Regional nutritional profile and antioxidant activity of *Gelidium sesquipedale* from the Moroccan Atlantic coast

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Abstract

Gelidium sesquipedale is a red seaweed exploited in Morocco for its agar-agar quality. Samples were examined across the Moroccan Atlantic coast to evaluate their nutritional composition and antioxidant activity. The objective was to find out the regional impact on the biochemical properties of this seaweed. The obtained results have shown that the region factor does not affect the carbohydrate content. However, protein content was higher in the western sites (15.77% in Lahdida and 16.79% in Sidi-Rahal) compared to those from the southern sites (14.08% in Sidi El Ghazi and 14.76% in Amgriou). Regarding the phenolic contents, the results are similar in the west (5.69 and 5.38 mg gallic acid equivalent (GAE)/g respectively in Sidi-Rahal and Lahdida) but differ in the south (6.22 mg GAE /g in Amgriou and 4.78 mg GAE /g in Sidi El Ghazi) due to stranding phenomena in Amgriou. Moreover, this seaweed exhibits the highest radical scavenging capacity (DPPH) and Ferric Reducing Antioxidant Power (FRAP) values in the south (90.41 and 114.33 µg/mL in Amgriou; 103.76 and 110.5 µg/mL in Sidi El Ghazi). Hence, the exploitation of *Gelidium sesquipedale* harvested on the western and southern coasts of Morocco should extend beyond the sole production of agar-agar.

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Introduction

Red algae or Rhodophyceae constitute the most important algal biomass in the world. Their global production accounts for around 60% of total algae production.[1] They are mainly intended for the extraction of agar and carrageenan but also include additional substances with dietary, biological, and beneficial properties. The amounts of these compounds differ depending on species, seasons, and geographical locations.[2] In 2020, Moroccan algal production reached slightly over 22 thousand tons, with *Gelidium sesquipedale* accounting for 93% of this total as the main seaweed harvest along the Moroccan Atlantic coast.[3] This production has two purposes: about 20% of it is intended for export in its raw state, while the rest is transformed locally into agar by the only transformation plant located in Kenitra.

Gelidium sesquipedale is a valuable economic resource for Morocco where it is harvested. Sustainable harvesting practices are crucial to ensure the long-term viability of *Gelidium sesquipedale* and other seaweed species. Overharvesting can lead to ecological imbalances and affect the marine ecosystem. Responsible management and monitoring are essential to preserve these valuable marine resources.

Gelidium sesquipedale, a red alga classified in the order Gelidiales and the family Gelidiaceae, is considered a valuable natural repository of essential biomolecules. Polysaccharides extracted from algal biomass form a highly intricate family known for their texturizing properties such as gelling, thickening, emulsifying, and

stabilizing.[4,5] These agars find numerous applications across various sectors of the food and pharmaceutical industries. Moreover, other interesting molecules are found in *Gelidium sesquipedale* proteins, lipids, polyphenols, and antioxidant activity capacity.[6,7,8]

The variability of algal composition in aquatic ecosystems is significantly influenced by geographical location.[9] Algae are highly responsive to environmental conditions, including abiotic factors like temperature, sunlight, nutrient availability, and water chemistry.[10] Since different regions of the world have unique climatic and geographic characteristics, they create diverse habitats that support varying algal communities. For instance, tropical regions with warm temperatures and abundant sunlight foster a wide array of algae species, resulting in a rich and diverse algal composition.

Conversely, colder temperate regions harbor different species of algae adapted to lower temperatures and nutrient levels.[11] Additionally, human activities and pollution in specific geographic areas can further alter algal communities, leading to shifts in species distribution.[12]

In our study, we aim to carry out an in-depth assessment of the physicochemical traits defining *Gelidium sesquipedale* at the national level, marking the first example of such an investigation. Additionally, we explored the influence of different regions by evaluating four categories of samples collected from the main harvesting Moroccan Atlantic coasts, namely: Amgriou site near Laâyoune city (27° 68' N - 13° 16' W) and Sidi El Ghazi site near Boujdour city (34° 46' 52" N; Longitude 5° 40' 28" W), two sites in the south-west of Morocco that are approximately 1000 km farther away from the first sites; Lahdida site near El-Jadida city (33° 24' 27,56" N - 08° 08' 55,55" W) and Sidi-Rahal site near Casablanca city (33° 38' 45,85" N - 07° 28' 23,88" W), two locations in the west of Morocco situated at a distance of around 30 km from each other and sharing a nearly identical marine environment.

Materials and Methods

Materials

The samples used in this study were sun-dried raw algal masses collected from the selected coasts at the end of July 2021. At the Quality Control Laboratory - Fisheries Technology Department of the Higher Institute of Maritime Fisheries (ISPM) in Agadir city, the algae underwent further sorting to isolate only the *Gelidium sesquipedale* species and remove impurities such as corals and sand. The cleaned algae were then dried in an oven for 48 hours. For analysis, some samples were dried at 30°C to determine polyphenol content, while others were dried at 60°C to assess other algal components. The dried thallus was then crushed.

Chemicals

Folin-Ciocalteu's reagent, Gallic Acid, Sulphuric Acid 98%, and bovine serum albumin (BSA) were brought from Loba Chimie PVT LTD (India), and 2,2-Diphenyl-1-picrylhydrazyl-free radical (DPPH) 98% powder from ALTA AESARA (USA). Coomassie Brillant Blue G 250 was purchased from SERVA (USA). Na_2CO_3, phenol 5%, Trichloroacetic Acid (TCA), NaOH, methanol, HCl, and Ferric Reducing Antioxidant Power (FRAP) assay were provided from Panreac (EU).

Moisture content and water activity

The moisture content was obtained by drying each sample at 105 °C for 24 hours in a drying oven (Shanghai Boxum, China).[13] Water activity was measured by a Portable Water Activity Analyzer (Hygropalm HP23, Switzerland).

Ash content

The ashes were assayed by physical mineralization using a muffle furnace (Nabertherm, Germany), at 550°C for 6 hours.[14]

Macronutrient contents

Total lipid content

Total algal lipids were extracted using the method described by Bligh and Dyer,[15] with few modifications. The dry powder of the sample was suspended in a mixture of chloroform/methanol/water (4:2:1). The mixture was vortexed for 5 min and centrifuged. The lower layer containing the lipid extract was collected, evaporated, and weighed.[16]

Total carbohydrate content

The total carbohydrate content in dried biomass was estimated using the Phenol-sulphuric acid method.[17] Using a UV/visible single beam spectrophotometer (Akribis Scientific Limited, UK). The extraction was performed using 2.5N HCl,[18] and the absorbance of the sample was determined at 490 nm. The results were calculated based on the obtained glucose standard curve ($y=0.0006x$; $r^2=0.99152$) performed with glucose concentrations between 0 and 1 g/L.

Total protein content

Algae protein content was obtained by the Bradford method[19] with a few modifications. Samples of dried vegetal material were digested in 1 N NaOH for 18 h with occasional shaking.[20] The protein content was determined based on a standard curve ($y=0.001x$; $r^2=0.959$) of BSA at 595 nm.

Extract phenolic preparation

The dried powdered samples were soaked in methanol/water (70:30) at a ratio of 1g/10 mL and mixed well using a vortex for 1 minute. The mixtures were then centrifuged at 1400 rpm for 20 minutes and filtered through filter paper.[21]

Total phenolic content

To measure the total phenolic content (TPC), an aliquot of 0.1 mL of the extracts of MeOH extract was mixed with 0.5 mL of Folin–Ciocalteu reagent. The mixture was allowed to stand for 5 minutes before adding 0.4 mL of aqueous solution of Na_2CO_3 7.5%. The solution was then incubated at room temperature for 20 minutes. The absorbance of the blue color produced was determined at 765 nm using a spectrophotometer.[22] A calibration curve of gallic acid was plotted to determine TPC, which was expressed as milligrams of gallic acid equivalents (GAE) per gram of extract ($y=0.0004x$; $r^2=0.96842$).

Antioxidant activity analysis

Radical scavenging capacity

One mL of algal methanol extract was placed in a test tube and added to 2.5 mL of 0.3 mM DPPH solution.[21] The mixture was

vortexed for 1 min and allowed for 30 min in the dark. The absorbance was determined at 518 nm with the spectrophotometer. The percentage of inhibition (I %) was measured using the Eq. (1).

$$I\ \% = [A_0 - A_1/A0] \times 100 \quad (1)$$

where A_0 and A_1 are respectively the control and sample absorbance.

Graphs of scavenging effect percentage depending on extract concentrations were established for each sample (Figure 1A). The extract concentration providing 50% inhibition (IC_{50}) was concluded from these graphs.

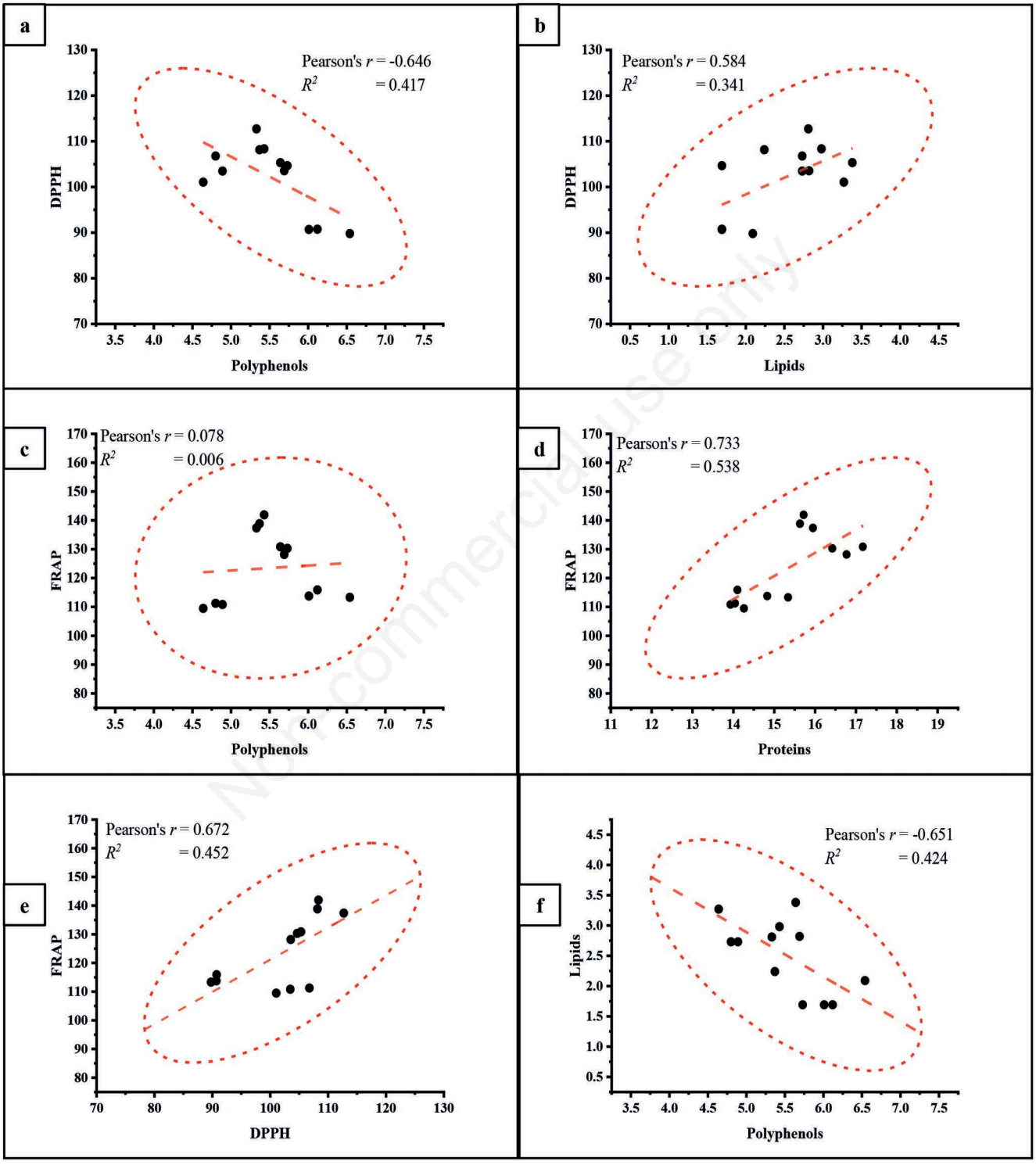

Figure 1. Illustrations of the most significant correlations and absence of correlation between the antioxidant activities and nutrients. a) DPPH-Polyphenols; b) DPPH-Lipids; c) FRAP-Polyphenols; d) FRAP-Protein; e) FRAP-DPPH; f) Lipids-Polyphenols.

Ferric reducing antioxidant power

Various concentrations of extract of each sample were added with 2.5 mL of a 0.2 M phosphate buffer solution (pH=6.6) and 2.5 mL of a 1% potassium ferricyanide solution ($K_3Fe(CN)_6$) and then incubated at 50°C for 20 min. The reaction was stopped by adding TCA solution (10% w/v). The whole was centrifuged at 6000 g for 10 min; 2.5 mL of the supernatant of each mixture was then diluted with 2.5 mL of distilled water and 0.5 mL of the ferric chloride solution (0.1% w/v). The absorbance was measured at 700 nm[23] and the concentration corresponding to 50% inhibition (IC_{50}) was extracted from graphs expressing the absorbance as a function of inhibition concentrations for the four samples (Figure 2 B).

Statistical analysis

All results are reported as the mean±standard deviation. The data were analyzed through pairwise multiple comparison procedures specifically Duncan's multiple range comparison test (DMRT) with SPSS statistics v.26, with a 5% level of probability to demonstrate statistical significance difference. The results of

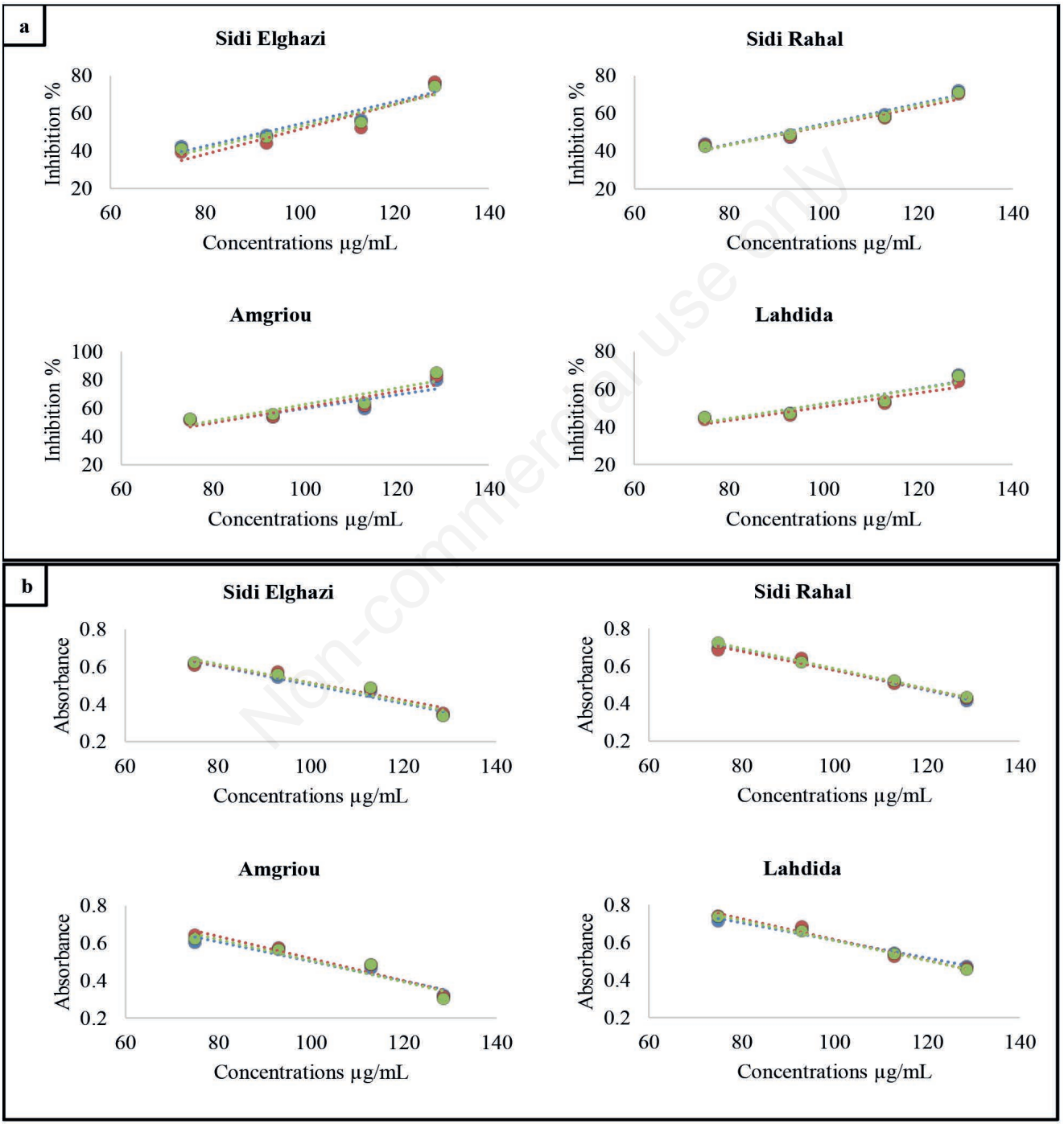

Figure 2. Variation of inhibition percentage (a) for DPPH test and absorbance for FRAP assay (b) as a function of the extract concentrations.

the following analysis (lipids, proteins, carbohydrates, and phenolic contents) were expressed as a percentage of the total dry weight (d.w.).

Results

Moisture and water activity

The moisture content of *Gelidium sesquipedale* ranged from 8.33% to 12.84% d.w., with the highest moisture content found at Lahdida and the lowest at Sidi El Ghazi. The water activity values of the dried seaweed samples align with their humidity levels as presented in Table 1. Overall, this species seems to have a low water presence, which contributes to the excellent preservation of the seaweed powder.

Ash

Our analysis of *Gelidium sesquipedale* revealed that the ash contents of the samples collected from the southern zones (26.01% d.w. Amgriou; 24.16% d.w. Sidi El Ghazi) were significantly higher compared to the other two zones, Sidi-Rahal and Lahdida. The samples from Sidi-Rahal and Lahdida exhibited relatively important ash content as well, with values of approximately 22.82% and 20.74% d.w., respectively (Table 1). These findings are consistent with the results conducted on *Gelidium sesquipedale* from the coasts of Spain (22% d.w.),[6] and higher than those found for *Gelidium corneum* (11.30% d.w.).[10]

Macronutrients

Total lipid

The results obtained from this study regarding the total fat content in the analyzed samples ranged from 1.82% to 2.91% d.w. (Table 1). The findings indicated that the lipid content of the studied *Gelidium* species varied slightly but significantly along the Moroccan coast. The highest lipid content was observed in Sidi El Ghazi, while the lowest was found in Amgriou, both located in the southern region. This disparity could be attributed to the specific climatic conditions of these regions.[24,25] On the other hand, the lipid content in the western region sites, Sidi-Rahal and Lahdida, showed similarities.

The lipid contents of the studied *Gelidium sesquipedale* are consistent with those reported in other studies, which state that red algae generally have low lipid content ranging from 1% to 5% dry weight.[10,26] Although the overall lipid content does not exceed 7%, it is considered significant due to the richness of the product in polyunsaturated fatty acids (PUFAs), particularly omega-3.[27]

Total carbohydrate

According to the analyses presented, it was found that the contents of total sugars of the extracts obtained from different harvesting regions were comparable and represented the main component of the seaweed studied, ranging from 53.94% to 59.15% d.w. (Table 1). Red algae, especially *Gelidium sesquipedale,* are generally known for their high content of polysaccharides (such as agar and carrageenan), which can reach levels of 40% to 50% d.w.[28] These relatively high contents of polysaccharides are a major constituent of the cell wall in red algae.[29] Algal polysaccharides have garnered significant attention from the scientific community due to their exceptional functional and bioactive properties.[30]

Total protein

In this study, *Gelidium sesquipedale* exhibited similar protein content in the southern region sites, (14.08% in Sidi El Ghazi and 14.76% in Amgriou). However, it demonstrated higher protein content in Lahdida and Sidi Rahal, with values of 15.77 and 16.79% of d.w., respectively (Table 1). These values are comparable to those found by Gomes-Dias *et al.*[31] for *G. sesquipedale* (14.52% d.w.) and respectively in the two species *Gelidium corneum* and *Gelidium microdon* which correspond to 14.61% and 15.18 % d.w.[10] In general, red algae contain a higher amount of protein than green and brown algae, ranging from 10 to 50%.[32] They have a variety of amino acids which have great pharmaceutical potential.[33,7] Thus, the introduction of red algae into a daily diet is considered a nutraceutical food since their protein content is similar to or higher than that of legumes and soybeans.[34]

Total polyphenol

In Amgriou, the Rhodophyceae *Gelidium sesquipedale* presented the highest phenolic content compared to other collection sites, whereas the lowest content was observed in Sidi El Ghazi (Table 2). This variation in TPC may be attributed to differences in the environmental conditions at these two southern collection points. At the Amgriou site, seaweed is collected after stranding. However, the TPC values in the western region sites, Sidi Rahal, and Lahdida, showed similarities. In general, red algae are able to synthesize phenolic components with values up to 6 mg GAE/g dry weight.[35]

Antioxidant activities

At the western sites, results align with the TPC of samples from that region. Nevertheless, *Gelidium sesquipedale* revealed the best DPPH and FRAP values in the southern region (90.41 and 114.33 µg/mL in Amgriou; 103.76 and 110.5 µg/mL in Sidi El Ghazi). Despite their low content of free polyphenols, the samples from Sidi El Ghazi show a good reducing value (FRAP) as indicated by Ortiz-

Table 1. Moisture, water activity (WA), ash and macronutrient contents of *Gelidium sesquipedale* collected from the studied Moroccan Atlantic coasts.

Site	WA	Moisture %	Ashes % (d.w.)	Lipids % (d.w.)	Carbohydrates % (d.w.)	Proteins % (d.w.)
Amgriou	0.498±0.012^{a}	11.11±0.47^{a}	26.10±0.48^{a}	1.82±0.23^{c}	58.26±3.76^{a}	14.76±0.62^{c}
Sidi El Ghazi	0.489±0.002^{a}	8.33±1.85^{b}	24.16±0.50^{b}	2.91±0.31^{a}	53.94±1.87^{a}	14.08±0.16^{c}
Sidi- Rahal	0.501±0.002^{a}	11.32±0.54^{a}	20.74±0.23^{c}	2.63±0.86^{b}	56.63±8.21^{a}	16.79±0.37^{a}
Lahdida	0.507±0.018^{a}	12.84±1.35^{a}	22.82±0.24^{d}	2.68±0.39^{b}	59.15±0.59^{a}	15.77±0.16^{b}

*Means followed by the same letters in each column are not significantly different according to Duncan's test at α=5%.

Viedma *et al.*[26] This may explain that other factors contributed to the antioxidant power as well as phenolic substances in red algae such R-phycoerythrin (R-PE) and R-phycocyanin (R-PC) attached to the thylakoid membranes.[36]

Discussion

Characterization of the composition of *Gelidium* sp. collected from the Moroccan Atlantic coast is a prerequisite for the full exploration of its bio-compounds. They are mainly rich in polysaccharides, little affected by the region factor with a rate of 57.00% of d.w. Slight similar results were found by Tang *et al.*[37] in *Gelidium amansii* (54.17% d.w.). In contrast, Nil *et al.*[38] reported the highest value of total sugar (87.63%) in *Gelidium* sp. collected from the Algeria coast. In the second order, we found proteins with almost similar concentrations for the dry algal mass coming from the same region (western sites: 16.79% and 15.77%; southern sites: 14.76% and 14.08%). Concerning lipids, the rates are relatively homogeneous in the western sites, while there is a notable difference at the level of the southern collection points, with 1.82% in Amgriou and 2.91% in Sidi El Ghazi. Generally, algae contain a minor lipids content,[4] which is in agreement with our study. Regarding polyphenols, a similar trend to lipids was observed for the influence of regional factors, where different values were found in Amgriou 6.22 mg GAE/g and Sidi El Ghazi 4.78 mg GAE/g, while similar values were observed in Sidi-Rahal and Lahdida. Comparing to result found by Matos *et al.*[8] respectively 0.86 mg GAE/g and 0.7 mg GAE/g using ethanolic and water extract were found. Methanolic extract samples in this study exhibited the highest phenolic content. However, with ethanolic extract Xu *et al.*[39] reported 16 mg GAE/g for the same species.

Samples from southern sites revealed significant antioxidant activity (90.41 and 103.76 µg/mL for DPPH; 114.33 and 110.51 µg/mL for FRAP). Thus, Moroccan *Gelidium sesquipedale* proves to be a valuable reservoir of bio-compounds.

Bivariate correlation analysis employing the Pearson coefficient was applied to all variables to enhance comprehension of the relationships among various parameters. Table 3 and Figure 1 show multiple significant correlation findings at the 0.05 or 0.01 significance levels. Specifically, DPPH IC_{50} revealed positive and significant correlations with lipids and FRAP IC_{50}, while demonstrating a negative correlation with TPC (r=0.584*, r=0.646*, and r=-0.646*, respectively). Also, TPC and lipids were negatively correlated (r=-0.625*). That means the polyphenols positively impact the inhibition capacity of the studied seaweeds, which are in accordance with previous studies.[40] Whereas the lipids have a negative influence on this capacity. In addition, FRAP IC_{50} presented a highly significant and positive correlation with proteins, with a correlation coefficient of r=0.733**. So, the proteins exert a negative effect on the inhibition power. Furthermore, there was a positive and significant correlation between DPPH IC_{50} and FRAP IC_{50} (r=0.646*). These correlations confirm that polyphenols, lipids, and proteins are the main constituents contributing to the antioxidant activity of this seaweed.

Conclusions

Gelidium sesquipedale from the two regions studied have interesting potential nutrients. They are mainly rich in polysaccharides, which are not much affected by the region factor They are mainly rich in polysaccharides, which are not much affected by the region factor with a rate of 57.00% d.w. In the second order, we found proteins with almost similar concentrations for the dry algal mass coming from the same region (western sites: 16.79% and 15.77%; southern sites: 14.76% and 14.08%). Regarding lipids, the levels are relatively consistent in the western sites, while there is a notable difference at the southern collection points, with 1.82% in Amgriou and 2.91% in Sidi El Ghazi. As for polyphenols, a similar pattern to lipids was noticed for the influence of the region factor, where different values were found in Amgriou 6.22% and Sidi El Ghazi 4.78%, while similar values were observed in Sidi-Rahal and Lahdida. The samples of these southern sites revealed respectively an important antioxidant activity (90.41 and 103.76 µg/mL for DPPH; and 114.33 and 110.51 µg/mL for FRAP). Indeed, Moroccan

Table 2. Total phenolic content (TPC) and antioxidant activity of *Gelidium sesquipedale* collected from the studied Moroccan Atlantic coasts.

Site	TPC mg GAE/g (d.w.)	DPPH IC_{50} (µg/mL)	FRAP IC_{50} (µg/mL)
Amgriou	6.22±0.25^{a}	90.41±0.54^{c}	114.33±1.36^{c}
Sidi El Ghazi	4.78±0.12^{d}	103.76±2.87^{b}	110.51±0.92^{d}
Sidi- Rahal	5.69±0.04^{b}	104.51±0.89^{b}	129.78±1.41^{b}
Lahdida	5.38±0.04^{c}	109.74±2.57^{a}	139.38±2.31^{a}

*Means followed by the same letters in each column are not significantly different according to Duncan's test at α=5%.

Table 3. Matrix of correlation coefficients between *Gelidium sesquipedale* antioxidant activity and nutrients examined in this study.

	Polyphenols	Sugars	Lipids	Proteins	DPPH	FRAP
Polyphenols	1					
Sugars	0.365	1				
Lipids	**-0.625***	-0.341	1			
Proteins	0.328	0.206	0.193	1		
DPPH	**-0.646***	-0.026	**0.584***	0.365	1	
FRAP	0.078	0.321	0.161	**0.733****	**0.646***	1

*Correlation is significant at 0.05 level (bilateral); **correlation is significant at 0.01 level (bilateral).

Gelidium sesquipedale proves to be a valuable reservoir of bio-compounds. Indeed, their production should not be limited to agar-agar production as a gelling product. A complete exploitation of the plant will be ideal to explore its pharmaceutical and nutritional benefits.

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