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Original Work

Prophylactic antioxidants and phenolics of seagrass and seaweed species: A seasonal variation study in a Southern Indian Ocean Island, Mauritius

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ABSTRACT: The seasonal variations in the polyphenolic contents and potential antioxidant activities of seven seaweed species (*Padina gymnospora, Gracilaria salicornia*, *Palisada papillosa*, *Galaxaura rugosa*, *Enteromorpha intestinalis*, *Codium arabicum* and *Dictyosphaeria cavernosa)* and five seagrass species (*Syringodium isoetifolium, Halodule uninervis, Thalassodendron ciliatum, Halophila ovalis* and *Halophila stipulacea*) were assessed. In summer, the highest total phenolic content was recorded in the seaweed *P. gymnospora* and the lowest in *G. rugosa*. The total phenolic contents in the seagrass species were significantly higher than those observed in the seaweed species during both seasons. The highest flavonoid concentrations (FC) were observed in the seaweed species *E. intestinalis* in winter and in the seaweed *P. gymnospora* in summer. All tested species had higher FC in winter. The highest antioxidant activity (assessed using the Ferric ion reducing antioxidant power) was in the seaweed *P. gymnospora* during summer (FRAP: 9.7 ± 0.3 $X10^{-3}$ Fe^{2+}mM/g DW). However, the seaweed *P. gymnospora* extract and the extracts from all 5 seagrass species had significantly different ($p<0.01$) antioxidant activities (assessed using the Trolox equivalent antioxidant capacity assay) in winter compared to the summer season. The collective data are indicative of the potential of Mauritian seaweeds and seagrasses as possible sources of secondary metabolites for pharmaceuticals. Further analysis using bio-efficacy models are warranted to justify the phytoceutical capacity of the seaweeds and seagrasses.

KEY WORDS: ***Antioxidant; Total Phenols; Total Flavonoids; FRAP; TEAC; Seagrass; Seaweed; Seasonal variation; Mauritius***

INTRODUCTION

The human body is constantly exposed to free radicals, reactive oxygen species (ROS) and reactive nitrogen species (RNS). These include the hydroxyl radical ($OH^{\cdot}$), superoxide radical ($O_2^{\cdot-}$), peroxyl radical ($ROO^{\cdot}$), alkoxyl radical (RO) and nitric oxide ($NO^{\cdot}$). Hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), singlet oxygen ($1O_2$), ozone (O_3) and peroxynitrite ($ONOO^{\cdot}$) are not free radicals but can easily mediate damage to biological molecules. *In vivo* sources of free radicals arise from the reduction of molecular oxygen during respiration and from the synthesis of complex biochemical compounds[1,2]. External factors such as radiation, cigarette smoke, pollutants[3] and lipid peroxidation[4] contribute to a great extent to the formation of free radicals, ROS and RNS. Within the body, the production of ROS and antioxidant defenses are approximately balanced. When this balance is tipped in favor of ROS production, one of the outcomes is oxidative stress.. The latter contributes to cellular dysfunctioning by damaging DNA, proteins, lipids, and other biomolecules leading to numerous

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disorders and diseases such as Alzheimer's and Parkinson's diseases, cancer, stroke and diabetes among others[5,6]. Fortunately, the body has evolved intricate defense systems to reduce the cumulative load of ROS and RNS within cells[7]. These include protection afforded by the antioxidant enzymes superoxide dismutase, catalase, and glutathione peroxidase; the antioxidant response elements (ARE), low molecular weight antioxidants; some endogenously produced compounds (glutathione, NADH, carnosine, uric acid, melatonin, α-lipoic acid, bilirubin)[8,9] and others provided through dietary intake (ascorbic acid, tocopherols, ergothioneine, carotenoids, ubiquinol, quinones, phenolics). The implication of ROS/RNS, inflammatory processes and aberrant signal transduction pathways in degenerative diseases have led to suggest that antioxidants /anti-inflammatory agents and modulators of cell signaling can represent potential therapeutic applications. New avenues of research have focused at identifying novel therapeutic agents in particular plant based phenolics that could potentially disrupt the perpetual cycle of events involved in the etiology and progression of these detrimental processes. The tantalizing epidemiological links observed between diets high in fruits, vegetables, green and black tea decrease risks of cancer and other degenerative diseases[10] further emphasize the potential role of phenolic antioxidants as preventive or curative measures.

Seaweeds and seagrasses are reported to contain a plethory of antioxidant compounds with potent prophylactic benefits[11,12]. A number of seagrass species has been identified to have predominant growth inhibitory activity against human pathogens[13] and seaweeds have been reported to be a potential source of anti-cancer[14] agents. Seaweeds, also referred to as macroalgae, account for more than 150,000 species that are derived from the subtropical and tropical intertidal regions[15] and seagrasses account for more than 50 species with many still to be discovered[16]. Seagrasses are very often confused with seaweeds. As compared to seaweeds, which are thallophytes, seagrasses are monocotyledons that is, flowering plants. Seaweeds are classified in terms of their pigments, nutrient contents and chemical composition as Rhodophyta, Phaeophyta or Chlorophyta whereas seagrasses have no real taxonomical classification. They are said to be a polyphyletic group, thus, the 12 genera of seagrass cannot represent a "natural" taxon[17].

Very few studies have assessed the prophylactic activities of seagrasses as compared to seaweeds. Most of the works on seaweeds have emphasized on the characterization of antioxidant activities, (India, Korea) and the use of different antioxidant screening methods, (Japan) amongst others. Works on seagrass were mainly focused on phytochemical profiles[18], bioactivities in selected species and antioxidant capacities. Seaweed and seagrass both have a very high economical interest with US$ 4.8 billion in 2009 for seaweed in terms of world exportation[19] and approximately US$ 48.7 million for seagrass[20] in terms of ecological benefits. In the island of Mauritius, 435 seaweeds[21] and some 6 seagrass species[22] have been described with only preliminary works conducted on the phenolic and antioxidant profile of macroalgae[23].

The present study examined the phenolic content and the antioxidant capacities of seven seaweed and five seagrass species on a seasonal basis in the lagoon of Mauritius. The seaweeds and seagrasses were chosen according to their availability and their commercial importance. This study aims to contribute to the ongoing construction of a prophylactic antioxidant capacity bank in both terrestrial and marine species and providing a basis for potential industrial applications in the pharmaceutical, medical and food sectors.

METHODOLOGY

Collection of seaweed and seagrass species

The seven seaweed species studied were: *Padina gymnospora, Gracilaria salicornia*, *Palisada papillosa*, *Galaxaura rugosa*, *Enteromorpha intestinalis*, *Codium arabicum, Dictyosphaeria cavernosa* and the five seagrass species were: *Syringodium isoetifolium, Halodule uninervis, Thalassodendron ciliatum, Halophila ovalis* and *Halophila stipulacea*. The samples were freshly collected from four different sites in the West and North of Mauritius as shown in **Table 1**. The samples were kept in seawater in the dark and conveyed to the laboratory where they were washed thoroughly with tap water and preserved at -80°C till further analyses.

Physio-chemical parameters record

The physio-chemical parameters recorded were: temperature, pH, dissolved oxygen salinity and light intensity and they were taken only on the day of sample collection on all sites. Only in Albion, temperature and light intensity were recorded by an underwater data logger (HOBO pendant data logger). Temperature and light intensity were logged every 10-15 mins from August to November 2011 at Albion site only due to limited number of underwater data loggers.

Table 1: Collection sites and GPS Coordinates of the studied species

Species		Collecting Sites	GPS Coordinates
Seagrass	*Thalassodendron cilliatum*	Pointe aux Cannoniers	20°00'058"S 057°33'263"E
	Syringodium isoetifolium	Mont Choisy	20°00'455"S 057°33'271"E
	Halodule uninervis	Mont Choisy	20°00'455"S 057°33'271"E
	Halophila ovalis	Albion	20°12'32.83"S 057°24'13.04"E
	Halophila stipulacea	Albion	20°12'32.83"S 057°24'13.04"E
Seaweed	*Padina gymnospora*	Albion	20°12'31.35"S 057°24'13.89"E
	Gracilaria salicornia	Albion	20°12'31.35"S 057°24'13.89"E
	Palisada papillosa	Albion	20°12'31.35"S 057°24'13.89"E
	Galaxaura rugosa	Tombeau Bay	20°06'25.69"S 057°30'53.15"E
	Enteromorpha intestinalis	Pointe aux Cannoniers	20°00'10.88"S 057°34'9.10"E
	Codium arabicum	Pointe aux Cannoniers	20°00'10.88"S 057°34'9.10"E
	Dictyosphaeria cavernosa	Albion	20°12'31.35"S 057°24'13.89"E

Preparation of seaweed and seagrass extracts

50g fresh weight of each sample was homogenized using a Waring blender in methanol/water (80:20, v/v) (2x100 ml), was left to macerate for 72 hr at 4°C and then filtered. The residue was extracted in acetone/water (70/30, v/v) (2x100ml) for 72 hr at 4°C, followed by an exhaustive extraction in 100% acetone (2x100 ml) for 24 hr at 4°C. Extraction solvents were removed from collected filtrates *in vacuo* at 37°C. The extracts were then centrifuged and washed with dichloromethane to remove lipids and chlorophylls. The final aqueous extracts were concentrated *in vacuo*. Extracts were separated into two aliquots, one for flavonoid content analysis and the other for quantitative phenolic determination and antioxidant activity evaluation.

Determination of total phenolic content (TPC)

Phenolic contents of crude extracts were estimated using a method adapted from Singleton and Rossi[24]. 0.25 ml of extract was mixed with equal portion of Folin-Ciocalteu reagent, followed by 3.5 ml distilled deionized water. 1 ml of 20% sodium carbonate (Na_2CO_3) solution was then added after 3 minutes. The content of the test tube was mixed thoroughly and was then incubated at 40°C for 40 minutes. Absorbance of all samples was measured at 685 nm using a spectrophotometer (Unicam Instruments, Cambridge, UK). Phenolic contents are expressed as mg GAE g^{-1} dry weight of sample and data were expressed as means ± standard deviation of mean (±SD) from independent experiment performed in triplicates.

Determination of total flavonoid content (TFC)

The Aluminium Chloride ($AlCl_3$) method adapted from Lamaison and Carnet[25] was used to determine the TFC of crude extracts of test seaweeds and seagrasses. 1 ml methanolic extract was mixed with equal portion of 2% aluminium chloride ($AlCl_3.6H_2O$). The solution was incubated at room temperature for 10 minutes. Absorbance was then read at 440 nm using a spectrophotometer (Unicam Instruments, Cambridge, UK). Results are expressed in µg Quercetin g^{-1} dry weight of sample and data were expressed as means ± standard deviation of mean (±SD) from independent experiment performed in triplicates.

Determination of antioxidant activities

Ferric Reducing Antioxidant Power Assay

The reducing power determination of the samples was carried according to the method described by Benzie and Strain[26]. The principle of this assay is

based on the ability of extracts to reduce Fe(III)-2,4,6-Tri(2-pyridyl)-striazine (TPTZ) complex to Fe (II)-TPTZ, which result in a dark blue coloration related linearly to the amount of antioxidant present. Freshly prepared FRAP reagent consists of 20 ml of 10mM TPTZ solution in 40 mM hydrochloric acid (HCl) and 20 ml of 20 mM ferric chloride in 200 ml of 0.25M acetate buffer (pH 3.6) at 37°C. An aliquot of each sample (50 µl) was mixed with 150 µl of distilled water, followed by 1.5 ml of FRAP reagent. The resulting coloration was read at 593 nm after 4 minutes incubation. Result was expressed as µmol Fe^{2+} g^{-1} dry weight and data were expressed as means ± standard deviation of mean (±SD) from independent experiment performed in triplicates.

Trolox Equivalent Antioxidant Capacity Assay

The total antioxidant activity of the crude seaweed and seagrass extracts were assessed using the TEAC assay according to the method of Campos and Lissi[27]. This assay measures the ability of the antioxidant substances found in the extract to scavenge the 2,2′-azino-bis (3,ethyl benz-thiazoline-6-sulfonic acid) radical cation ($ABTS^{\bullet+}$) relative to the standard amounts of synthetic antioxidant Trolox, which is a water-soluble vitamin E analogue. $ABTS^{+}$ solution was generated by a reaction between ABTS (0.5 mM) and activated MnO_2 (1 mM) in phosphate buffer (0.1 M, pH 7). To 3 ml of $ABTS^{+}$ radical solution, 0.5 ml aqueous extract was added and the decay in absorbance was monitored at 734 nm for 15 minutes on a spectrophotometer (Unicam Instruments, Cambridge, UK). Results are expressed as µmol Trolox equivalent g^{-1} dry weight of sample and data were expressed as means ± standard deviation of mean (±SD) from independent experiment performed in triplicates.

Statistical analysis

The seaweeds and seagrass extracts were analysed for phenolic contents and antioxidant activities using Spectronic Unicam spectrophotometer (Unicam Instruments, Cambridge, United Kingdom) interfacing with Unicam UV-Visible Spectrometry Vision 32-bit (version 1.22) and expressed as per dry weight. Means differences were determined by two-way ANOVA and was followed by Tukey's HSD comparison test using STATISTICA software (version 10.0). Correlations between antioxidant activities (FRAP and TEAC) and total phenol content and flavonoids were computed as Pearson's correlation coefficient (r) using SPSS (version 16.0).

RESULT

Physico-chemical parameters

The temperature varied from site to site with a higher temperature in summer at Mont Choisy (29.6 ± 0.1°C) (**Table 2**). The pH was slightly alkaline in winter at all collection sites and was more or less neutral in summer except for Albion and Tombeau Bay whereby the pH remained slightly alkaline in both seasons. Dissolved oxygen remained relatively unchanged during winter and summer and salinity showed no significant variation seasonally. As winter moved towards the end, an increase in temperature from 24.53 ± 0.57°C to 27.55 ± 1.14ºC was observed **(Figure 1)**. Also, light intensity was noted to increase constantly within this transition.

Table 2: Physico-chemical parameters at the four sampling sites in winter (August 2011) and summer (November 2011). The values are expressed as mean ± SD (n=3). N.D = not determined

	Sampling Sites							
Physical	**Pointe Aux Cannoniers**		**Mont Choisy**		**Tombeau Bay**		**Albion**	
Parameters	**Winter**	**Summer**	**Winter**	**Summer**	**Winter**	**Summer**	**Winter**	**Summer**
Temperature (°C)	23.5±1.0	29.2±1.0	24.0±1.0	29.6±0.1	24.5±0.5	26.9±0.4	24.5±0.5	27.5±1.1
pH	8.20±0.2	7.94±0.2	8.32±0.3	7.11±0.3	7.89±0.46	8.10±0.17	8.40±0.2	8.43±0.2
Dissolved Oxygen (mg/L)	6.2±0.2	6.0±0.1	6.2±0.2	6.0±1.0	N.D	N.D	6.0±0.2	5.6±0.2
Salinity (ppt)	35.0±0.1	35.5±0.1	35.0±0.1	35.4±0.2	35.2±0.12	35.1±0.06	35.7±0.3	35.8±1.0

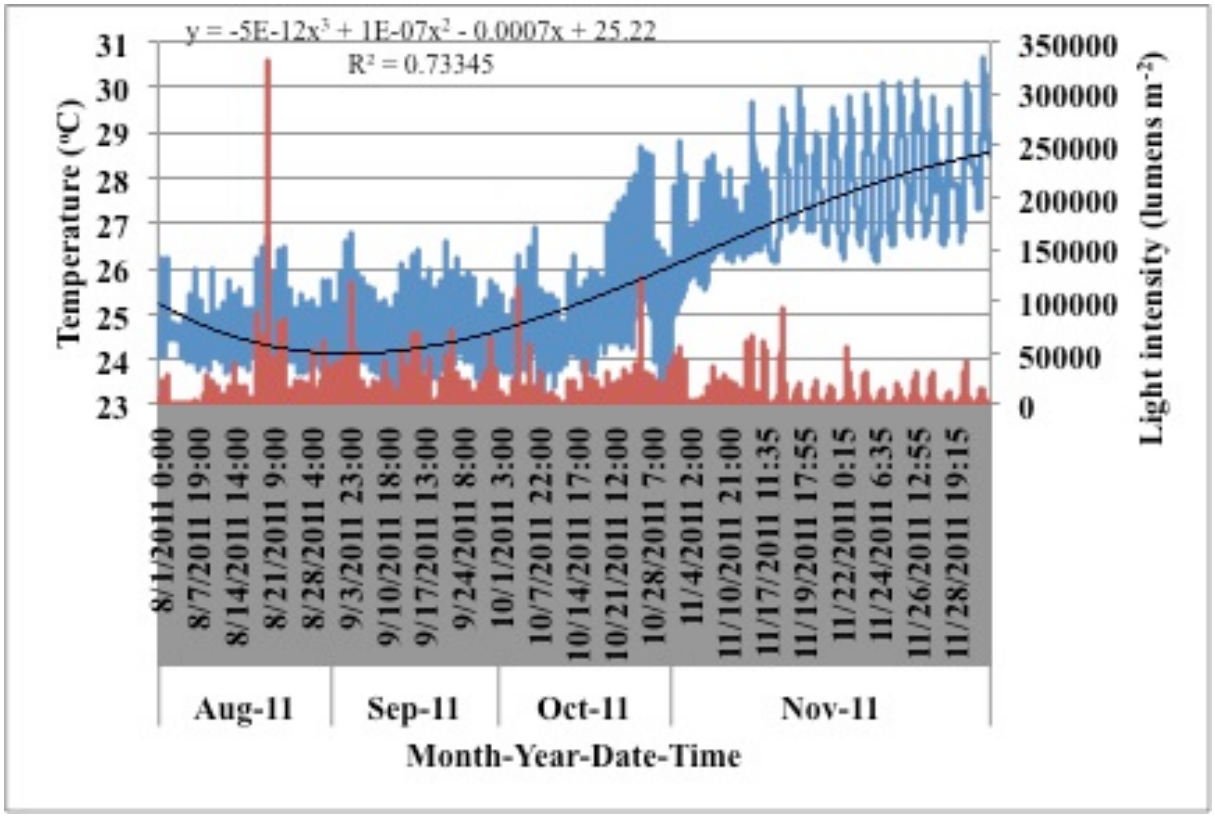

Figure 1: Temperature and light intensity records (every 5-15 minutes) using the Hobo Temperature and light data logger at Albion from August to November 2011. The August and November 2011 mean±SD temperature data were 24.53±0.57°C (2976 data points) and 27.55±1.14°C (5493 data points), respectively

Total phenol content of seaweeds and seagrasses

TPC was higher in *Padina gymnospora* in summer whereas *Thalassodendron ciliatum* contained the greatest level in winter. FC was maximum in *Enteromorpha intestinalis* in winter while *Padina gymnospora* contained the highest concentration in summer compared to the other species.

TPC was observed to vary in summer and in winter (**Figure 2**). Tukey tests showed that the TPCs were not significantly different between winter and summer in almost all species with the exception of *Padina gymnospora* and *Halodule uninervis* which showed a very high significant difference ($p<0.001$) between its winter and summer contents (TPC). It was also observed that TPC was dominant in seagrasses in both winter and summer as compared to the seaweed species with the exception of *Padina gymnospora* (119.3 ± 0.2 mg Gallic acid / g DW in summer).

Total flavonoid content in seaweeds and seagrasses

FC was observed to be much higher in winter for most species of seaweeds and seagrasses. The Tukey test revealed that there were very high significant differences in FC values for winter and summer in three species of seaweed namely *Padina gymnospora, Enteromorpha intestinalis* and *Palisada papillosa* ($p<0.01$, $p<0.001$ and $p<0.001$ respectively) and two species of seagrass namely *Halodule uninervis* and *Halophila ovalis.* The highest flavonoid concentration was observed in *E. intestinalis* (13.0 ± 1.0 mg Quercetin / g DW) in winter and in *P. gymnospora* (2.1 ± 0.0 mg Quercetin / g DW) in summer whereas the lowest flavonoid level occurring in both winter and summer was in the seaweed *G. rugosa* (0.1 ± 0.0 mg Quercetin / g DW for both) (**Figure 2).**

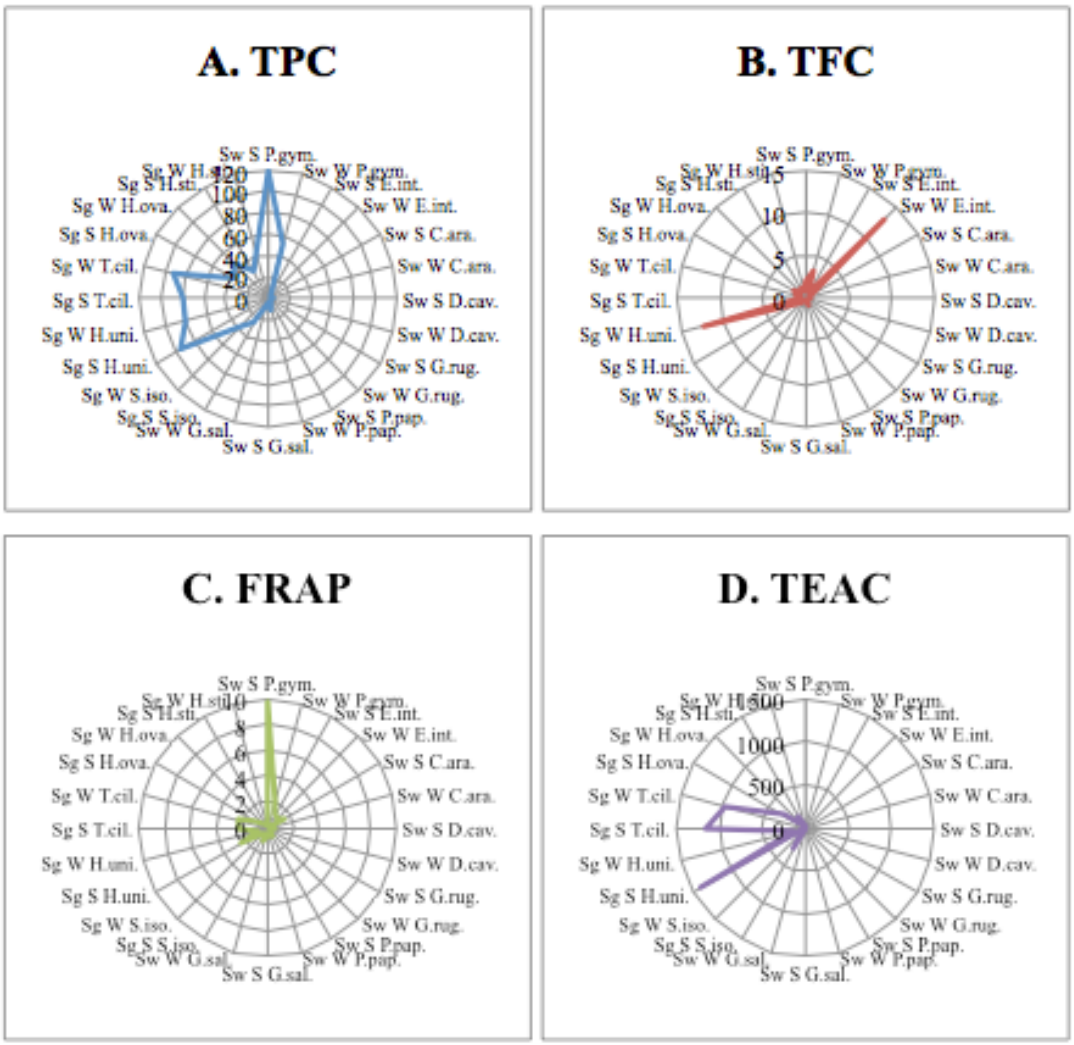

Figure 2: Radar-diagrams showing (A): total phenol contents (mg Gallic acid / g DW), (B): flavonoid contents (mg Quercetin / g DW) and antioxidant activities using (C): FRAP assay (X10⁻³ Fe^{2+} mM/g DW) and (D): TEAC assay (Trolox mM/g DW) of seven species of seaweeds and five species of seagrasses in winter and summer. Data represents Mean ± SD (n=3). The abbreviations on the diagrams represents: Sw S P.gym = Seaweed Summer *Padina gymnosphora*, Sw W P.gym = Seaweed Winter *Padina gymnosphora*, Sw S E.int = Seaweed Summer *Enteromorpha intestinalis*, Sw W E.int = Seaweed Winter *Enteromorpha intestinalis*, Sw S C.ara = Seaweed Summer *Codium arabicum*, Sw W C.ara = Seaweed Winter *Codium arabicum*, Sw S D.cav = Seaweed Summer *Dictyospharia cavernosa*, Sw W D.cav = Seaweed Winter *Dictyospharia cavernosa*, Sw S G.rug = Seaweed Summer *Galaxaura rugosa*, Sw W G.rug = Seaweed Winter *Galaxaura rugosa*, Sw S P.pap = Seaweed Summer *Palisada papillosa*, Sw W P.pap = Seaweed Winter *Palisada papillosa, Sw S G.sal*= Seaweed Summer *Gracilaria salicornia*, Sw W G.sal = Seaweed Winter *Gracilaria salicornia*, Sg S S.iso = Seagrass Summer *Syringodium isoetifolium*, Sg W S.iso = Seagrass Winter *Syringodium isoetifolium*, Sg S H.uni = Seagrass Summer *Halodule uninervis*, Sg W H.uni = Seagrass Winter *Halodule uninervis*, Sg S T.cil= Seagrass Summer *Thalassodendron ciliatum*, Sg W T.cil = Seagrass Winter *Thalassodendron ciliatum*, Sg S H.ova = Seagrass Summer *Halophila ovalis*, Sg W H.ova = Seagrass Winter *Halophila ovalis*, Sg S H.sti = Seagrass Summer *Halophila stipulacea*, Sg W H.sti= Seagrass Winter *Halophila stipulacea*

Antioxidant activities in seaweeds and seagrasses

Antioxidant activities were observed to be higher in summer (FRAP assay) with the highest potency being recorded in *P. gymnospora* (9.7 ± 0.3 X10⁻³ Fe^{2+}mM/g DW). Both *P. gymnospora* and *T. ciliatum* had the highest antioxidant activities in winter (2.3 ± 0.1 X10⁻³ Fe^{2+} mM/g DW and 2.3 ± 0.2 X10⁻³ Fe^{2+}mM/g DW respectively). The lowest antioxidant activities observed in winter were in the seaweed *C. arabicum* and the seagrass *H. stipulacea* (0.2 ± 0.0 X10⁻³ Fe^{2+} mM/g DW for both) and in *G. rugosa* and *H. stipulacea* (0.3 ± 0.1 and 0.3 ± 0.0 X10⁻³ Fe^{2+} mM/g DW respectively) in summer. The antioxidant activities in winter and

summer (FRAP assay) showed no significant difference between the species with the exception of *P. gymnospora, C. arabicum* and *H. uninervis* which had a very high significant difference ($p<0.001$) between its antioxidant activities seasonally.

The Tukey test revealed that all the seaweed species had no significant difference in the antioxidant activities (TEAC assay) in winter and summer with the exception of *P. gymnospora* whereby its antioxidant activities were significantly different ($p<0.01$) in both seasons. The antioxidant activities of the seagrass species were significantly different in winter and summer ($p<0.001$ for all). The TEAC assay showed that antioxidant activities in the seagrass species were very much higher than that in the seaweed species and that summer was the season where the antioxidant activities were more potent. The highest antioxidant activities were recorded in the seagrass species *H. uninervis* ($1405.8 \pm 41.6 \times 10^{-3}$ Trolox mM/g DW) in summer and *T. ciliatum* ($964.7 \pm 2.4 \times 10^{-3}$ Trolox mM/g DW) in winter.

Relationship between polyphenolic contents and antioxidant activities in the seagrass and seaweed species.

As data were not normally distributed, Pearson's correlation test was used to determine the relationship between the polyphenolic contents and their antioxidant activities seasonally (**Table 3)**.

Table 3: Pearson Correlation Table for the seagrass and seaweed species during winter and summer

Pearson Correlation Test								
Season	Winter				Summer			
Parameters	FRAP		TEAC		FRAP		TEAC	
	Correlation Coefficient, p	Correlation Coefficent,r	Correlation Coefficient, p	Correlation Coefficent,r	Correlation Coefficient, p	Correlation Coefficent,r	Correlation Coefficient, p	Correlation Coefficent,r
Padina gymnosphora								
TPC	0.843	0.244	0.165	-0.967	0.704	0.448	0.188	-0.957
FC	0.142	0.975	0.537	-0.665	0.448	0.762	0.964	-0.560
Entheromorpha intestinalis								
TPC	0.629	-0.550	0.924	0.119	0.868	-0.206	0.654	0.518
FC	0.245	0.927	0.691	-0.466	0.204	-0.949	0.419	0.792
Codium arabicum								
TPC	0.982	0.280	0.110	0.985	0.856	0.224	0.142	-0.975
FC	0.973	0.319	0.079	0.992	0.214	0.944	0.500	-0.708
Dictyospharia cavernosa								
TPC	0.542	0.659	0.587	0.605	0.750	-0.383	0.024	0.999
FC	0.350	-0.853	0.305	-0.888	0.466	0.744	0.260	-0.918
Galaxaura rugosa								
TPC	0.992	0.013	0.835	0.256	0.556	0.642	0.638	-0.538
FC	0.331	-0.868	0.505	0.702	0.029	0.999	0.834	0.258
Palisada papilosa								
TPC	0.315	-0.880	0.933	0.105	0.382	-0.825	0.553	-0.646
FC	0.622	-0.559	0.626	0.554	0.325	-0.872	0.497	-0.711
Gracilaria salicornia								
TPC	0.338	-0.820	0.084	-0.991	0.131	-0.979	0.719	0.427
FC	0.642	0.533	0.935	-0.102	0.120	-1.000	0.861	0.216
Syringodium isoetifolium								
TPC	0.247	-0.925	0.273	0.909	0.509	-0.697	0.827	0.268
FC	0.460	0.997	0.474	-0.735	0.964	0.057	0.719	0.428
Halodule uninervis								
TPC	0.569	-0.627	0.923	0.120	0.863	-0.213	0.783	0.334
FC	0.528	0.675	0.021	0.999	0.912	0.138	0.912	-0.012
Thalassodendron ciliatum								
TPC	0.112	0.985	0.611	0.574	0.113	-0.984	0.743	0.393
FC	0.463	0.747	0.036	0.998	0.984	-0.025	0.160	-0.968
Halophila ovalis								
TPC	0.263	0.916	0.259	0.919	0.411	0.799	0.530	0.673
FC	0.468	-0.742	0.472	-0.737	0.168	0.966	0.286	0.901
Halophila stipulacea								
TPC	0.346	0.856	0.193	0.955	0.867	0.207	0.046	-0.997
FC	0.225	-0.938	0.764	-0.362	0.662	-0.507	0.159	0.969

There were no strong positive correlations between TPC and antioxidant activities during winter and summer in the FRAP assay. The strongest positive correlations were with *T. ciliatum* ($r=0.985$, $p=0.112$) during winter and in *H. ovalis* ($r=0.799$, $p=0.411$) during summer which were both seagrass species. A striking positive correlation between TPC and antioxidant activities was observed in the

seaweed specie *D. cavernosa* (r=0.999, p=0.024) during summer and a strong negative correlation was calculated in the seagrass *H. stipulacea* (r=-0.997, p=0.046) during summer itself.

Strong positive correlations were observed between FC and antioxidant activities in the seagrass species. The FC in *S. isoetifolium* was very closely related to the FRAP (r=0.997, p=0.460) during winter. A strong correlation between FC content and TEAC values was also evident in two seagrass species, *H. uninervis* (r=0.999, p=0.021) and *T. ciliatum* (r=0.998, p=0.036) during winter. During summer, the FC in *G. rugosa* correlated highly with FRAP data (r=0.999, p=0.029) while a very strong negative correlation was observed between the FC and the FRAP activities in *G. salicornia* (r=-1.000, p=0.120).

DISCUSSION

In the present study, five seagrass and seven seaweed species were quantitatively assessed for their total phenol, flavonoid contents and antioxidant activities. Our results demonstrate that the total phenolic content was significantly higher in the seagrasses than in the seaweeds in both winter and summer thereby impacting on their antioxidant propensities. The seaweed *Padina gymnospora* [TPC: 119.3 ± 2.0 mg Gallic acid/g DW (Summer), TEAC: 22.1 ± 1.0 Trolox mM/g DW (Summer)], and the seagrasses *Thalassodendron ciliatum* [TPC: 91.3 ± 8.5 mg Gallic acid/g DW (Winter), TEAC: 1166.5 ± 17.6 Trolox mM/g DW (Summer)] and *Halodule uninervis* [TPC: 94.2 ± 2.1 mg Gallic acid/g DW (Summer), TEAC: 1405.8 ± 41.6 Trolox mM/g DW (Summer)] were the three species with the highest phenolics and most potent antioxidant activities.

In a study assessing the phenolic content and antioxidant profile of shallow water seaweeds from Mauritius, Somanah *et al.*[28] reported that *Padina gymnospora* was relatively poor in phenols with low antioxidant propensity. In that study, samples were collected between the months of September and November at a depth of less than 1m. The high level of phenols observed in the same species in this study may be influenced by a relatively higher temperature (24.5±0.5 °C in winter and 26.9±0.4 °C in summer) and/or solar irradiance at the collection site. Temperature rise generally results from an increase in sunlight intensity. Due to their intrinsic properties, phenolic compounds have been reported to exert protective effects against UV radiation as evidenced in red and brown algae[29] where they exist in the form of mycosporine. Furthermore, it has been reported that the level of phenolic compounds of algae usually increases with excessive exposure to UV radiations[30].

The TPC data obtained for both seaweeds and seagrasses in this study were relatively higher compared to studies conducted for instance by Athiperumalsami *et al*[13,19]. Folin-Ciocalteau assay is based on the principle of oxidation-reduction reaction. Sugars, amino acids and other phenolic derivatives (flavanols, flavonoids, flavones, phenolic acids and flavanones) can favourably interfere with the reaction thus, giving an overestimation of the total phenolic contents[31]. Moreover, the TPC data for *Syringodium isoetifolium* in the present study (25.8 ± 1.4 mg Gallic acid/g DW) was found to be inconsistent with those reported by Rengasamy *et al*[32] (3.94 ± 0.265 mg Gallic acid/g DW). Different stress factors may influence the physiology and biochemistry of seagrasses. According to Berns[33], salinity variation effect on *Thalassia testudinum* and *Ruppia maritima* induces changes in physical responses causing an increase in stress in the seagrass. The studied site where both *Halophila* species were collected had a very high salinity gradient in both winter and summer as compared to the other sites. Literature data show that total phenols were increased with salinity stress in the following mangrove species: *Ceriops roxburghiana*, *Crithmum maritimum* and *Aegiceras corniculatum*. Several other reports have shown higher levels of polyphenols in different tissue types under increasing salinity stress[34,35]. The stressed environment is believed to be responsible for primary producers to produce these secondary products as an adaptive mechanism against stress-induced oxidative kinase.

The determination of flavonoids in the seagrasses was conducted using the Aluminium Chloride method with quercetin as standard. Low concentrations of flavonoids during both winter and summer were found in all species except in the seagrass *Halodule uninervis* and the seaweed *Enteromorpha intestinalis* which had a significantly higher ($p > 0.001$) flavonoid content as compared to the other test species in winter only (3.4 ± 0.2 mg Quercetin / g DW and 12.4 ± 2.6 mg Quercetin / g DW, respectively). Factors generally contributing to phenolic variations and *in extenso* to flavonoid composition can include treatment mode of samples prior to extraction. Chilling and lyophilizing have been pointed out to be the causes of reduction of phenolic yields notably flavonoids: 39% by chilling and 87% by freeze-drying in the seagrass *Posidonia oceanica*[36]. In addition, phenolic and flavonoid contents have been reported to vary due to seasonal changes (i.e.transition from winter to summer) and the degree of maturation of the plant parts. It is noteworthy that the biosysnthesis of flavonols has been documented to be light-dependent and can also be affected by temperature variation[37-38].

Flavonoids have been emphasized to interrupt the propagation of autoxidation of free radicals by contributing a hydrogen atom from several hydroxyl (OH) bases that are attached outside the benzene rings, resulting in the formation of stable free radical that does not initiate or propagate further oxidation processes[39]. These groups of polyphenolic compounds are very important in plants as they make up their defensive mechanisms[40]. In the context of human health they provide the prospect to be used as adjunct therapy to modulate markers of oxidative stress. Frankel and Meyer[41] suggested the use of a multi-method approach to determine the antioxidant effect and action mechanism of an extract since no one method can predict its total antioxidant efficiency. Thus, two independent methods; FRAP and TEAC differing in biological action mechanisms were used to provide an indicative mechanistic insight of the antioxidant actions of the extracts under study. It was noted that the brown seaweed *Padina gymnospora* had a very high antioxidant action in both seasons when both FRAP and TEAC assays were used. FRAP assay works best in acidic condition (pH 3.6). On the other hand, compounds such as proteins, thiols and water-soluble compounds such as carotenoids are not detected by the FRAP method, as they are involved in radical quenching[42-44] which may, to some extent, explain the important antioxidant activity of *Padina gymnospora* as compared to the other species tested in this study. Parameters such as dissolved oxygen content and salinity are known to be potential elements that could influence the antioxidant activity[45]. From the study of Somanah *et al.*[28], *Padina gymnospora* had a lower antioxidant capacity compared to this study. It is also noted that both antioxidant propensities obtained in the seaweed species in the present study and that of Somanah *et al*[28] were significantly different whereby the former showed higher antioxidant statuses in the TEAC assay. Among the seagrass species, the highest antioxidant activities occurred in *Halodule uninervis* ($1405.8 \pm 41.6 \times 10^{-3}$ Trolox mM/g DW) in summer and in *Thalassodendron ciliatum* ($964.7 \pm 2.4 \times 10^{-3}$ Trolox mM/g DW) in winter and showed to be higher than those of the seagrass species tested in the study of Athiperumalsami *et al*[13,19]. It is plausible that the pigments found in the seaweeds and seagrass species could have contributed to the increase of the antioxidant level. It has been demonstrated that naturals products from marine algae such as phycoerythrobilin, chlorophyll *a*, chlorophyll *b* and fucoxanthin which are accessory pigments have established antioxidant activities[46] thus, reasonably contributing to the overall synergistic antioxidant capacity in *Padina gymnospora* and the seagrasses. Flavonoids can potentially influence the antioxidant capacity in plant based extracts as reported by literature data. Chaillou *et al*[47] suggested that the antioxidant activity of flavonoids is strongly structure dependent. In this study, however, flavonoid levels seem to be relatively too low to be linked directly to the observed activities. As such TPC, encompassing the major polyphenolic classes, would most probably be the major contributor to the observed antioxidant activities. This is supported by the statistically significant correlations obtained by linear regression analysis (**Table 3**).

CONCLUSION

This study highlights that seagrass species were richer sources of natural antioxidants than seaweeds. Relatively important phenol levels linked to potent antioxidant activities were observed during summer for both seaweeds and seagrasses, the latter being more potent than the seaweeds. The data generated, though preliminary, therefore contributes to the development of the data base on Mauritian marine organisms presenting potential pharmaceutical and medical applications. Further studies using bio-efficacy models are necessary to justify the bioactivity of the secondary metabolites extracted from the seaweed and seagrass species investigated.

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