Li reported the activity of aromatic polyketides isolated from marine sponge-derived fungus *Aspergillus versicolor*.[36] They displayed significantly higher antioxidant capacity than that of butylated hydroxytoluene (BHT). Sugiyama *et al*. reported that marine sponge-derived yeast was capable of producing antioxidative indole derivatives.[37]

The results obtained by the statistical analysis for the correlation between assays indicate a relationship between phenolic compound concentration in methanol/butanol extracts and their ferric reducing capacities. Therefore, it is suggested that the presence of phenolic compounds in the extracts contributes significantly to their antioxidant potential. This result is in agreement with previous reports that ferric reducing potential can be related to phenolic content.[38] Antioxidant properties of phenolic compounds are directly linked to their structure. According to Rice-Evans *et al*., phenolics are composed of one (or more) aromatic rings bearing one or more hydroxyl groups and are, therefore, potentially able to quench free radicals by forming resonance-stabilized phenoxyl radicals.[39] This explains the higher correlation between antioxidant assays and total phenolic content for methanol/butanol extracts.

CONCLUSION

Marine natural compounds, specifically those of sponge origin, offer a wide variety of compounds, many of them with antioxidant properties. In this research, we highlight the antioxidant properties of 123 sponge extracts from Mauritius waters. These antioxidant capacities open up future research about the correlation between the types of compounds present in the extracts to the various biological properties exhibited by marine sponges.

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