

## Valorization of *Gelidium corneum* Industrial Residue as a Source of Antioxidant and Antifungal Agents

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### Abstract

The industrial agar extraction of the red alga *Gelidium corneum* generates substantial residue, a biomass with considerable potential for valorization into a sustainable bioproduct. This investigation aimed to characterize the phytochemical composition and bioactivities of extracts from the residue in comparison to the whole alga. For this purpose, extracts were prepared from both the residue and whole alga using various solvents. The antioxidant capacity of the extracts was then evaluated using DPPH, ABTS, and total antioxidant capacity assays. Additionally, their antifungal activity was tested against 4 storage fungi (*Fusarium graminearum*, *Fusarium oxysporum*, *Aspergillus flavus* and *Penicillium chrysogenum*), with efficacy compared to the commercial fungicide Azoxystrobin. Our analysis revealed that the 90% methanolic extracts were particularly effective, as they were rich in total phenolic compounds (TPC: 13 mg GAEs/g in residue and 14.9 mg GAEs/g in whole alga), total flavonoid content (TFC: 5.90 mg QEs/g in residue and 7.07 mg QEs/g in whole alga), and condensed tannins (CTC: 2.90 mg CEs/g in residue and 5.92 mg CEs/g in whole alga). These extracts exhibited potent antioxidant activity and their notable antifungal activity was comparable to Azoxystrobin. The observed bioactivities were consistently correlated with the high phenolic content. These findings suggest that *G. corneum* industrial residue is a promising and sustainable source of natural antioxidant and antifungal agents for applications in food preservation and related industries.

**Keywords:** *G. corneum* alga, By-products, Antioxidant, Antifungal, Phytopigment, Total phenolic content, Total flavonoid content

### Introduction

The issue of oxidation and microbial contamination represents one of the most prevalent challenges of food preservation, contributing to diminished quality, safety concerns, and a shortened shelf life of the products [1]. Furthermore, some fungal

species including *Aspergillus spp.* and *Penicillium spp.* have the potential to produce mycotoxins, making them toxigenic [2]. In order to ensure the safety of these food items, it is essential to implement control strategies that exhibit effective antimicrobial properties, the ability to inhibit mycotoxin formation, and the capacity to

counteract oxidative stress through antioxidative mechanisms [3]. Synthetic preservatives, which have been used in foods for decades, could lead to negative effects on both consumers and the environment [4]. This situation has prompted the scientific community to explore alternative compounds derived from natural resources [5].

Recently, marine algae have been recognized as valuable sources of naturally occurring antimicrobial and antioxidant agents, potentially replacing synthetic chemicals for various applications in food [6]. Among these marine algae, red algae stand out as the largest group, containing a diverse range of bioactive compounds such as polysaccharides, lipids, polyphenols, and steroids. These compounds offer promising alternatives to synthetic products for the food industry [7]. Notably, *G. corneum*, a species of red algae, contains a wide range of beneficial bioactive compounds and serves as the primary raw material for agar production. The *G. corneum* algae serves as the primary raw material for agar production. Remarkably, it constitutes approximately 90% of the marine algae harvested in Morocco [8]. The process of extracting agar from *G. corneum* generates a substantial quantity of by-products estimated to be around 870 tons per year, leading to challenges related to their storage, conversion, or disposal. These challenges have both environmental and economic implications. These by-products hold significant potential as natural sources of bioactive compounds. Therefore, the extraction of antioxidant and antimicrobial compounds from these residues could represent a significant advancement in preserving environmental balance. Concerning the utilization of solid waste generated during industrial agar production, previous research conducted by Aboulkas *et al.* [9], has explored the controlled pyrolysis process as a means to produce bio-oil and bio-char from algae waste. Furthermore, Tûma *et al.* [10], conducted an analysis in which the residues were biologically upgraded to poly-3-hydroxybutyrate (P3HB) through the saccharification of their carbohydrate portion into elemental simple sugars. To the best of our knowledge, no study has yet explored the antioxidant properties of extracts from different solvents and their antifungal effects against post-harvest fungi in residues derived from *G. corneum*, in comparison with the whole alga. This area presents a promising opportunity for thorough

research, aiming to enhance the quality of food products and extend their shelf life during storage.

The present research was undertaken to (1) assess the bioactive compounds in *G. corneum* industrial residues by comparing their phytochemical profile to that of the whole alga, (2) Evaluate the *in vitro* antioxidant and antifungal activity of extracts from both the residue and the whole alga, (3) identify the specific bioactive compounds associated with these activities.

## Materials and methods

### Preparation of residue and alga extracts

The whole *G. corneum* alga and its industrial residue obtained after agar-agar extraction were provided by the SETEXAM company (located in Kenitra, Morocco). This red alga has been identified and extensively studied by SETEXAM [11]. The materials were prepared by washing, shade-drying for 8 days at room temperature, and grinding them into a fine powder. Subsequently, extracts were obtained through maceration by soaking 10 g of dried powder in 100 mL of solvent for 48 h at 25 °C [12]. For extraction purpose, 3 different solvents were employed including methanol (MeOH) in a ratio of MeOH: H<sub>2</sub>O (90:10), ethanol (EtOH, 99.5%), and acetone (Ace, 100%). These solvents were used to extract bioactive compounds from both whole alga and its industrial residue.

### Phytopigment analysis

#### Chlorophyll analysis

The quantification of chlorophyll a in both *G. corneum* alga and its residue was carried out using the spectrophotometric method described by El-Din *et al.* [13]. Briefly, 500 mg of the alga and its residue were ground with 10 mL of acetone using a pestle and mortar. The absorbance (A) was then measured with a spectrophotometer, and the chlorophyll a concentration was calculated using the following formula:

$$\text{Chlorophyll}_a (\mu\text{g mL}^{-1}) = 11,75 \times A_{662} - 2.35 \times A_{645}$$

#### Phycobiliproteins analysis

The contents of phycobiliproteins (R-phycoerythrin, and R-phycoyanin) in *G. corneum* alga and its residue were determined according to the methods described by Castejón *et al.* [14]. Five g of each sample were ground in 50 mL of phosphate buffer (0.1

M, pH 6.8) through repeated cycles and then centrifuged at 4,000× g for 12 min at 4 °C. The resulting clear supernatant was carefully collected to measure the phycobiliproteins content. The concentrations of R-phycoerythrin, and R-phycoerythrin, and R-phycoerythrin were calculated using the equations below:

$$R - \text{phycoerythrin (mg mL}^{-1}\text{)} = 0,1247 [(A_{564} - A_{730}) - 0,4583 (A_{618} - A_{730})]$$

$$R - \text{phycoerythrin (mg mL}^{-1}\text{)} = 0,154 (A_{618} - A_{730})$$

$$\text{Total amount (mg mL}^{-1}\text{)} = R - \text{phycoerythrin} + R - \text{phycoerythrin}$$

### Phytochemical analysis

#### Total Phenolic Content (TPC)

The Folin-Ciocalteu method was used to assess the total phenolic content (TPC) in the extracts [15]. Each diluted extract at 1 mg mL<sup>-1</sup> was combined with 0.25 mL of Folin-Ciocalteu reagent for 3 min. Following this, 1 mL of 7.5% sodium carbonate solution was added, and the mixture was left to incubate in darkness at room temperature for 30 min. A standard gallic acid curve (0 to 0.2 mg mL<sup>-1</sup>) was similarly prepared. The absorbance was measured at 760 nm using a spectrophotometer. The average of 3 readings was calculated, and the total phenolic content was expressed as milligrams of gallic acid equivalent per gram of dry extract (mg GAE/g) for both the algae and residue extracts.

#### Total Flavonoid Content (TFC)

The total flavonoid content (TFC) was determined following the method by Chang *et al.* [16]. Each diluted extract at 1 mg mL<sup>-1</sup> (0.5 mL) was mixed with 1.5 mL of ethanol (95%), 0.1 mL of aluminum chloride (10%), 0.1 mL of 1M potassium acetate, and 2.8 mL of distilled water. The mixture was left to incubate in darkness at room temperature for 30 min. Afterward, the absorbance was measured at 415 nm using a spectrophotometer. A standard calibration curve with quercetin (ranging from 0 to 0.2 mg mL<sup>-1</sup>) was utilized. The total flavonoid content was quantified in mg of quercetin equivalent per g of dry extract (mg QEs/g).

#### Condensed Tannins Content (CTC)

The condensed tannin content (CTC) was determined using the vanillin-HCl assay following Broadhurst and Jones [17]. Each extract (0.5 mL) was mixed with vanillin reagent (4% w/v in methanol) (3

mL) and concentrated HCl (37%) (1.5 mL). After incubation in darkness at room temperature for 15 min, the absorbance was measured at 500 nm. A standard curve with concentrations of catechin ranging from 0 to 0.14 mg mL<sup>-1</sup> was used. The condensed tannin content was expressed as milligrams of catechin equivalents per gram of dry extract (mg CEs/g).

### Antioxidant activity

#### DPPH free radical scavenging activity analysis

The free radical scavenging activity of the 3 extracts derived from *G. corneum* alga and its residue was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The quantitative estimation of DPPH was performed according to Nehete *et al.* [18]. Specifically, 1 mL each of the 3 extracts at varying concentrations was mixed with 1 mL of a DPPH solution (0.1 mM). After a 20 min incubation in the dark, the absorbance (A) of the mixtures was determined at 517 nm using a spectrophotometer and subsequently compared with standards (ascorbic acid). The percentage of inhibition was calculated using the following equation.

$$I (\%) = \left( \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \right) \times 100$$

The IC<sub>50</sub> value representing the concentration at which 50% of DPPH is inhibited, was determined graphically through linear regression of I% versus concentrations.

#### ABTS radical scavenging activity

The 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) free radical scavenging activity of the 3 extracts was determined according to Re *et al.* [19]. The ABTS<sup>•+</sup> radical was generated by reacting 7 mM of ABTS and 2.45 mM potassium persulfate for 16 h in darkness at room temperature. The resulting ABTS<sup>•+</sup> solution was diluted with ethanol to achieve an absorbance of 0.70 ± 0.02 at 734 nm. Subsequently, 2 mL of the diluted ABTS<sup>•+</sup> solution was added to 0.1 mL of various extracts (MeOH 90%, EtOH and Ace) at different concentrations. The absorbance was measured at 734 nm using a spectrophotometer after 6 min of incubation. The ABTS<sup>•+</sup> free radical scavenging activity of the

extracts was calculated using the formula given in the DPPH assay, with ascorbic acid used as a standard.

#### **Total Antioxidant Capacity (TAC)**

The Total Antioxidant Capacity (TAC) of different extracts was evaluated using a prepared solution consisting of 1 mL containing 4 mM ammonium molybdate, 28 mM sodium phosphate, and 0.6 M sulfuric acid. This solution was mixed with 0.1 mL of the diluted extracts (1 mg mL<sup>-1</sup>). The resulting mixture was incubated at 95 °C for 90 min. Following incubation, the absorbance of the solutions was measured at 695 nm, with a negative control containing 0.1 mL of water used [20]. The results were expressed in milligrams of ascorbic acid equivalents per gram of dry extract (mg EAA/g) for both algae and residue extracts.

#### **Antifungal activity in vitro**

##### **Fungal material used**

Four fungal isolates, *Fusarium graminearum*, *Fusarium oxysporum*, *Aspergillus flavus*, and *Penicillium chrysogenum*, were selected to investigate the antifungal activity of the extracts. These species are commonly found in seeds and are known to produce various mycotoxins. The strains were isolated from stored chickpea seeds in the laboratory of phytopathology of food legumes in INRA-Morocco. The identification of the species was based on their morphological characteristics observed under a microscope, using identification key [21].

##### **Determination of Minimal Inhibitory Concentration (MIC)**

The antifungal activity of *G. corneum* alga and its residue was assessed by determining the Minimal Inhibitory Concentration (MIC) of each extract using the micro-dilution method according to Bouaziz *et al.* [22]. Each extract was dissolved in a 1% dimethyl sulfoxide (DMSO) solution and filtered through 0.22 µm pore Syringe Filters. The test was conducted in sterile 96-well microplates with final concentration of 12.5, 6.25, 3.13, 1.56, 0.78, and 0.39 mg mL<sup>-1</sup>. In each well, 20 µL of a fungal spore suspension (1×10<sup>6</sup> spores/mL) was added to achieve a final concentration of 1×10<sup>5</sup> spores/mL. The negative control consisted of a fungal suspension in Sabouraud Dextrose Broth with

1% DMSO, while azoxystrobin fungicide served as a positive control.

The plates were covered and incubated at 22 °C under a 12-hour photoperiod for 72 h. Absorbance was measured using a microplate reader at 492 nm before and after the incubation period. The Growth Inhibition Percentage (GIP) was calculated using the following formula:

$$\text{GIP (\%)} = \frac{[(\text{ACTf} - \text{ACT0}) - (\text{ATtf} - \text{ATt0})] \times 100}{(\text{ACTf} - \text{ACT0})}$$

where ACT0 and ATt0 represent the initial absorbances of the control (C) and the treatment (T) extracts, respectively, and ACTf and ATtf represent the final absorbances [23].

The MIC represents the lowest concentration at which 100% of the fungi are inhibited. Each test was replicated in triplicate, and the entire experiment was performed twice.

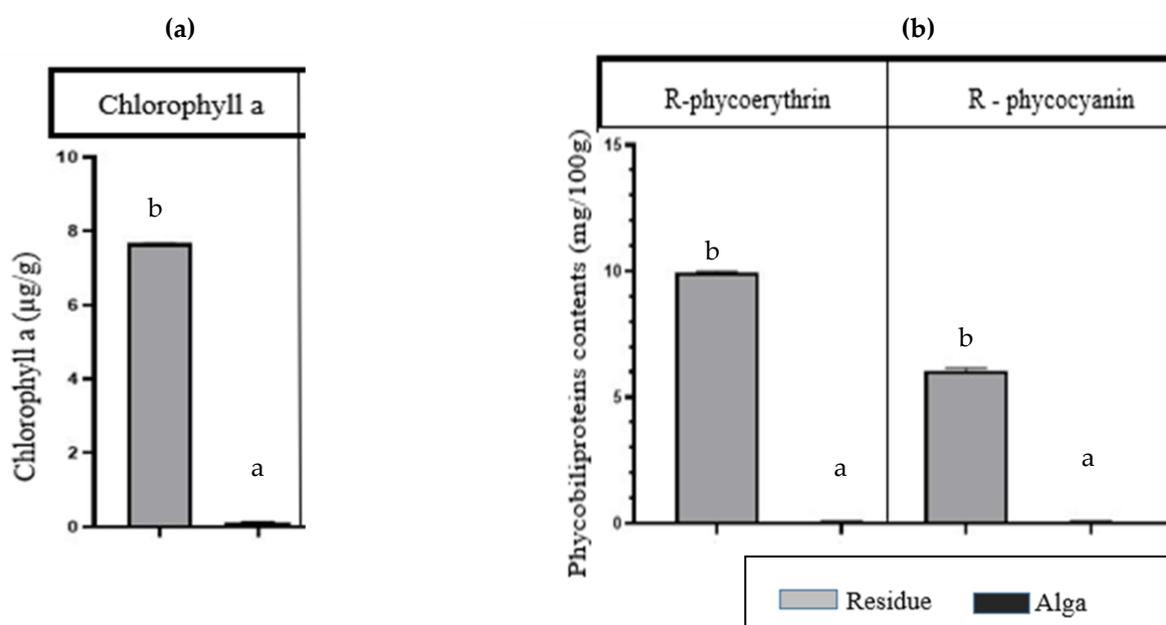
##### **Determination of Minimal Fungicidal Concentration (MFC)**

The MFC was determined to evaluate the fungicidal or fungistatic properties of *G. corneum* alga and its residue. Briefly, 20 µL of supernatant from wells showing complete inhibition of fungal growth at the Minimum Inhibitory Concentration (MIC) were sub-cultured in 200 µL of Sabouraud Dextrose Broth (SDB) in new cell culture plates. These plates were then incubated at 22 °C under a 12-hour photoperiod for 72 h. The MFC was defined as the lowest concentration where no visible fungal growth was observed in the well, examined under a binocular microscope [24]. The MFC/MIC ratio was calculated, with an agent classified as fungicidal when the MFC/MIC ratio was ≤ 4 and as fungistatic when the MFC/MIC ratio was > 4 [25].

##### **Data analysis**

Statistical analyses were performed using SPSS version 22.0 (IBM, Armonk, NY, USA). Variations in phytopigment content, Total Phenolic Content (TPC), Total Flavonoid Content (TFC), Condensed Tannin Content (CTC), and antioxidant activity within *G. corneum* alga and its residue were assessed via 1-way analysis of variance (ANOVA). ANOVA and Tukey's multiple range tests were employed to determine

differences among samples and between their respective extracts, with statistical significance level at  $p < 0.05$ . Furthermore, to elucidate the relationship between phytochemical contents (TPC, TFC and CTC) and antioxidant as well as antifungal activity, Pearson's correlation coefficients were calculated using SPSS version 22.0 (IBM, Armonk, NY, USA).



**Figure 1** Phytopigment analysis of the whole alga and its residue. (a) Concentration of chlorophyll a; (b) concentrations of phycobiliproteins (R-phycoerythrin and R-phycoyanin). Values with different letters (a, b) indicate significant differences between the whole alga and its residue ( $p < 0.05$ ).

#### Quantitative determination of Total Phenolic Content (TPC)

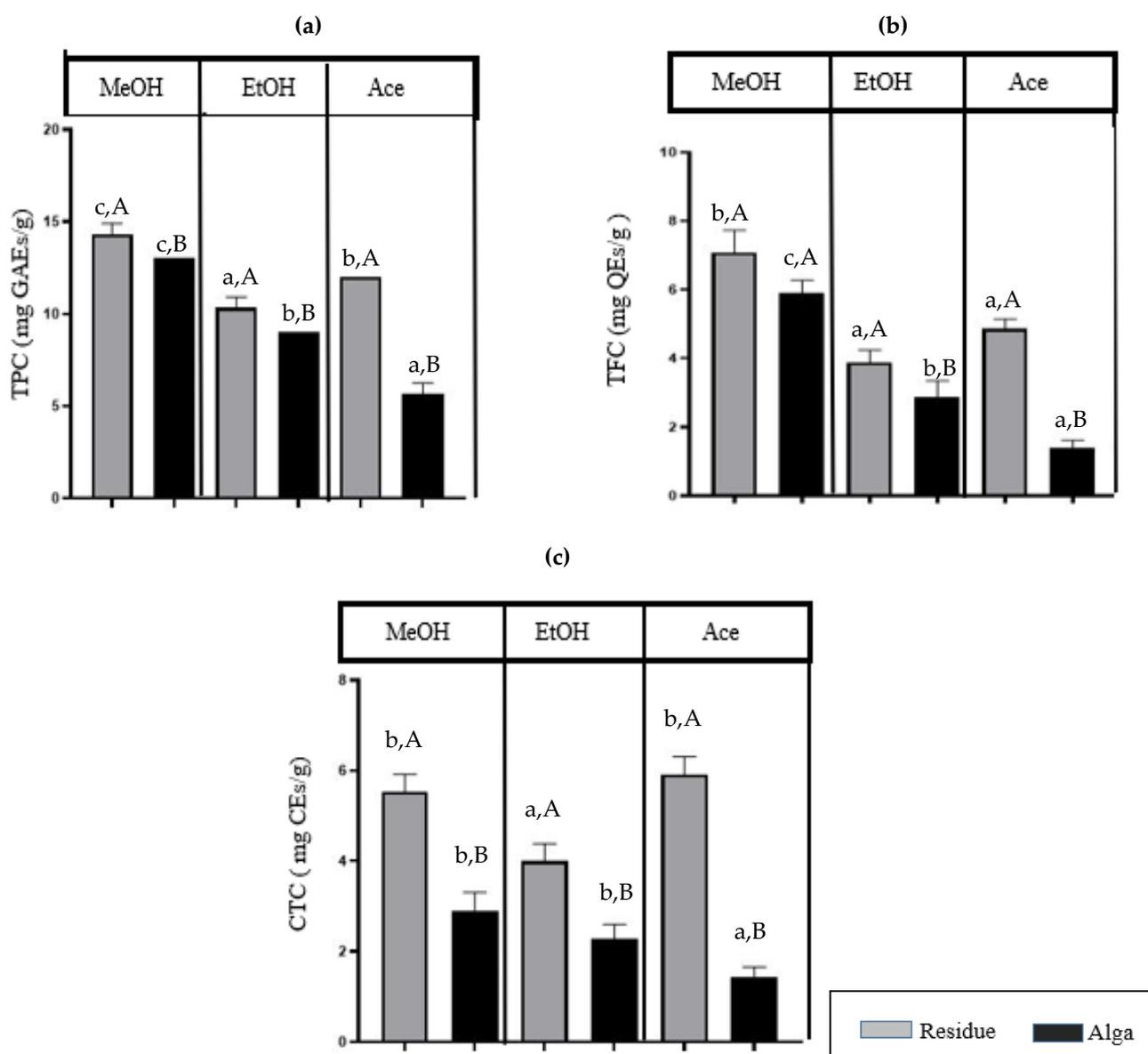
Quantitative analysis showed that methanolic (90%) extracts yielded the highest concentrations of total phenolic compounds (TPC), flavonoids (TFC), and condensed tannins (CTC), compared to ethanolic and acetic extracts (Figures 2(a) - 2(c)). Interestingly, the TPC and TFC levels in the residue (13 mg GAEs/g and 5.90 mg QEs/g) were close to those of the whole alga (14.9 mg GAEs/g and 7.07 mg QEs/g), while CTC was significantly lower in the residue (2.90 mg CEs/g) than in the alga (5.92 mg CEs/g). These values are higher than those reported for other red algae such as

## Results and discussion

### Phytopigments contents

The phytopigment analysis revealed that the whole alga contains the phytopigment chlorophyll a, with a concentration of 6.018 µg/g (Figure 1a). In addition, the alga contains significant amounts of 2 essential phycobiliproteins: Phycoerythrins (9.93 mg/100 g) and phycocyanins (6.03 mg/100 g), respectively (Figure 1(b)). Notably, these phytopigments are absent in the residue, most likely due to their removal during the agar extraction process.

*Gracilaria edulis* (4.1 mg GAEs/g) and *Acanthophora spicifera* (3.55 mg GAEs/g) [26], highlighting the potential of both *G. corneum* alga and its residue as valuable sources of phenolic compounds. The high polarity of methanol (90%) likely explains its efficiency in extracting these compounds. From an economic and environmental perspective, the recovery of such bioactive compounds from residues is particularly promising.



**Figure 2** Variation in phytochemical contents. (a) Concentration of total Phenolic Content (TPC), (b) Concentration of Total Flavonoid Content (TFC), (c) Concentration of Condensed Tannin Content (CTC), in *G. corneum* alga and its residue. The values presented are the means ( $n = 3$ ), and the error bars represent the standard errors. Different letters on the bars indicate significant difference at a level of  $p < 0.05$  according to Tukey's HSD test. Specifically, means with different lowercase letters (a - c) indicate the significant differences between the extracts of each sample, while means marked with different capital letters (A - B) indicate the significant differences between the same extract of the 2 samples.

### Antioxidant activity

The antioxidant properties of methanolic, ethanolic, and acetic extracts from both *G. corneum* alga and its residue were assessed using DPPH, ABTS, and TAC assays (Table 1). Lower  $IC_{50}$  values indicate higher antioxidant efficacy in DPPH and ABTS scavenging tests, while higher values indicate greater antioxidant potential in the TAC assay. Methanolic extracts from both the alga and residue demonstrated the

most substantial antioxidant activity using DPPH, ABTS, and TAC methods, compared to the (EtOH) and (Ace) extracts. The methanolic extract of the alga exhibited the highest antiradical capacity, with  $IC_{50}$  values of 0.436 and 1.311  $mg\ mL^{-1}$  for DPPH and ABTS, respectively. The residue displayed a moderate antiradical capacity, with  $IC_{50}$  values of 0.997 and 1.525  $mg\ mL^{-1}$  for DPPH and ABTS, respectively. These values were compared to the  $IC_{50}$  values of ascorbic acid

used as a standard (0.004 mg mL<sup>-1</sup> for DPPH and IC<sub>50</sub>: 0.131 mg mL<sup>-1</sup> for ABTS).

Furthermore, the results showed that the methanolic extract of *G. corneum* alga and its residue demonstrated a very strong TAC of 136.098 ± 1.977 and 116.737 ± 1.596 mg AAE/g, respectively. After agar extraction, the antioxidant activity in the residue declined. This decrease can be attributed to the removal of specific bioactive compounds present in the whole alga, such as chlorophyll a, phycoerythrin, and phycocyanin [27]. Nevertheless, significant antioxidant activity persisted in the residues, with IC<sub>50</sub> values still

much lower than those reported for other red algae, such as *Kappaphycus alvarezii*, which exhibited an IC<sub>50</sub> value of 4.28 mg/mL in methanol extract [28].

Overall, the methanolic extracts (90%) of both *G. corneum* alga and its residue exhibited the highest antioxidant activity across all tested methods. This strong activity can be attributed to the high phenolic content extracted in this solvent, since phenolic compounds are well known for their ability to neutralize free radicals through hydrogen atom transfer from their hydroxyl groups [29].

**Table 1** The antioxidant activities (IC<sub>50</sub> DPPH), (IC<sub>50</sub> ABTS), and Total Antioxidant Capacity (TAC) of *G. corneum* alga and its residue.

	Extracts	DPPH IC <sub>50</sub> (mg mL <sup>-1</sup> )	ABTS IC <sub>50</sub> (mg mL <sup>-1</sup> )	TAC (mg AAE/g Extract)
<b>Alga</b>	Methanolic (90%)	0.436 ± 0.001 <sup>a,A</sup>	1.311 ± 0.005 <sup>a,A</sup>	136.098 ± 1.141 <sup>c,A</sup>
	Ethanollic	0.783 ± 0.003 <sup>c,A</sup>	2.555 ± 0.004 <sup>c,A</sup>	109.645 ± 0.630 <sup>a,A</sup>
	Acetonic	0.669 ± 0.000 <sup>b,A</sup>	1.439 ± 0.009 <sup>b,A</sup>	119.928 ± 0.431 <sup>b,A</sup>
<b>Residue</b>	Methanolic (90%)	0.997 ± 0.002 <sup>a,B</sup>	1.525 ± 0.064 <sup>a,B</sup>	116.737 ± 0.921 <sup>c,B</sup>
	Ethanollic	1.633 ± 0.002 <sup>b,B</sup>	3.002 ± 0.041 <sup>b,B</sup>	76.808 ± 0.368 <sup>b,B</sup>
	Acetonic	1.762 ± 0.007 <sup>c,B</sup>	3.510 ± 0.004 <sup>c,B</sup>	71.489 ± 0.491 <sup>a,B</sup>
<b>Positive control</b>	Ascorbic acid	0.004 ± 0.000*	0.131 ± 0.000*	-

The values presented are the means ± standard deviations (n = 3). Different letters indicate significant differences at a significance level of  $p < 0.05$ , as determined by Tukey's HSD test. Specifically, means with different small lowercase letters (a - c) indicate the significant differences between the extracts of each sample, while means marked with different capital letters (A - B) indicate the significant differences between the same extract of the 2 samples.

### Antifungal activity

The antifungal activity of *G. corneum* alga and its residues was evaluated in vitro against four different fungi by determining the MIC and MFC values (Table 2). The results revealed that the whole alga exhibited stronger antifungal activity than the residues. Among the tested solvents, methanolic extracts (90%) from both the whole alga and its residues showed the highest activity compared to ethanollic and acetonic extracts, as indicated by their lower MIC values. The methanolic extract of the whole alga displayed the strongest activity, with MIC values ranging from 1.56 to 3.13 mg/mL, while methanolic extracts from the residues showed MIC values between 3.13 and 6.25 mg/mL. Notably, the fungicide used as a reference

(Azoxystrobin) exhibited similar MIC values (1.56 - 3.13 mg/mL) to those observed for the methanolic extracts of both the alga and its residues, highlighting comparable antifungal efficacy. Additionally, all extracts exhibited an MFC/MIC ratio below 4, indicating fungicidal rather than fungistatic effects.

The persistence of antifungal activity in the residue even after agar-agar extraction highlights their potential as a valuable source of bioactive compounds. Both the whole alga and its residues significantly inhibited the growth of several storage fungi, with activities approaching that of Azoxystrobin. These findings suggest that the high antifungal activity observed in methanolic extracts is primarily linked to their elevated levels of phenolic compounds, flavonoids,

and tannins. Phenolic constituents are known to exert antifungal effects through multiple mechanisms, including interference with hyphal growth, disruption of fungal cell walls, and impairment of metabolic processes, ultimately leading to mycelial death [30]. The hydroxyl groups in phenolic compounds may also

contribute by interacting with fungal cell membranes, altering their permeability and stability [31]. Similar reports have highlighted the pivotal role of phenolic compounds as antifungal agents in various algal and plant extracts [32].

**Table 2** Minimal inhibitory concentration (MIC) and (MFC) values of *G. corneum* alga and its residue against *F. graminearum*, *F. oxysporum*, *A. flavus*, and *P. chrysogenum* strains in comparison to chemical fungicide (Azoxystrobin).

Strains	Extracts	Alga			Residue			Azoxystrobin
		MIC	MFC	Ratio MFC/MIC	MIC	MFC	Ratio MFC/MIC	MIC
<i>F. graminearum</i>	Methanol (90%)	1.56	3.13	2	3.13	3.13	1	1.56
	Ethanol	3.13	3.13	1	6.25	6.25	1	
	Acetone	3.13	3.13	1	12.5	12.5	1	
<i>F. oxysporum</i>	Methanol (90%)	3.13	3.13	1	3.13	6.25	2	3.13
	Ethanol	6.25	6.25	1	6.25	12.5	2	
	Acetone	3.13	3.13	1	12.5	12.5	1	
<i>P. chrysogenum</i>	Methanol (90%)	3.13	3.13	1	6.25	6.25	1	1.56
	Ethanol	6.25	6.25	1	6.25	6.25	1	
	Acetone	3.13	6.25	2	12.5	12.5	1	
<i>A. flavus</i>	Methanol (90%)	3.13	3.13	1	6.25	6.25	1	1.56
	Ethanol	6.25	6.25	1	6.25	.25	1	
	Acetone	3.13	3.13	1	12.5	12.5	1	

The values display the minimum inhibitory concentration (MIC), minimum fungicidal concentration (MFC), and their corresponding MFC/MIC ratio.

#### Correlations between TPC, TFC, CTC content, antioxidant (IC<sub>50</sub> DPPH, IC<sub>50</sub> ABTS, TAC) and antifungal activity (MIC)

Pearson's correlation analysis was conducted to investigate the relationships among phytochemical content, antioxidant activity, and antifungal activity in *G. corneum* alga and its residues (Tables 3 and 4). Both the whole alga and residue showed a significant negative correlation between total phenolic content (TPC) and IC<sub>50</sub> values for DPPH (-0.966) and ABTS (-0.840) scavenging activities, while a strong positive correlation was observed between TPC and total antioxidant capacity (TAC) (0.983). It is important to note that in DPPH and ABTS assays, lower IC<sub>50</sub> values indicate stronger antioxidant efficacy, whereas in the TAC assay, higher values reflect greater antioxidant potential. Thus, the negative correlation observed between phenolic

content and IC<sub>50</sub> values confirms that increasing phenolic levels enhance antioxidant activity.

This relationship was particularly evident for flavonoids, which showed high significance in both the whole alga and residue. An increase in TPC and TFC was therefore associated with enhanced antioxidant activity, as reflected by lower IC<sub>50</sub> values for DPPH and ABTS, and higher TAC values. These results suggest that TPC and TFC are major contributors to the antioxidant activity of *G. corneum* alga and its residue extracts. Such associations are consistent with previous studies reporting the central role of phenolic compounds in the radical scavenging capacity of marine algae [33,34].

Regarding antifungal activity, significant negative correlations were observed between MIC values of the tested fungal strains (*F. graminearum*, *F. oxysporum*, *A.*

*flavus* and *P. chrysogenum*) and the phytochemical contents (TPC, TFC, and CTC) of both alga and residues (**Table 4**). Since higher antifungal efficacy corresponds to lower MIC values, these results indicate that higher levels of these bioactive compounds are associated with stronger antifungal effects. Both flavonoids and condensed tannins likely contributed to the inhibition of fungal growth, reinforcing their role as antifungal agents.

Altogether, the strong correlations observed confirm that phenolic compounds, flavonoids, and tannins are the main contributors to the dual antioxidant and antifungal activities of *G. corneum* alga and its residues. These findings agree with previous reports demonstrating similar relationships in other marine algae [35].

**Table 3** Pearson's correlation coefficients values showing relationship between the chemical content and antioxidant IC50 DPPH and IC50 ABTS, Total Antioxidant Capacity (TAC) of alga and residue extracts.

	DPPH	ABTS	TAC
<b>Alga</b>			
TPC	-0.966**	-0.840**	0.983**
TFC	-0.959**	-0.766*	0.946**
CTC	-0.578	-0.898**	0.623
<b>Residue</b>			
TPC	-0.870**	-0.950**	0.934**
TFC	-0.849**	-0.931**	0.928**
CTC	0.909**	-0.803**	0.848**

The correlation is significant at \*:  $p < 0.05$ ; \*\*:  $p < 0.01$ . Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and Condensed Tannins Content (CTC).

**Table 4** Pearson's correlation coefficients values showing relationship between the chemical content, and antifungal activity (MIC) of alga and residue extracts.

	<i>F. graminearum</i>	<i>F. oxysporum</i>	<i>P. chrysogenum</i>	<i>A. flavus</i>
<b>Alga</b>				
TPC	-0.886**	-0.793*	-0.793*	-0.793*
TFC	-0.918**	-0.710*	-0.710*	-0.710*
CTC	-0.306	-0.919**	-0.919**	-0.919**
<b>Residue</b>				
TPC	-0.967**	-0.967**	-0.835**	-0.835**
TFC	-0.915**	-0.915**	-0.743*	-0.743*
CTC	-0.908**	0.908**	-0.824**	-0.824**

The correlation is significant at \*:  $p < 0.05$ ; \*\*:  $p < 0.01$ . Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and Condensed Tannins Content (CTC).

## Conclusions

In conclusion, our study highlights the significant antioxidant and antifungal potential, particularly against storage fungi, of methanolic extract (90%) of *G. corneum* residue, which are rich in phenolic compounds comparable to its whole alga. The phenolic compounds, and specifically flavonoids, played a significant role in the biological activity of these residues. These findings underline the potential of *G. corneum* residue as natural sources abundant in bioactive compounds, possessing antioxidant and antifungal properties. Moreover, these residues could be incorporated into stored products in the industrial sector to enhance both their quality and preservation. Further investigations should include *in vivo* tests to evaluate the biological, antioxidant, and antifungal activity of residues against a wide range of fungi.

## Declaration of generative AI in scientific writing

The authors declare that no generative artificial intelligence (AI) tools were used in the preparation of this manuscript. All content, interpretations, and conclusions were developed entirely by the authors.

## CRedit author statement

**Hajare Errati:** Conceptualization; Methodology; Writing- original draft. **Sarra Aouzal:** Data Curation; Investigation; **Rania Benjamaa:** Formal Analysis. **Lamyae Et-tazy:** Investigation; **Lahoucine Hilali:** Methodology; Supervision. **Salim Lebbar:** Resources; Investigation. **Bouchaib Bencharki:** Resources. **Sanae Krimi Bencheqroun:** Supervision; Conceptualization; Validation; Writing- Review & Editing.

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