Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 29(3): 3267 – 3275 (2025)
www.ejabf.journals.ekb.eg

Phytochemicals Screening, Antioxidant Properties and Antibacterial Activity of *Chondrus crispus* Fractions

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ARTICLE INFO

Article History:
Received: April 30, 2025
Accepted: June 20, 2025
Online: June 29, 2025

Keywords:
Chondrus crispus,
Fractions,
Antibacterial,
Antioxidant

ABSTRACT

The hexane, ethanol, and methanolic extracts of *Chondrus crispus* were subjected to phytochemical screening before antioxidant properties and antibacterial activity were assessed. The aim of this study was to evaluate the phytochemical screening, antioxidants, and antibacterial activity of *Chondrus crispus* fractions. The antioxidant activity was determined by measuring total phenolic content (TPC), 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP). The paper disc method was used to evaluate the antibacterial activity of two microorganisms, *Bacillus cereus* and *Pseudomonas aeruginosa*. The fractions contained a total of six substances that were classified as phenolics, flavonoids, alkaloids, terpenoids, tannins, and saponins. Total phenol and antioxidant activity (FRAP and DPPH) were the highest in methanol extract (41.67 mg GAE/100g, 91.67.81 percent, and 63.92mg TE/100g, respectively). At the methanol fraction, the highest zones of inhibition against *Pseudomonas aeruginosa* and *Bacillus cereus* were 13.86 and 15.10mm, respectively. It was determined that the solvent fraction has a significant impact on the bioactive component of the *Chondrus crispus* extract and can be utilized to prevent oxidative damage and manage infectious illnesses.

INTRODUCTION

Pathogenic bacteria's resistance to current antibiotics has spread throughout the world **(Benbott *et al.*, 2012)**. Nowadays, bacterial illnesses frequently result in the creation of biofilm. The majority of the time, biofilms cause diseases to spread, and they appear to be responsible for over 60% of infections **(Foster, 2017)**. Global maritime flora is highly diverse; around 90% of marine species have not yet been named, and little is known about the bioactivities of their constituents **(Sigwart *et al.*, 2021)**. *C. crispus* has been used historically since 400 BC for food in the islands of the North Atlantic Ocean and since 600 BC for medicinal uses in China **(Fleurence & Levine, 2016; Valero *et al.*, 2017)**. Carrageenan **(Tanoeiro *et al.*, 2023)** from *C. crispus*, commonly referred to as Irish moss, has an average molecular weight of 400–560 kDa. On the other hand, semi-refined carrageenan has a molecular weight of 615 kDa **(Imeson, 2009)**. The usual molecular weight of food-grade carrageenan is 200–800 kDa, while it can vary up to

7000 kDa **(Khotimchenko *et al.*, 2020; Premarathna *et al.*, 2024)**. Extract from seaweed is a rich source of chemicals with biological activity. According to **Blount *et al.* (2007)**, this skill was created as a protection against the many creatures that live and interact in the same complicated habitat. Over the past three decades, there has been a significant increase in the finding of metabolites from algae that have biological action, according to **Smit (2004)**. Numerous unique biological activities, including antibacterial activity, are demonstrated by these compounds **(Etahiri *et al.*, 2001; Etahiri *et al.*, 2007)**. This study aimed to determine the biological activity of *Chondrus crispus* fractions.

MATERIALS AND METHODS

1-Preparation of extraction

Amazon, an American corporation, provided the *Chondrus crispus* sample powder. Gradient elution of 100ml of hexane, ethanol, and methanol, respectively, was used to finish the fractionation process. The mixture was agitated for 24 hours and then evaporated at 40^0C using a rotary evaporator. Until additional analysis, the resulting fractions were stored in the freezer **(Purwayantie & Dewi, 2019)**.

2-Phytochemical analysis

Following normal procedures, one gram of *Chondrus crispus* hexane, ethanol, and methanol extracts were diluted in ten milliliters of solvent and put through a preliminary phytochemical screening **(Harborne, 1998)**.

3- Determination of total phenolic content

The Folin-Ciocalteu method was used to estimate the total phenolic content **(Karen & Vernon, 1977)**. The gallic acid was used as the standard. A 96-deep well plate (Eppendorf, Hamburg, Germany) was used to oxidize a 100-µL sample of *Chondrus crispus* extract using 500µL of diluted Folin-Ciocalteu reagent. After 5 minutes, 1 milliliter of 7.5% w/v sodium carbonate was added to neutralize the mixture. It was then incubated for 120 minutes before the absorbance at 765 nm was measured.

4- Ferric reducing antioxidant power (FRAP) assay

Trolox was used as the standard in a modified version of the FRAP assay method **(Benzie & Strain, 1996)** to measure antioxidant activity. 300 mM acetate buffer, pH 3.6 (3.1 g) sodium acetate trihydrate, and 16 mL glacial acetic acid were combined to make one liter of distilled water to create the FRAP reagent. To create the working reagent, 10 mM TPTZ (2,4,6-tri(2-pyridyl)-s-triazine) was combined with 40 mM HCl and 20 mM FeCl3 6H2O in a 10:1:1 ratio with the acetate buffer. Assays involved mixing 100µL of sample, standard, or blank with 1900µL of freshly warmed FRAP reagent in wells of a 96-deep well plate (Eppendorf, Hamburg, Germany) and incubating for 30 minutes before measuring absorbance at 595nm.

5- DPPH radical scavenging activity assay

The antioxidant activity was measured using the (DPPH) radical scavenging method described in the study of **Brands-Williams (1995)**. A standard DPPH solution was made by dissolving 40mg of DPPH in 100mL of methanol, and it was stored at -20°C until it was required. An absorbance of 0.70 ± 0.01 at 517nm was obtained by adjusting the absorbance using a UV-visible spectrophotometer by combining 350µL of the standard solution with 350µL of methanol.

6-Antibacterial assay

In the experiment, *Pseudomonas aeruginosa* and *Bacillus cereus* were employed. In the antibacterial assay, Mueller Hinton agar was utilized. To reach a concentration of 200mg/ mL, *Chondrus crispus* extracts were dissolved in acetone, ethanol, and methanol. The disc diffusion method, as previously described by **Kamali Gardea *et al.* (2015)**, was used to perform antibacterial experiments. The discs' surrounding zones of inhibition were measured in millimeters. The mean diameter of the inhibitory zones was computed after the experiment was conducted three times.

7- Statistical analysis

The means and standard deviation (SD) of three measurements are used to express the results. Using SPSS version 25, analysis of variance (ANOVA) and Duncan test were used to assess the significance of differences between mean values. A difference was deemed significant when its probability value was less than 0.05.

RESULTS

Table 1. Analysis of phytochemicals of *Chondrus crispus* fractions

Solvent	Phenols	Flavonoids	Alkaloids	Terpenoids	Tannins	Saponins
Hexane	+	+	+	-	-	+
Ethanol	+	+	+	-	+	+
Methanol	+	+	+	+	+	+

+: presence of phytochemicals, −: absence of phytochemicals

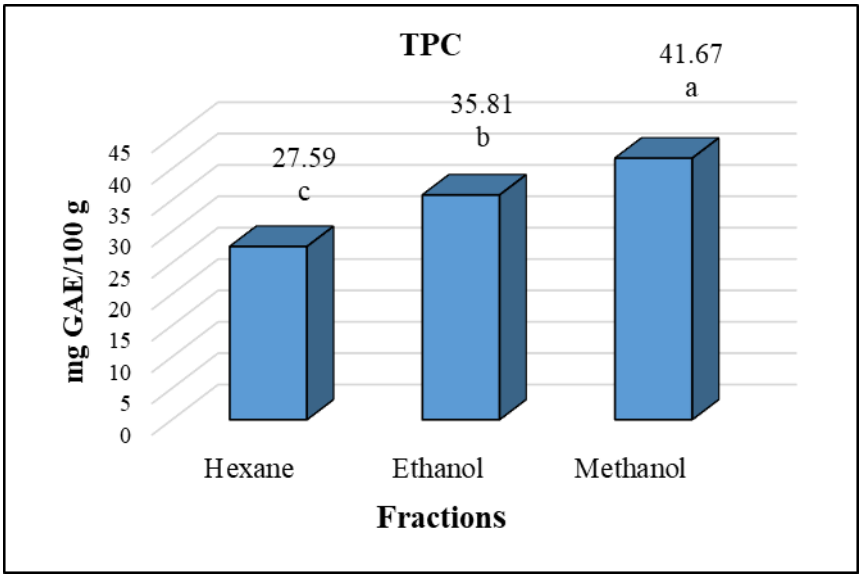

Fig. 1. Total phenolic content of *Chondrus crispus* fractions with different solvent
[a–c] Different letters indicate significant difference ($P< 0.05$)

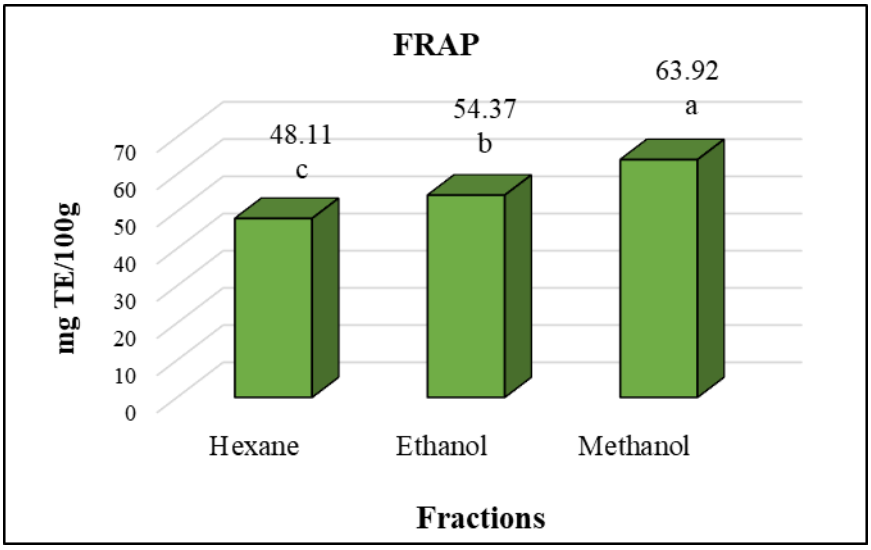

Fig. 2. Ferric reducing antioxidant power of *Chondrus crispus* fractions with different solvents
[a–c] Different letters indicate significant difference ($P< 0.05$)

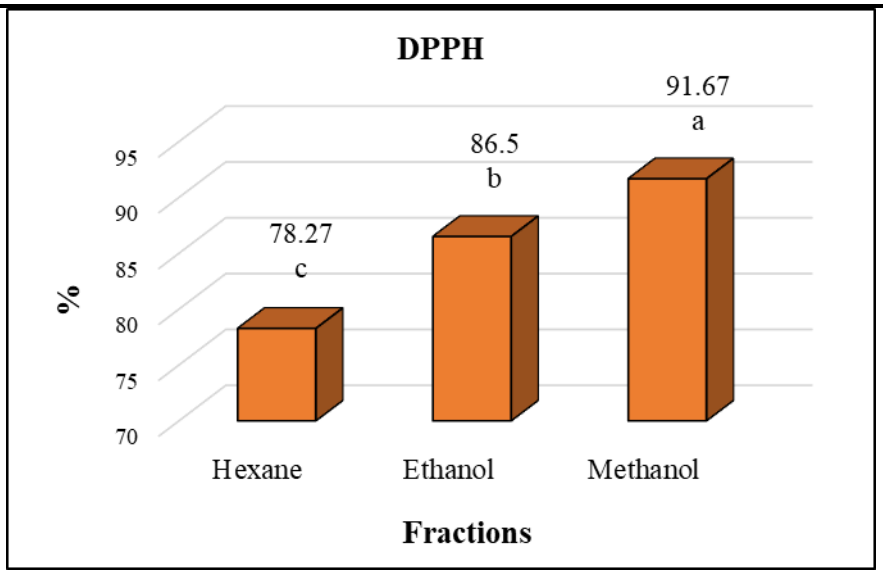

Fig. 3. DPPH radical scavenging activity of *Chondrus crispus* fractions with different solvents

[a–c] Different letters indicate significant difference ($P< 0.05$)

Table 2. Antibacterial activity of *Chondrus crispus* fractions

Fractions	**Minimum inhibitory concentration (200mg/ml)**	
	Bacillus cereus	***Pseudomonas aeruginosa***
Hexane	8.00 ± 1.00 [c]	6.15 ± 1.00 [c]
Ethanol	11.45 ± 1.20 [b]	9.51 ± 0.50 [b]
Methanol	15.10 ± 0.55 [a]	13.86 ± 1.00 [a]

[a–c] Different letters indicate significant difference ($P< 0.05$)

DISCUSSION

1- Phytochemical analysis

The crude extracts of *Chondrus crispus* (Table 1) showed phenolics, flavonoids, alkaloids, terpenoids, tannins, and saponins in methanol, ethanol, and hexane extracts, according to phytochemical screening. It is well known that the phytochemicals under evaluation have both physiological and therapeutic properties. Antibacterial, antioxidant, anti-inflammatory, antiallergic, antimutagenic, and vasodilatory properties have been described for flavonoids, alkaloids, and terpenoids **(Addai *et al.*, 2022)**. Tannins and triterpenoids demonstrated antibacterial and antioxidant qualities, while saponins showed hypocholesterolemic and antidiabetic effects (21). According to this study's testing,

Chondrus crispus contains biologically significant phytochemicals that add to their medicinal value and, as a result, provide possible sources for beneficial medications.

2-Total phenol content and antioxidants activity

Fig. (1) displays the content of phenolic compounds and antioxidant activity in the various fraction extracts of *Chondrus crispus* extract (hexane, ethanol, and methanol). In comparison to ethanol and hexane, which had phenolic contents of 35.81 and 27.59 mg/GAE/100g, respectively, methanol extract had the greatest phenolic level (41.67 mg/GAE/100g). In *Chondrus crispus* extract, the ethanol fraction had a higher concentration of phenolic chemicals than the methanol fraction. The polarity of solvents has an indirect function in the extraction process since it might make antioxidant compounds more soluble **(Sousa *et al.*, 2007)**. There is no way to develop a conventional solvent that can be used to extract several antioxidants from plants sample. The phenolic concentration was also found to be influenced by the solvent polarity and the kind of sample or materials employed **(Addai *et al.*, 2022)**. The Fe3+-Fe2+ conversions in the presence of a sample of *Chondrus crispus* extract were analyzed to determine the degree of reductive capacity. FRAP values for three distinct solvents are displayed in Fig (2). The range of the results was 48.11–63.92 mg TE/100g. The FRAP values of the various solvents showed significant variations ($P< 0.05$). The most effective solvent for identifying extracts with greater antioxidant activity was methanol. The ethanol-derived FRAP value was substantially ($P< 0.05$) higher than the hexane-derived extract. The findings demonstrated that *Chondrus crispus* methanol extract had significantly ($P< 0.05$) greater scavenging activity than acetone and ethanol. The findings in Fig. (2) show that antioxidant activity was affected by the extraction solvents; methanol generally had the highest extraction recovery. Methanol outperformed all other organic solvents in producing extracts with high antioxidant activation in *Chondrus crispus*, with ethanol coming in second ($P> 0.05$) with 91.67 and 86.50 percent, respectively. The total phenolics content and the sample extract's ability to scavenge radicals have been correlated **(Abdille *et al.*, 2005; Abbas & Yazea, 2025)**, and other investigations have found a strong link between phenolic compounds and antioxidant activity using the DPPH method **(Zwaid *et al.*, 2025)**. *Chondrus crispus* extract demonstrated high antioxidant activity in the present investigation, presumably due to its phenolic composition **(Zwaid *et al.*, 2025)**.

3-Antibacterial activity

Anti-infective agents may be abundant in natural products. The present study evaluated the antibacterial activity of *Chondrus crispus* extracts against pathogenic strains of *Pseudomonas aeruginosa* and *Bacillus cereus* bacteria (Table 2). Depending on the type of bacteria employed, different *Chondrus crispus* extracts have different levels of antibacterial activity. *Bacillus cereus* was the most resistant organism and the most vulnerable. The inhibitory zone's diameter ranged between 15.10 and 13.86mm. *Bacillus cereus* had the highest antibacterial activity of the *Chondrus crispus* extract, whilst

Pseudomonas aeruginosa exhibited the lowest activity. Furthermore, the *Chondrus crispus* extract's higher total phenolic contents might be connected to its antibacterial properties (Fig. 1). This result is in line with other earlier studies that showed that the phenolic content of natural product extracts inhibited Gram-positive bacteria more than Gram-negative ones. Generally speaking, phenolic compounds (phenolic acids, flavonoids, and anthocyanins) can interfere with the function of bacterial cell membranes, delaying bacterial growth and reproduction. Other phenolic chemicals that contribute to pathogen inactivation include enzyme inactivation, protein and cell wall binding, and intercalation into the cell wall and/or DNA **(Saeed *et al.*, 2020; Prasetyaningsih *et al.*, 2025)**. The extraction yield was affected by temperature, pH, polarity, concentration, solubility, extraction solvents, and the extraction procedure **(Benbott *et al.*, 2012)**.

CONCLUSION

According to the study, total phenol concentration was crucial for antioxidants (FRAP and DPPH) and antibacterial activity. Due to its higher total phenol concentration, the methanol fraction from *Chondrus crispus* extract was found to have the maximum antioxidant and antibacterial activity. These findings suggest that extracts from *Chondrus crispus* contain a variety of bioactive chemicals, a high concentration of phenol, and potent antibacterial and antioxidant properties.

REFERENCES

Abbas, D. and Yazea, N. (2025). GC-MS Identification and Antibacterial Activity of Palmaria Palmata Algae Extract Against Some Pathogenic Bacteria. Egyptian Journal of Aquatic Biology and Fisheries, 29(2): 1543-1550.

Abdille, M.H., Singh, R.P., Jayaprakasha, G.K and Jena, B.S. (2005). Antioxidant activity of the extracts from Dillenia indica fruits. Food Chemistry, 90(4): 891-96.

Addai, Z. R., Abood, M. S. and Hlail, S. H. (2022). GC-MS Profiling, Antioxidants and Antimicrobial Activity of Prickly Pear (*Opuntiaficus-indica*) Pulp Extract. Pharmacogn J, 14(2): 262-267.

Addai, Z. R., Abood, M. S. and Hlail, S. H. (2022). Evaluation of Antioxidant and Antimicrobial Activities of *Stachys* Leaves Extract. Trop J Nat Prod Res, 6(9):1469-1473.

Benzie, I. F. and Strain, J. J. (1996) The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical biochemistry*, *239*(1): 70-76.

Blunt, J.W.; Copp, B. R.; Munro, M. H.; Northcote, P.T. and Prinsep, M.R. (2007). Marine Natural Products. Natural Product Reports, 24(1): 31-86.

Brand-Williams, W. (1999). Use of a free radical method to evaluate antioxidant activity. Food Sci. Technol., *28*:1231-1237.

Benbott, A., Yahyia, A. and Belaidi, A. (2012). Assessment of the antibacterial activity of crude alkaloids extracted from seeds and roots of the plant *Peganum harmala* L. J. Nat. Prod. Plant Resour, 2: 568-73

Dewi, YSK. (2019). Purwayantie S. Phythochemical and antioxidant activity from fruit of Kulim (Scorodocarpusborneensis Becc.), in: Proceeding of the International Conference on Food and Agriculture.

Etahiri, S.; Butel-ponce, V.; Caux, C. and Guyot, M. (2001). New bromoditerpenes from red *Sphaerococcus coronopifolius*. Journal of Natural Products, 64:1024-1027.

Etahiri, S.; El Kouri, A.; Bultel-Ponce, V.; Guyot, M. and Assobhei, O. (2007). Antibacterial bromophenol from the marine red algae *Pterosiphonia complanata*. Natural Product communications, 2(7):749-752.

Fleurence, J. and Levine, I. (2016). *Seaweed in health and disease prevention*. Academic Press.

Foster, T.J. (2017). Antibiotic resistance in *Staphylococcus aureus*. Current status and future prospects. FEMS Microbiology Reviews 41: 430– 449

Harborne, J.B. (1998). Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis, 3rd ed. New York: Chapman and Hall Int Ed: 234−45.

Imeson, A. P. (2009). Carrageenan and furcellaran. In *Handbook of hydrocolloids* (pp. 164-185). Woodhead Publishing.

Kamali, M.; Khosroyar, S. and Mohammadi, A. (2015). Antibacterial activity of various extracts from Dracocephalumkotschyi against food pathogenic microorganisms. Int.J. PharmTech Res, 8:158- 163.

Karen, S. and Vernon LS. (1977) Total Phenol Analysis: Automation and Comparison with Manual Methods. *Am J Enol Vitic*, 28:49-55.

Khotimchenko, M. (2020). Pectin polymers for colon-targeted antitumor drug delivery. International journal of biological macromolecules, 158: 1110-1124.

Prasetyaningsih, A.; Nugroho, L. H.; Kristiani, E. B. E.; Hutomo, S. and Sasongko, G. (2025). Antioxidant and Antimicrobial Potential of Sargassum spp. Extract from Gunungkidul Regency, Indonesia Using Triphasic Extraction Method. Egyptian Journal of Aquatic Biology & Fisheries, 29(1): 55 – 85

Premarathna, A. D.; Ahmed, T. A.; Kulshreshtha, G.; Humayun, S.; Darko, C. N. S.; Rjabovs, V. and Hincke, M. T. (2024). Polysaccharides from red seaweeds: Effect of extraction methods on physicochemical characteristics and antioxidant activities. Food Hydrocolloids, 147: 109307.

Saeed, A.; Abotaleb, S.; Alam, N.; ELMehalawy, A. and Gheda, S. (2020). In vitro assessment of antimicrobial, antioxidant and anticancer activities of some marine macroalgae. Egyptian Journal of Botany, 60(1):81-96.

Sigwart, J. D.; Blasiak, R.; Jaspars, M.; Jouffray, J. B. and Tasdemir, D. (2021). Unlocking the potential of marine biodiscovery. Natural Product Reports, 38(7): 1235-1242.

Smit, A.J. (2004). Medicinal and pharmaceutical uses of seaweed natural products. Journal of Applied Phycology. 16:245-262.

Valero, M.; Guillemin, M. L.; Destombe, C.; Jacquemin, B.; Gachon, C. M.; Badis, Y. and Faugeron, S. (2017). Perspectives on domestication research for sustainable seaweed aquaculture. Perspectives in Phycology, 4(1): 33-46.

Zwaid, S.K.; Addai, Z.R. and Ayal, A.W.R. (2025). Study of Chemical Content and Antioxidant Activity of *Palmaria palmata*, *Chondrus crispus* Algae and *Raphanus sativus* Leaves Extracts. Egyptian Journal of Aquatic Biology & Fisheries, *29*(3).