

Seasonal Variation in Nutritional Composition and Antioxidant Potential of *Gelidium corneum* from the Doukkala Coast, Morocco

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Abstract. The coastal areas of Doukkala, Morocco, are recognized for its abundant populations of *Gelidium corneum*, an acknowledged red alga used for agar production. This study focused the valorization of *G. corneum* beyond agar extraction, focusing on its nutritional composition, antioxidant properties, and socio-economic significance. Seasonal changes in biochemical composition were analyzed by measuring ash, crude protein, total lipid, and carbohydrate contents throughout the year. The antioxidant capacity of aqueous algal extracts was evaluated seasonally using DPPH, ABTS, and FRAP assays, along with total phenolic content (TPC). Results indicated that the biomass contained substantial amounts of protein (up to $19.22 \pm 0.13\%$) and carbohydrates (up to $43.9 \pm 0.07\%$), while lipid content remained relatively low (up to $2.37 \pm 0.7\%$), particularly in autumn. Moderate antioxidant activity was observed, with DPPH inhibition reaching $14.44 \pm 0.12\%$, ABTS inhibition $20.55 \pm 0.18\%$, FRAP 1.66 ± 0.35 mg AAE g^{-1} , and TPC 12.34 ± 0.28 mg GAE g^{-1} . These findings underscore the value of *G. corneum* as a natural resource with both nutritional and functional potential, supporting its prospective use in food applications while contributing to the socio-economic development of the Doukkala region.

1 Introduction

The Doukkala region of Morocco is well known for its abundant agarophyte *G. corneum*, a red alga valued worldwide for the production of high-quality agar [1]. In recent years, this species has attracted increasing scientific and industrial attention due to its wide range of potential applications [2,3]. Beyond agar extraction, *G. corneum* has been explored for its uses in the pharmaceutical, cosmetic, nutraceutical, bioremediation, biofuel, biofertilizer, biostimulant, biomaterial, and nanocrystal sectors [4,5]. This cartilaginous red alga, belonging to the division Rhodophyta. It can reach heights of 20–30 cm and typically forms

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dense tufts. However, the increasing exploitation of *G. corneum* raises concerns about the sustainability of its natural stocks, as this species is not cultivated but harvested from wild populations [4]. Such practices exert additional pressure on already stressed marine ecosystems, which are further impacted by climate change [6]. Environmental factors can induce physiological stress, leading to metabolic adjustments that alter the synthesis of primary and secondary metabolites [7]. These processes may generate reactive oxygen species (ROS), highly reactive molecules whose excessive accumulation disrupts the balance between free radical production and antioxidant defenses, resulting in oxidative stress. Organisms mitigate this stress through antioxidant compounds that neutralize or eliminate these radicals [8]. Macroalgae are particularly rich in such antioxidants, which not only protect the algae themselves but also provide health benefits when consumed by humans. In response to the growing demand for natural products, the study was carried out with the intention of assessing the nutritional composition of *G. corneum*, evaluating the seasonal variation of its main components, in order to confirm its potential. A series of analyses were carried out to determine the biochemical profile. Additionally, the antioxidant activity of *G. corneum* obtained in different seasons was measured to better understand the potential as a food source.

2 Material and Methods

2.1 Algal material

G. corneum was collected along the rocky coast of Doukkala, Morocco, at four sites (Jorf Lasfar, Moulay Abdellah, Sidi Bouzid, and El Jadida), once per season during the year 2019. The freshly collected material was immediately transported to the laboratory. Inside, samples were rinsed to remove epiphytic organisms [6]. Subsequently, *G. corneum* samples were dried and ground into a fine powder, which was stored in dry, light-protected glass bottles until further use (Fig. 1).

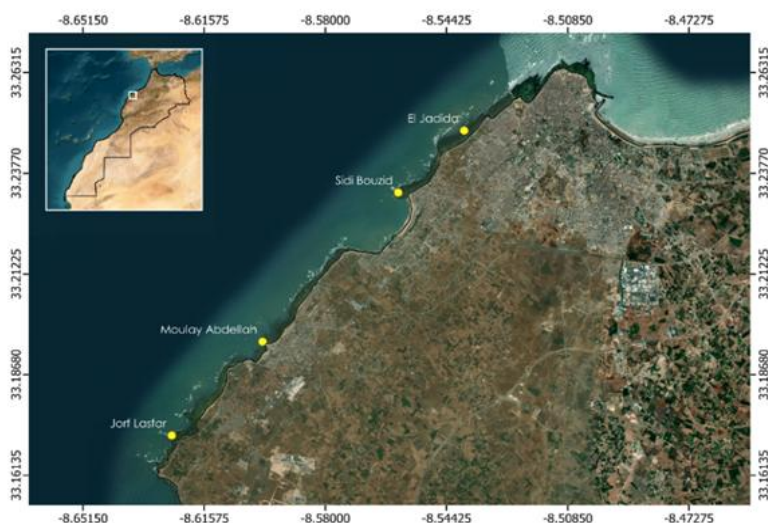


Fig. 1. Location of the four sampling sites along the Atlantic coast of Doukkala

2.2 Preparation of Algal Extracts

2.2.1 Aqueous Extraction

Water-soluble compounds were extracted by combining 10 g of lyophilized *Gelidium corneum* powder with 500 mL of ultrapure water. The mixture was heated at 100 °C for 2 h with manual stirring every 30 min. The supernatant was filtered and centrifuged at 3000 rpm for 30 min. The resulting filtrate was stored at -24 °C for 24 h, lyophilized (CHRIST Alpha 1-2 LD PLUS), and kept in the dark until use.

2.2.2 Agar Extraction

Agar was extracted following the procedure of Martínez-Sanz et al. [9]. Forty grams of dried algal powder were immersed in 400 mL of distilled water, heated to 100 °C for 2 h, and filtered. The filtrate was precipitated with 96% ethanol for 24 h, and the obtained gel was dried at 60 °C, and the agar yield was determined as a percentage of the dry biomass. A second extraction was performed after an alkaline pretreatment. For this step, 40 g of dried algal powder were soaked in 400 mL of 10% NaOH solution at 90 °C for 2 h, filtered, and the residue was rinsed with distilled water before undergoing the same hot-water extraction procedure described above.

2.3 Biochemical Composition Analysis

2.3.1 Determination of Ash, Moisture Content

The ash and Moisture contents were measured following AOAC procedures. Approximately 1 g of ground fresh biomass (3 mm particle size) was oven-dried overnight at 100 °C until constant weight. The dry matter content was calculated as:

$$\%DM = \frac{\text{Weight after drying}}{\text{Initial dry weight}} \times 100$$

Ash content was obtained by incinerating the dried samples in a muffle furnace at 550 °C for 5 h until a constant weight was reached:

$$\%Ash = \frac{\text{Ash weight}}{\text{Initial dry weight}} \times 100$$

2.3.2 Total Sugar Determination

Total sugars were determined using the phenol-sulfuric acid method [10]. Briefly, 200 μL of sample was mixed with 200 μL of 5% phenol solution, followed by 1 mL of concentrated sulfuric acid (97%). After 30 min at room temperature, absorbance was measured at 490 nm using a Camspec M106 spectrophotometer.

2.3.3 Total Lipid Content

Total lipids were extracted according to the method of Barnathan et al. [11], using a $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (1:1, v/v) solvent system, as described by Bligh and Dyer. One gram of *G. corneum* powder was macerated in 20 mL of solvent for 48 h, filtered, and the organic phase was washed with distilled water (40% of the final solvent volume) to remove salts. The filtrate was evaporated at 40 °C under reduced pressure to yield crude lipid extract (CLE). The lipid percentage was calculated as:

$$\%Lipids = \frac{Lipid\ mass}{Lipid\ mass + Dry\ mass} \times 100$$

2.3.4 Total Protein Content

Soluble proteins were determined using the Bradford assay [12]. Equal volumes (500 μL) of extract and Bradford reagent were mixed and incubated for 5 min at room temperature. Absorbance was then measured at 595 nm.

2.4 Evaluation of Antioxidant Activity

The antioxidant potential of *G. corneum* extracts was evaluated through several complementary in vitro assays:

2.4.1 DPPH Radical Scavenging Activity

The free radical scavenging activity was determined according to the protocol described by Brand-Williams et al. [13], where the stable DPPH radical was employed. And the absorbance at 517 nm was recorded.

2.4.2 Ferric Reducing Antioxidant Power (FRAP)

Determined according to method of Dorman et al. [14], this assay ranks the extracts by their strength of reducing Fe^{3+} to Fe^{2+} . The absorbance was determined at 700 nm, and the data were represented as mg of ascorbic acid equivalents per gram of the dried weight (mg AAE/g DW).

2.4.3 Total Antioxidant Capacity (TAC)

Assessed using the phosphomolybdenum method of Prieto et al. [15], which is predicated on the reduction of Mo(VI) to Mo(V) under acidic conditions. Absorbance was noted at 695 nm and results were reported as mg AAE/g DW.

2.4.4 ABTS Radical Scavenging Assay

According to Nenadis et al. [16], Agreed that ABTS radicals were formed by the oxidation of ABTS with potassium persulfate. Absorbance was detected at the radical scavenging activity of extracts mixed with the ABTS solution at a wavelength of 734 nm.

2.4.5 Total Phenolic Content (TPC)

Determined using the the Folin–Ciocalteu method [17]. Extracts were treated with the Folin–Ciocalteu [18] reagent, and absorbance reading at 755 nm. The results were expressed as mg gallic acid equivalents per gram of extract (mg GAE/g).

2.4.6 Agar analysis by infrared spectroscopy

FTIR spectroscopy was utilized to characterize and identify functional groups in agar extracts. The recording of spectra was done using an infrared spectrometer wherein the data were processed with The Unscrambler software (Camo, USA) which made it possible to identify the differences in the spectra among samples that were collected monthly.

2.5 Statistical Analysis

All experiments were performed in triplicate, and results are presented as mean ± standard deviation (SD) (n = 3). Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey’s post hoc test, with statistical analyses conducted using SPSS version 26.

3 Results

3.1 Seasonal Variation in the Biochemical Composition of *G. corneum*

The biochemical composition of *G. corneum* was analyzed for each season across the four collection sites along the Doukkala coast during 2019. The results for moisture, ash, protein, lipid, and carbohydrate contents are presented in Table 1.

Table 1. Seasonal variation in the biochemical composition of *G. corneum* collected along the Doukkala coast.

	Winter	Spring	Summer	Autumn
Moisture (% FW)	51,36±0,35 ^c	55,32±0,42 ^d	50,02±0,03 ^b	67,14±0,06 ^a
Ash (% DW)	16,68±0,14 ^b	14,38±0,21 ^b	13,38±0,31 ^c	12,23±0,04 ^a
Protein (% DW)	13,4±0,49 ^c	14,4±0,49 ^c	17,44±0,5 ^b	19,22±0,13 ^a
Lipids (% DW)	1,33±0,42 ^a	1,5±0,63 ^a	2,16±0,19 ^a	2,37±0,17 ^a
Carbohydrates (% DW)	41,4±0,56 ^c	43,85±0,07 ^d	31,38±0,44 ^b	36,45±0,83 ^a

Different lowercase letters indicate significant differences at $p < 0.05$, whereas identical letters indicate no significant difference for each evaluated parameter.

The average moisture content of the biomass was 55.96 ± 0.215 % FW, with autumn showing the highest value (67.14 ± 0.06 %) and summer the lowest (50.02 ± 0.03 %), the difference between summer and autumn was significant when compared with spring biomass ($p < 0.05$). The ash content of *G. corneum* showed the highest mean value of 16.68 ± 0.14 % DW in winter, with significant differences between summer and autumn. There was a significant variation of total protein content through the seasons, the autumn peak being at 19.22 ± 0.13 % and the winter trough at 13.4 ± 0.49 %, with significant differences between spring and both summer and autumn. The study also indicated that the total lipid contents were quite low, not exceeding the range that is usual for red algae, with the least being 1.33 ± 0.42 % in winter and the most being 2.37 ± 0.17 % in autumn. The soluble carbohydrate

content was highest in spring (43.85 ± 0.07 %), and while it was lowest in summer (31.38 ± 0.44 %). The winter values were significantly different from those of the autumn biomass.

Table 2 presents agar yields of *G. corneum* with and without alkaline pretreatment.

Table 2. Agar yield of *G. corneum* without and with alkaline pretreatments.

	Winter	Spring	Summer	Autumn
Yield without alkaline pretreatment	6,72±0,21 ^c	13,52±0,11 ^d	15,47±0,86 ^b	9,73±0,56 ^a
Yield with alkaline pretreatment	3,43±0,42 ^c	7,51±0,30 ^d	8,75±0,60 ^b	5,27±0,34 ^a

Agar yield ranged from 6.72 to 15.47 % DW, with a peak observed in summer for extraction without alkaline pretreatment (15.47 ± 0.86 % DW). Significant seasonal variations were observed. Samples pretreated with a 10 % NaOH solution showed a significant reduction in extraction yields, with a maximum of 8.75 ± 0.60 % in summer. FT-IR analysis was performed to confirm the presence of the chemical bonds typically found in agar in the extracts obtained by the primary method during all four seasons. It was also used to detect any potential impurities in the agar FT-IR analysis of extracted agar was performed from 500 to 4000 cm^{-1} (Figure 2). As in all polysaccharide form, agar is mainly composed by β -1,3-linked galactopyranose as detected by the key peak at 890 cm^{-1} , and the 3,6-anhydro-galactose residue at 930 cm^{-1} as reported by various studies. A robust two peaks at 1069 cm^{-1} and 1038 cm^{-1} were noticed, corresponding to C-O and C-C stretching vibrations of the pyranose ring. A momentous peak at 1629 cm^{-1} was credited to N-H scums allied with peptide bonds in proteins. Lastly, a broad peak corresponding to O-H was detected at 3255 cm^{-1} , stretching in all aqueous and lyophilized agar extracts across the two periods.

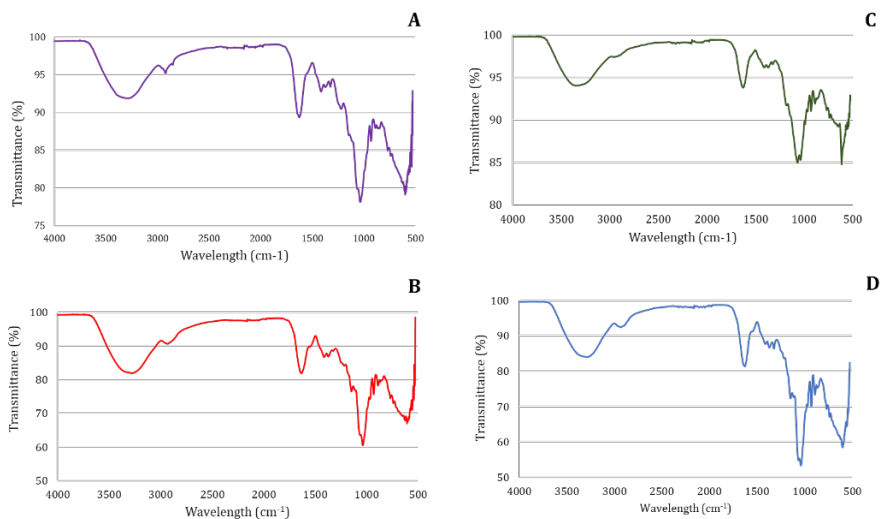


Fig. 2. : Spectra of agar extracted from *G. corneum* collected on the Doukkala coast during the four seasons of 2019, obtained by infrared spectrometry (A: Winter; B: Spring; C: Summer; D: Autumn).

3.2 Antioxidant Potential of *G.corneum*

Figure 3 presents the seasonal variation of antioxidant activity of aqueous extracts of *G. corneum*.

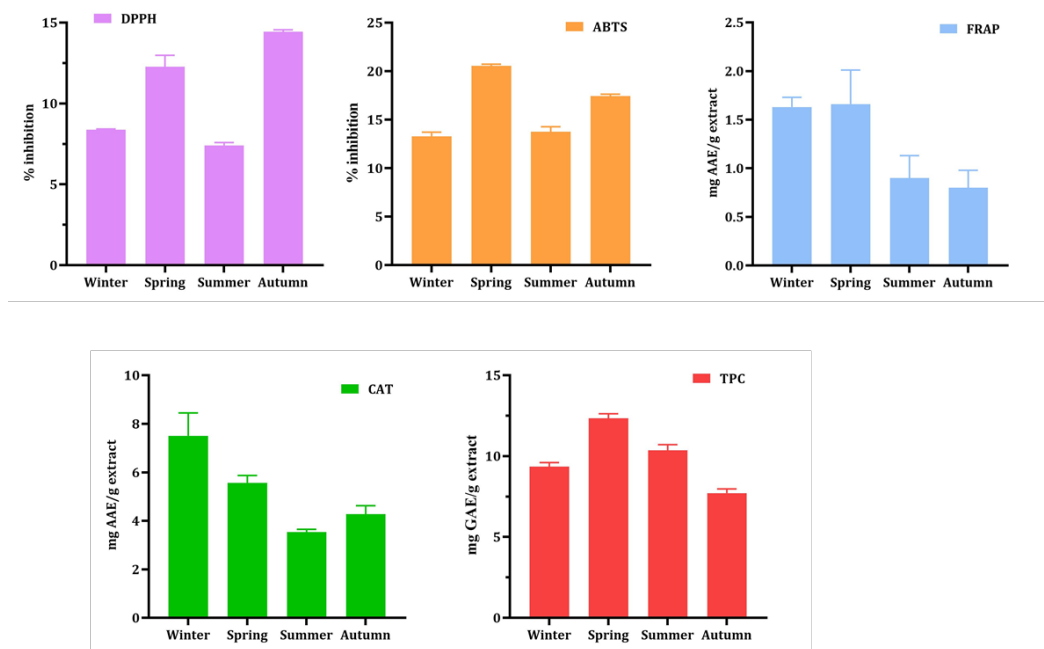


Fig. 3. Seasonal variation of antioxidant activity of *G.corneum* collected along the Doukkala coast.

The results indicate that maximal inhibition in the DPPH assay was observed in autumn, with a value of 14.44 ± 0.12 %. The ABTS assay revealed significantly higher inhibition in spring compared to other seasons. In the FRAP assay, spring biomass exhibited the highest antioxidant activity (1.66 ± 0.35 mg AAE/g extract). Similarly, the TPC test revealed a maximum of 12.34 ± 0.28 mg GAE/g extract in the spring, whereas the autumn total had the minimum value (7.71 ± 0.28 mg GAE/g extract) with the highest CAT assay values being 12.34 ± 0.28 % with the notable winter season the highest antioxidant activity. These results demonstrate significant seasonal variation in the antioxidant properties of *G. corneum* extracts, highlighting the potential of this species as a source of natural antioxidants

4 Discussion

Marine macroalgae are known to undergo metabolic changes throughout the year, leading to seasonal variations in their biochemical composition. These fluctuations are influenced by environmental factors such as temperature, pH, and light intensity [4]. Understanding such variations is essential for identifying the most suitable harvest periods, especially when the biomass is intended for specific applications that depend on its bioactive compounds. Earlier studies have demonstrated that parameters such as solar radiation, seawater temperature, and depth can significantly affect the chemical composition of *G. corneum* [19].

In this work, *G. corneum* showed slight but statistically significant seasonal changes in water content. Moisture is an important quality indicator, as it influences both product stability and storage life, excessive moisture can encourage microbial growth. The highest moisture value recorded ($67.14 \pm 0.06\%$) was greater than those reported [20,21].

The ash content of *G. corneum* fell within the typical range reported for red algae (5.8–46.2 %) [22], though it was slightly lower than in other *Gelidium* sp. In this study, the highest ash value ($16.68 \pm 0.14\%$) reflects a moderate mineral content compared with *Gracilaria vermiculophylla* and *G. pusillum* [11]. Lipid content was consistent with values generally observed in marine macroalgae (1–6 %), particularly in red species (1–4 %). Despite the typically low lipid levels in red algae, *G. corneum* showed a relatively higher amount than some edible species such as *Himanthalia elongata* ($0.97 \pm 0.07\%$) [23].

Red algae also constitute a significant protein source. The protein content observed in *G. corneum* (up to 19.22 %) was similar to *G. microdon* (15.18 %) [24]. As an agar-producing species, *G. corneum* also exhibited high carbohydrate levels (up to 43.85 %), exceeding those found in other red algae like *P. palmata* (25 %). Industrially, this species is mainly exploited for its high-quality agar. In the current study, agar yields ranged from 6.72–15.47 % DW without pretreatment and from 3.43–8.75 % DW after strong alkaline pretreatment with 10 % NaOH. Such treatment is known to lower yields considerably [25]. However, other authors have reported that mild pre-extraction using alkalis or acids can sometimes improve agar recovery [26]. The lower yields observed here might be explained by excessive degradation during the 10 % NaOH treatment, which may have caused agar diffusion into the aqueous phase and reduced extraction efficiency [2].

Concerning antioxidant potential, *G. corneum* showed moderate activity, comparable to the findings of Murugan et al. [27], yet superior to those obtained for aqueous extracts of other macroalgae such as *Caulerpa chemnitzia*. The total phenolic content was in the same order of magnitude as other red algae, generally remaining below 10 mg GAE/g extract [28]. Although aqueous extracts of *G. corneum* did not display strong antioxidant properties, noticeable seasonal differences were observed.

5 Conclusion

This study highlights the potential for diversifying the utilization of *G. corneum* beyond agar extraction. By gaining a better understanding of the seasonal variations in its chemical composition and antioxidant properties, new avenues for valorization can be explored, particularly in the food and cosmetic industries. Such an approach could help reduce the pressure on natural *G. corneum* populations while providing more sustainable socio-economic opportunities, including stable employment for individuals working in this sector. Therefore, by optimizing the year-round use of *G. corneum* resources, it is possible to effectively balance economic development with environmental preservation.

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