

Nutritional and antioxidant potential of *Gelidium corneum* from the El Jadida coast, Morocco

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Abstract

Gelidium corneum is a common red macroalgae (Rhodophyta) along the Atlantic coast of Morocco, particularly in El Jadida surrounding coast areas. It is recognized for its industrial importance as an excellent-grade agar producer but also with growing interest because of its bioactive compounds with nutritional and pharmacotherapeutic values. The goal of the current investigation is to evaluate this marine species based on its macronutrient and secondary metabolite composition and antioxidant capacity. Chemical examination of powdered algal material revealed appreciable levels of protein ($26 \pm 0.0007\%$), carbohydrate ($55 \pm 0.006\%$), and lipid ($6 \pm 0.001\%$). Our algal sample proved to be nutritionally beneficial as a consequence of its high alkaloid, flavonoid, coumarin, and tannin content.

Algal biomass has dose-dependent activity towards scavenging DPPH free radicals with high antioxidant activity. *G. corneum* from the El Jadida region has interesting functional properties in addition to nutritional value as expressed through its macronutrient and secondary metabolite content, and thus these marine products are worthy candidates for sustainable progress in the pharmaceutical, cosmetic, and food industries.

Keywords : macronutrients, *Gelidium corneum*, DPPH test, antioxidant activity, secondary metabolites, Morocco

پوخته

Gelidium corneum ماکروئالگایه کی سووره (Rhodophyta) که به شیوه یه کی به رفراوان به دریا یی که نارەکانی ئەتلەسی مەغریب بۆ بوو و تەو، بە تاییبەتی لە ناوچە ی ئیڵ جایدا. ئەم چلکناوە بە گرنگی پیشە سازییه که ی وه ک سەرچاوه ی ئاگاری کوالیتی بەرز ناسراوه، ههروهه جیگه ی سه رنجی گه شه سه ندوو به هۆی پیکهاته چالاکه زیندوو هکانی که تاییبەتمه ندی خۆراکی و چاره سه ری ده رمانییان هه یه. ئامانجی ئەم توێژینه وه یه

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هه لسه نگاندى ئەم جوړه دەرياييه به ديارىكردى پيکھاتهى خۇراكى گه وره و ميتابۆليتە لاوه كيبه كهى، ههروهه چالاكيبى دژه ئوكسىپنه ره كهى. شىكاريبه كانى مادهى چلكنه وشكراوه ئاستى بهرچاوى پړۆتين ($26 \pm 0.0007\%$)، كاربۆهيدرات ($55 \pm 0.006\%$)، و چهورى ($6 \pm 0.001\%$) ده رخست. نمونهى چلكنه كانمان پړؤفايلى خۇراكى نيشان دا به هوى دهوله مهندى به ئەلكالۆيد، فلاقۇنۆيد، كومارين و تانين.

بايو ماسهى چلكن تواناى وابهستهى زهمه ده رمانى هه به بۆ بيلايه نكردى رادىكاله ئازاده كانى DPPH، كه چالاكيبى دژه ئوكسىپنه رى سه رنجرا كيش ئاشكرا ده كات *G. corneum*. كه له ناوچهى ئيل جاديدا دروينه ده كړيت، تايه تمهندى كارايبى سه رنجرا كيشى هه به جگه له به هوى خۇراكى كه به هوى مادده خۇراكيه گه وره كان و ناوه پړۆكى ئاويته چالاكه زيندوووه كانيبه وه ده رپر دراوه، ئەمه ش واىكردوووه ئەم سه رچاوه ده ربايبانه به ليندەر بن بۆ گه شه پيدانى به رده وام له پيشه سازيبه كانى ده رمانسازى، جوانكارى و خۇراكا.

كيله ووشه: مادده خۇراكيه گه وره كان، *Gelidium corneum*، تاقىكردنه وهى DPPH، چالاكيبى دژه ئوكسىپنه، ميتابۆليتە لاوه كيبه كان

ملخص

طحالب "جيليديوم كورنيوم" هي طحلب أحمر كبير (رودوفيتا) منتشر على نطاق واسع على طول ساحل المحيط الأطلسي في المغرب، وخاصة في منطقة الجديدة. تشتهر هذه الطحلب بأهميتها الصناعية كمصدر للأجار عالي الجودة، كما أنها تحظى باهتمام متزايد بفضل مركباتها النشطة بيولوجياً ذات الخصائص الغذائية والعلاجية الدوائية. تهدف هذه الدراسة إلى تقييم هذا النوع البحري من خلال تحديد تركيب المغذيات الكبرى ونواتج الأيض الثانوية فيه، بالإضافة إلى نشاطه المضاد للأكسدة. كشفت تحاليل المواد الطحلبية المجففة عن مستويات عالية من البروتين ($26 \pm 0.0007\%$)، والكربوهيدرات ($55 \pm 0.006\%$)، والدهون ($6 \pm 0.001\%$). أظهرت عينة الطحالب لدينا قيمة غذائية عالية بفضل غناها بالقلويدات والفلافونويدات والكومارين والعفص. تتمتع الكتلة الحيوية الطحلبية بقدرة تعتمد على الجرعة على تحييد الجذور الحرة لـ DPPH، مما يكشف عن نشاط مضاد للأكسدة ملحوظ. يتميز طحالب *G. corneum*، التي تُحصد في منطقة الجديدة، بخصائص وظيفية مثيرة للاهتمام، بالإضافة إلى قيمتها الغذائية التي يُعبر عنها من خلال محتواها من المغذيات الكبرى والمركبات النشطة بيولوجياً، مما يجعل هذه الموارد البحرية واعدة للتنمية المستدامة في الصناعات الدوائية ومستحضرات التجميل والأغذية.

الكلمات المفتاحية: المغذيات الكبرى، طحالب *Gelidium corneum*، اختبار DPPH، النشاط المضاد للأكسدة،

المستقلبات الثانوية

Introduction

Renowned for being a natural source of bioactive molecules, red algae or rhodophytes are of numerous applications in the pharmaceutical, cosmetics, environmental, and food industries. One such alga, *Gelidium corneum*, is renowned for its economic importance as a source of agar-agar or commercially used as a gelling agent (Torres et al., 2019).

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It is composed of essential amino acids, polysaccharides, unsaturated fatty acids, phenolic compounds, pigments, and numerous minerals and vitamins. The complex gives this seaweed several biological activities, such as anti-inflammatory, antioxidant, anti-obesity, antidiabetic and antibacterial activities, that are being more and more sought after in the context of the sustainable exploitation of marine resources (Cherki et al., 2020; Cavaco et al., 2021; Taouam et al., 2024a; 2024b).

G. corneum has been recorded on temperate and tropical coasts around the world with unbroken populations along the Western European, Portuguese and Moroccan coasts. Nutritional analysis of *G. corneum* shows it to be rich in protein, minerals and fibre (Santos et al., 2018, Taouam et al., 2024a).

Morocco is a significant producer of this algae, and *G. corneum* is harvested mostly for industrial use in the production of agar-agar, which has been well researched. This potential also exists for therapeutic uses, with antioxidant, healing and photoprotective activities (Belattmania et al., 2021). Sustainable ecological management of the industry is required to meet the higher demands of foreign markets and preserve this valuable resource.

In order to improve and enrich our knowledge of *G. corneum* harvested in the Sidi Bouzide region (El Jadida), our aim is to explore the phytochemical composition in macronutrients and secondary metabolites of this alga, while highlighting its antioxidant property.

1. Materiel and methods

1.1 Sampling and preparation of algal materiel

G. corneum thallus was collected during July 2020 by an expert diver from Sidi Bouzid beach, 2 Km from town El Jadida of Morocco (33°13'52 "North, 8°32'51" West) (figure 1) at a depth of approximately 12m.

Figure 1. Geographical map of the harvesting location for *G. corneum* (Sidi Bouzid))

G. corneum thallus were washed and subsequently analyzed by a biologists of SITEXAM (company for the study and exploitation of algae and sea products) of El Jadida. The samples of algae were dried for 6 days in 25°C, then cut and ground into powder and kept in sterile glass vials, closed hermetically and protected from light and moisture until usage.

1.2 Macronutrients and humidity of algal samples

The experimental protocols used for the quantification of protein, carbohydrates, lipid and moisture in algal samples are shown in figures 2 and 3.

Figure 2: protocol for the analysis of macromolecules, (a) Dosage of the proteins (Bradford, 1976), (b) Dosage of carbohydrates (Dubois, 1959), and (c) lipid content (Folch, 1957)

Figure 3. Dosage of Moisture (William, 2000).

1-2- Determination of ash

Ash content was calculated from the percentage of moisture (%M), percentage of carbohydrates (%C), percentage of lipids (%L) and percentage of proteins (%P) using the following formula:

$$\text{Ash \%} = 100 - (\%M + \%C + \%L + \%P)$$

1-3- Energy value

Energy value was measured using calorific value of macronutrients with the following formula:

Energy value (Kcal/100g)= 9 x lipids + 4 x carbohydrates+4 x proteins

1-4- Antioxidant potential analysis

1-4-1- Preparation of algal extract

A ethanolic extract (EE) was isolated by subjecting 100 g algal powder to ethanol in the Soxhlet, for 8 h. Organic extract was made dry under reduced pressure at 30-35°C using a rotary evaporator. Once the extract was obtained, its color and yield (R%) were quantitated on the basis of the original quantity of dry seaweed (Garcia-Vaquero et al., 2020):

$R\% = (\text{mass (extract)}) / (\text{mass (algae)}) \times 100$

The obtained residue was measured and stored at -20°C in sterile Eppendorf tubes.

1-4-2- Phytochemical screening

Terpenoids: algal extract (EE) 5 ml, concentrated H₂SO₄ 3 ml and chloroform 2 ml was mixed (Adu et al., 2019). Formation of red-brown color at the interface is a sign of the presence of terpenoids.

Alkaloids: 3 ml of algal extract (EE) was combined with 1 ml of Dragendorff reagent (Yeh et al., 2014). Red precipitate formation indicates the presence of alkaloids.

Tannins: 5 ml of algal extract (EE) was combined with 1 ml of FeCl₃ 5 % solution (Santelices et al., 1991). Due to the presence of tannins, the reaction that causes blue-black coloration.

Flavonoids: magnesium and 1 ml of hydrochloric acid were added to algal extract (EE) (Mahurpawar, 2015). The formation of orange color that turns red indicates the presence of flavonoids.

Coumarins: 5 ml algal extract (EE) was placed in a test tube and afterward covered with filter paper soaked in a diluted NaOH (0.1N) solution coumarins (Mahurpawar, 2015). Filter paper was noted under a UV lamp at 254 nm. Yellow fluorescence color shows the occurrence of coumarins.

1-4-3 Antioxidant potential

The antioxidant potential of EE extract in different concentrations (0, 1, 2.5, 5, 10, 15, 20, 25 and 30 mg/ml), was estimated through DPPH (2, 2 diphenyl-1-picryl-hydrazyl) assay. Free radical scavenging activity was expressed as percentage inhibition and antioxidant potential of extracts was expressed as IC₅₀ values (sample inhibitory concentrations required for reducing 50% of free radicals).

DPPH solution was prepared by dissolving 2.3 mg of DPPH in 100 ml ethanol. 25 µl of extract of different concentrations and control (ascorbic acid, 0, 0.01, 0.02, 0.03, 0.04, 0.05 and 0.06 mg/ml) were used in 975 µl of DPPH solution. The absorbance was measured after 30 min of dark incubation at room temperature at 517 nm (Heo et al., 2006). Percentage DPPH radical inhibition by the samples was calculated based on the formula below:

$I (\%) = \frac{A(\text{blanc}) - A(\text{Ech})}{A(\text{blanc})} \times 100$

I %: DPPH inhibition percentage, A (blank): control absorbance, A (Ech): absorbance of the samples at different concentrations.

1-3 Statistical analysis

The experiments were conducted in triplicate. IC₅₀ was computed utilizing Excel 2016 software, where percentage inhibition was plotted against log concentration. The results were expressed as mean ± standard deviation.

2. Results and Discussion

2.1 Macronutrient content, moisture and ash

G. corneum possesses an interesting nutritional composition, yet fewer studies have focused on it compared to other red algae species (Cherki et al., 2020; Cavaco et al., 2021; El Majnaoui et al., 2024; Taouam et al. 2024a; b). Throughout this study, we intended to determine the nutritional composition of *G. corneum*, collected on Sidi Bouzid beach near El Jadida city (Morocco) in July, due to the fact that it has high summer nutritional value (Cavaco et al., 2021). The levels of carbohydrates, proteins, lipids, water, and ash content are presented in Figure 4.

Figure 4. Nutritional value of *Gelidium corneum* (100g)

The chemical composition analysis reveals that our alga under study comprises a high carbohydrate content (55 ± 0.06 % DW) forming the majority. Proteins comprise an intermediate content (26 ± 0.007 % DW), and lipids are present in less percentage (6 ± 0.001 % DW). Based on this macronutrient composition, the energy value of the alga is approximated at 378 kcal/100 g.

Several studies have confirmed the richness of *G. corneum* in macronutrients that are vital (lipids and proteins, and carbohydrates) though with varying levels significantly between species depending on where they originate, seasons of collection and environmental conditions (Bleakley and Hayes, 2017; Cherki et al., 2020; Cavaco et al., 2021).

Comparison with the carbohydrate content in our sample (55%) and that found in the same species shows consistency with Moroccan samples from other areas (53.94-60.77%) (Taouam et al., 2024a; El Manjaoui et al., 2024) and for other red algae species such as *Gelidium amansii* (54.17% DW), *Porphyra* sp. (58.74% DM), and *Pyropia vietnamensis* (60.36% DM) (Kavale et al., 2018; Tang et al., 2023; Arakaki et al., 2023). But lesser carbohydrate contents were recorded for *G. corneum* in Portugal and Spain (37%-44.8%) (Tum'as et al. 2020; Cavaco et al. 2021; Trigueros et al. (2021), whereas lesser values were recorded for other red algae, *Crassiphycus corneus* (36%) (Robledo and Freile-Pelegrin, 1997) and *Agarophyton vermiculophyllum* (48.9%) (Alfonso, 2021). However, Nil et al. (2016) reported the greatest total carbohydrate value (87.63%) in *Gelidium* sp. harvested off Algerian shores.

In addition, this peak in summer carbohydrate production typically captures a nutrient reserve that will be utilized in winter for protein synthesis (Kraan, 2013). As far as protein is concerned, our findings indicated a content of 25 ± 0.007 % DM. Such a high protein content provides *G. corneum* with nutritional value, provided its amino acid profile is balanced. This paper, thus, suggests its potential for use in animal or human diet as a source of protein. This protein content is higher compared to what has been documented for *G. corneum* of other Moroccan Atlantic coasts (14.08-20.6%) (El Manjaoui et al., 2024; Taouam et al., 2024a) and even of *G. corneum* from the Portuguese coast (16.25% DM) (Cavaco et al., 2021).

In terms of lipid content, it has been found that algae have low contents of lipids (1 to 6% dry matter), and the present result (6% DM) is at the upper end of this range. This is higher than the reported content in the same species harvested along other Moroccan coasts such as Dar Bouazza (1.23% DM), Amgriou (1.82% DM) and Sidi Rahal (2.63% DM) or even along the Portuguese coast (2.7% DM) (Cavaco et al., 2021; El Majnaoui et al., 2024; Taouam et al., 2024a and b).

Higher lipid content (7.07% DM) was, however, recorded by Rosemary et al. (2019) in *Gracilaria corticata* (red macroalgae).

In general, these differences in macronutrient percentages between studies are attributed to numerous factors, mainly environmental factors (salinity, temperature, availability of nutrients), harvesting season and algae growth phase. All these parameters have a significant influence on the biochemical composition of macroalgae, and therefore it is essential to standardize the cultivation conditions for optimum industrial valorization (Hodge et al., 1998 ; Nenadis et al., 2004).

Moisture determination showed an average content of dry matter to be $5 \pm 0.056\%$, which was lower than that obtained with *G. corneum* collected from various Moroccan Atlantic coastlines, Dar Bouaaza (9.3% DM), Sidi Rahal (11.32% DM), Amgiou (11.11% DM), and Lahdida (12.84% DM) and that from other red algae such as *Gelidiella acerosa* (8.71% DM) (Wang et al., 2010; Rasyid and Handayani, 2019; El Majnaoui et al., 2024; Taouam et al., 2024). These variations in water content can be attributed to several factors, such as drying method and drying period, but overall, our sample is not high in water content, which agrees with the good state of preservation of the seaweed powder.

Ash, or fixed mineral residue, is synonymous with the inorganic content of food and contributes immensely to the nutritional value of food (Redden et al., 2017). Ash content for our alga is $8 \pm 0.04\%$ DM. This value achieved here is comparable with those reported for *Gracilaria corticata* (8.10% DM) and *Hydropuntia edulis* (7.36–8.70% DM), though lower than those found in *G. corneum* from Portugal (14.12% DM), *G. pusillum* (21.2% DM), and *Gracilaria vermiculophylla* (24.5% DM) (Gamero-Vega et al., 2020; Cavaco et al., 2021; Afonso et al., 2021). But these values are within the normally reported range for red algae, i.e., 5.8 to 46.2% DM (Gamero-Vega et al., 2020). The ash value depends on the location and harvest period. In principle, the environmental conditions enhance the value of the harvesting sites for the batches of algae considered.

2.2 Phytochemical screening and antioxidant activity

In order for an individual to obtain an ethanolic extract (EE), ethanol was used to extract *G. corneum*. The analysis revealed a dark green color and 1.9% yield of EE extract. Phytochemical screening of the EE extract revealed the presence of tannins, flavonoids, coumarins, and alkaloids but not terpenoids (Table 1).

Table 1. Antioxidant substances present in the ethanolic extract (EE) of *G. corneum*.

Chemical substances	Tannins	Flavonoïds	Terpénoïds	Coumarins	Alcaloïds
Ethanolic extract	+++	-	+++		

(-) : absence ;

(+) : presence

Table to arrange (see the original version)

Phenolic compounds exhibit antioxidant properties. Our results indicate that *G. corneum* in general contains high levels of these compounds, which are chiefly responsible for the neutralization of free radicals owing to the presence of hydroxyl groups capable of sequestering reactive species (Bengueddour et al., 2013; Heo et al., 2006). Their yield, however, is significantly

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impacted by the polarity of extraction solvents, and this could be the reason why variations have been reported by other researchers.

Meanwhile, other research has indicated that *G.*'s antioxidant activity is reported in some studies. *corneum* is poor compared to other red algae despite their polyphenol- and alkaloid-content (Matos et al., 2019; Cavaco et al., 2021). A paradox like this may be a result of the chemical structure of the polyphenols present in the algae, the concentration used, or due to the presence of other bioactive compounds susceptible to affecting antioxidant activity by synergism or antagonism. Additional work is therefore needed to obtain proper characterization of these compounds, concentration and optimal extraction conditions, in an attempt to assess their efficacy as natural antioxidant agents in food, pharmaceutical or cosmetic applications.

Our DPPH findings show that ethanolic extract of *G. corneum* displays an antioxidant activity characterized by dose-dependent inhibition percentage (Figures 5A and 5B). Indeed, percentage inhibition was greater with increased ethanolic extract concentration. This result proves that *G. corneum* exhibits a satisfactory antioxidant activity ($IC_{50} = 1.57\text{mg/ml}$), but nevertheless less effective than ascorbic acid, used as a control ($IC_{50} = 0.093\text{ mg/ml}$) (Figure 6). This result is in agreement with several previous studies, which show that, excluding phenolic acids and alkaloids, other secondary metabolites from *G. corneum* (chlorophylls, carotenoids, phycocyanins and polysaccharides) may be involved in DPPH radical scavenging and stability and confirm our discovery by indicating that *G. corneum* possesses antioxidant activity which may be advantageous to human health (Matos et al., 2017; Torres et al., 2019; El Majnaoui and Kadmiri, 2021).

Figure 5. DPPH inhibition profile, A) in relation to *Gelidium corneum* ethanolic extract concentrations; B) in relation to ascorbic acid concentrations.

Figure 6. *Gelidium corneum* ethanolic extract and ascorbic acid antioxidant activity

To confirm and further investigate This research, it would be pertinent to complement the research in vitro and in vivo experiments using cellular or animal models in an attempt to further elucidate the role that the different secondary metabolites play in free radical neutralization as well as the influence of the latter on the biological mechanism of oxidative stress. This approach would also make it possible to determine the effectiveness of antioxidants in a physiological environment and assess their potential effect on human health.

Conclusion

The results derived confirm that *G. corneum* is an algae of nutritional and functional interest. Its carbohydrate, protein, and lipid content varies with environmental factors and place of harvesting. Phytochemical screening reveals a variety of secondary metabolites, namely flavonoids, tannins, coumarins and alkaloids, which confer antioxidant activity. This variability bears testimony to the need to further determine the effect of growth conditions on the nutritional value of this species.

These characteristics might suggest that *G. corneum* has potential applications in the cosmetic and nutraceutical industries. However, further studies, particularly in vivo, are still necessary in order to confirm further its phytochemical composition and biological activity, elucidate its mechanisms of action, and determine its therapeutic values.

Conflict of interest declaration

The authors declare that they have no financial or personal competing interests that would have influenced the design, conduct, analysis or interpretation of this scientific study.

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