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Research Article

Antioxidant enhancement of *Cystoseira barbata* extracts via nanoliposomal encapsulation

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Abstract: Seaweeds are well-known for their numerous health benefits, which include antioxidant, antimicrobial, antitumor, and anti-inflammatory properties. They produce secondary metabolites to withstand extreme conditions such as high temperatures, fluctuating salinity, intense sunlight, and varying oxygen levels. These compounds not only serve as protective mechanisms for the seaweeds but also hold potential for medicinal applications and other biologically active uses.
In this study, we investigated the effects of nanoliposomal encapsulation on the antioxidant capacity of extracts from *Cystoseira barbata*, collected from the Bursa/Mudanya coast. The extract was obtained from dried algal samples using ethanol solvent at a ratio of 1:1. The total phenolic content of the extract was analyzed, revealing the highest phenol concentration of 2.66 mg GAE/g. The DPPH assay was conducted to assess the antioxidant capacity of both free extract (FE) and the nanoliposomal encapsulated extract (NE). Inhibition (%) values showed a positive correlation with concentration, yielding values of 19.41 for FE and 31.33 for NE. Nanoliposomal encapsulation enhanced DPPH scavenging capacity by 61% compared to FE. Thus, the nanoliposomal encapsulation technique appears to be a promising method for enhancing the effectiveness of *C. barbata* extracts as antioxidant agents.

1. INTRODUCTION

Seaweeds, as photosynthetic organisms, play a vital role in the nutrition, reproduction, protection, and survival of many living organisms due to their rich biochemical composition. Even in ancient times, algae were used as sources of vitamins, minerals, proteins, food, fertilizers, and medicine (El Gamal, 2010; Mutlu-Durak *et al*., 2024). Today, seaweeds have become valuable raw materials used across various industries, including pharmaceuticals, food, cosmetics, and more (Kılınç *et al*., 2013; Bouzenad *et al*., 2024). Brown macroalgae, in particular, have attracted growing attention due to their abundance of bioactive compounds, such as polyphenols, sulfated polysaccharides, terpenoids, and flavonoids that exhibit antioxidant, antimicrobial, anticancer, and anti-inflammatory properties. Among them, the genus *Cystoseira*, which is widely distributed along the Mediterranean, Aegean, and Marmara coasts, is well known for its strong biological activities and industrial potential. *Cystoseira*

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barbata specifically has shown notable antioxidant and cytotoxic effects, largely attributed to its high content of phenolic compounds and fucoidans (Aly *et al*., 2024; Dini, 2023; Hejna *et al*., 2024; Mhadhebi *et al*., 2014; Sathya *et al*., 2017).

Previous research has indicated that *Cystoseira* sp. lipid extract may serve as an alternative source of antioxidants, anticancer, and antifungal agents in the food supplement, food, and cosmetic industries due to its phenolic compounds and sulfated polysaccharide fucoidan content (Goutzourelas *et al.*, 2023; Yegdaneh *et al*., 2016). Furthermore, the methanol extract was evaluated for its antibacterial activity against *E. coli* and *S. aureus*, and the activity was confirmed. Additionally, the ether extraction of *Cystoseira* sp. extract demonstrated cytotoxic activity in Duadi, Jurkat, and K562 tumor cell lines (Reyes *et al*., 2020).

Therefore, the TPC compounds found in *Cystoseira* sp. extracts may account for their remarkable antioxidant activities. A well-documented positive correlation exists between the total phenolic content of algae and their antioxidant capacity (Karawita *et al.*, 2005). Numerous researchers have explored the antioxidant properties of seaweeds, identifying several compounds with antioxidant potential. These include protective enzymes (Nakano *et al.*, 1995), ascorbic acid (Morgan *et al.*, 1980), lipophilic antioxidants (Takamatsu *et al.*, 2003), phlorotannins (Jiménez-Escrig *et al.*, 2001), and catechins (Yoshie *et al.*, 2000). These components are essential for understanding the broader health benefits associated with macroalgae. Phenolic compounds and flavonoids present in macroalgae play a significant role in their antioxidant activity. Antioxidants play a crucial role in combating reactive oxygen species, such as hydroxyl, superoxide, and peroxyl radicals. When present in excessive amounts, these species can lead to health issues, including cancer and aging (Aktar *et al.*, 2022). The molecules in macroalgae have the potential to alleviate oxidative stress by inhibiting the formation and chain reactions of free radicals (Anjali *et al.*, 2019; Mashjoor *et al.*, 2016; Parın *et al*., 2022; Sıcak *et al.*, 2017). Environmental factors such as light, oxygen, and humidity can rapidly impact these secondary metabolites. They are prone to oxidation, which leads to structural deterioration (Martín *et al.*, 2010; Sherry *et al.*, 2013). Encapsulation may provide a viable solution to these challenges. This technique can enhance bioavailability, enable controlled release, and ensure more precise targeting of bioactive compounds (Shoji &Nakashima, 2004).

The potential of macroalgae in treating medical disorders is limited by challenges in targeted substance delivery. To overcome this limitation, seaweeds can be encapsulated into colloidal systems such as liposomes, micelles, nanoparticles, noisomes, and nanospheres, enabling targeted delivery to affected cells, tissues, and organs (Kelman *et al*., 2012; Maghraby *et al*., 2022). Nanoliposomes offer a greater surface area compared to traditional liposomes, resulting in enhanced solubility and stability. This advancement leads to improved bioavailability and more precise delivery to targeted areas. The unique properties of nanoliposomes make them an asset in developing effective therapeutic formulations. (Khorasani *et al.*, 2018; Nizam *et al*., 2024; Savaghebi *et al.*, 2019; Taylor *et al.*, 2005). Our primary research objective was to investigate the impact of nanoliposomal encapsulation on the antioxidant capacities of brown macroalgae, specifically *C. barbata*. To the best of our knowledge, this is the first study to investigate nanoliposomal encapsulation of *C. barbata*. For this purpose, we assessed both the encapsulation process and the resulting antioxidant activity of *C. barbata* collected from the Marmara Sea.

2. MATERIALS and METHODS

2.1. Materials

Chemicals of analytical purity were used in the experiments. Ethanol was obtained from Isolab (Wertheim, Germany), Folin-Ciocalteu from Merck, gallic acid from Isolab, L-α-phosphatidylcholine (soybean lecithin, ≥30% enzymatic), 2,2-diphenyl-1-picrylhydrazyl (DPPH) from Sigma-Aldrich (Missouri, USA), and sodium carbonate from Sigma-Aldrich (Missouri, USA).

2.2. Sampling and Identification of the *Cystoseira barbata*

C. barbata was used as research material in this study. Samples were collected from the Marmara Sea. Marmara Sea samples were collected in June 2023 from the Güzelyalı coast of the Mudanya district (40.36209, 28.91222) and identified based on their morphological characteristics. The collected seaweeds were thoroughly washed with tap water, followed by distilled water to remove the dirt and sand. The cleaned biomass was dried under shade for 4-5 days at room temperature (RT), then ground and stored for further analysis in the fridge (4°C).

2.3. Preparation of Extracts and Their Nanoliposomal Formulations

Twenty grams of dried seaweed were incubated in 20 mL of 80% ethanol in a sonic water bath for 2.5 hours. After being filtered through filter paper, the ethanol solvent was evaporated in an evaporator. The extraction process was repeated three times. Then, the extracts were evaporated in the speed vacuum concentrator. The dry extracts were stored at -20°C for later analysis.

Nanoliposomes were prepared using a homogenization technique. A 1% (w/v) soy lecithin dispersion was prepared with an ultrasonic homogenizer (Optic Ivymen System - CY-500, Spain), operating at 60% amplitude and a constant pH for 10 minutes. The lipid vesicles encapsulating the extracts were subsequently combined using a simple physical mixing method in a 1:1 (v/v) ratio, following the methodology outlined by Yılmaz *et al*. (2023). For each formulation, 0.002 g of the extract was dissolved in 200 µL of Dimethyl Sulfoxide (DMSO) before being combined with the liposomes. To create the formulation, 4 mg of algal extract was dissolved in 400 µL of DMSO. This extract solution (200 µL) was then mixed with 200 µL of the liposome preparation and vortexed thoroughly.

2.4. Characterization of Nanoliposomal Formulations

Physical size characteristics were examined using the Malvern brand, ZS 501 model Particle Sizer dynamic light scattering (DLS) instrument (Malvern, Worcestershire, UK). From this data, the size distribution, mean particle size, and polydispersity index were expressed as an average of three trials. To determine the surface charge of the formulations, the Zeta (ζ) Potential (ZP) measurements were also measured with the Malvern ZetaSizer (Malvern brand ZS 501, Malvern, Worcestershire, UK) (Macit *et al*., 2021). Additionally, a field emission scanning electron microscope (FESEM) (Zeiss GEMINI 500, ZEISS, Germany) was used to analyze the vesicles morphologically.

The encapsulation efficiency (EE%) of the nanoliposome formulations was assessed using the method described by Baranauskaite *et al.* (2018), with minor modifications. To calculate the encapsulation efficiency (w/w%), the dry weight of the extract was first determined. The non-encapsulated extract was separated from the formulation through centrifugation at 3000 rpm for 1 hour at 4°C. The resulting pellet was then dried in an incubator and weighed to ascertain its final weight. The EE was calculated using the following formula:

$$EE\ (\%) = [W1\text{-}W2 / W1] \times 100$$

W1: weight of the ethanol extract added to load nanoliposomes

W2: weight of unincorporated ethanol extract

2.5. Determinations of Total Phenolic Content

The total phenolic content (TPC) of extracts was determined using the Folin-Ciocalteu method. The solutions, prepared from the ethanolic extracts with a concentration of 800 µg/mL, were used to determine the TPC amount. 25 µL of the prepared extract was taken, and 2.5 mL of 0.2 N Folin-Ciocalteu reagent was added. The mixture was left for 10 minutes. 5 mL of 20% sodium carbonate was added to the mixture and made up to 10 mL with autoclaved ultrapure water. The mixture was then allowed to stand at room temperature for 2 hours in the dark. The absorbance was measured at 765 nm using a UV-Vis spectrophotometer (VWR/UV-1600PC, Wayne, PA, USA) (Alagan *et al*., 2017). The concentration of total phenolic content in the

ethanol extract was expressed as mg gallic acid equivalents (GAE) per gram dry weight of the extract.

2.6. DPPH (1,1-Diphenyl-2-picryl-hydrazyl) Scavenging Capacity

The study by Leelavathi and Prasad (2014) was used to determine the ability of free extract in nanoliposomes to scavenge the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) with some modifications. A volume of 1 mL of the extract (800 µg/mL) was prepared in triplicate for each sample, and 1 mL of DPPH (Sigma, Germany) in a 0.15 mM methanolic solution was added and thoroughly mixed. After 60 min of incubation at room temperature in the dark, absorbance was measured at 517 nm in a UV/visible light spectrophotometer (VWR/UV-1600PC).

Radical scavenging activity was calculated by the following formula:

$$\%\text{Inhibition} = (A0 - \text{Asample})/A0 \times 100$$

Where: A0 – Absorbance of the blank, A sample – Absorbance of the sample. Results were expressed as mg of ascorbic acid equivalents (AA Eq) per g of dry extract. The analysis was performed in triplicate.

To evaluate the antioxidant capacity of an encapsulated extract within nanoliposomes using DPPH, we utilized the method developed by Caddeo *et al*. (2018). As a control, ascorbic acid was applied. The analyzed variables were performed in 3 replications. The findings of DPPH analyses were examined using JMP (Version 7.0, SAS Institute Inc., Carry, NC, USA). Mean changes were evaluated for significance with a least significant difference (LSD) test at a 5% significance value.

3. RESULTS

3.1. Analysis of Encapsulation Efficiency, Vesicle Size, Zeta Potential

The encapsulation efficiency of *C. barbata* was calculated as 46.53%. The empty nanoliposomes and extract-loaded nanoliposomes were characterized by their particle size distribution, polydispersity index, and zeta potential (Table 1). Measurement of size distribution and polydispersity of nanoliposomes provides information regarding their physical stability. A PDI value of less than 0.5 indicates that the nanoliposomes are uniformly sized and have a narrow size distribution. The average size of the extract-loaded nanoliposomes was 170.9 nm and the PDI value was 0.224 (Figure 1). The zeta potential of the extract-loaded nanoliposomes in this study was calculated as -51.3 mV (Figure 2) (Table 1). The extract-loaded nanoliposomes were also characterized by STEM technique (see Figure 3).

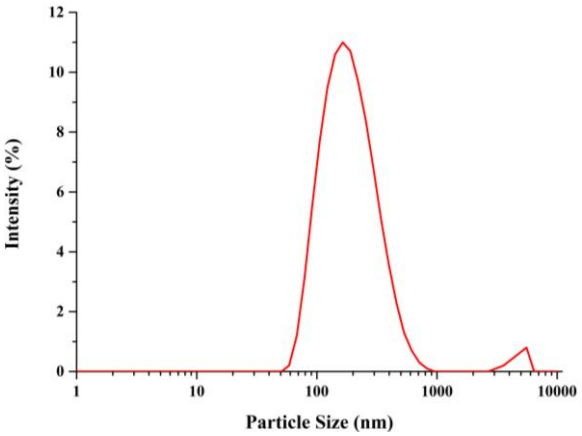

Figure 1. Size distribution of extract-loaded nanoliposomes.

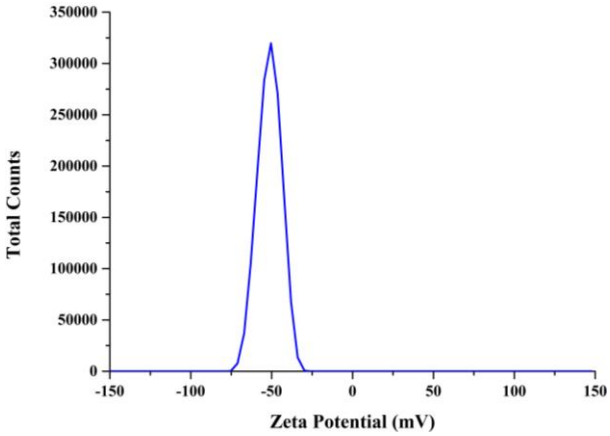

Figure 2. Zeta potential of extract-loaded nanoliposomes.

Table 1. Size, PDI, and Zeta Potential value of the nanoliposomes and extract-loaded nanoliposomes.

Particles	Size (nm)	Polydispersity Index (PDI)	Zeta Potential (mV)
Nanoliposome	142.2	0.288	-53.8
Extract-loaded nanoliposomes	170.9	0.224	-51.3

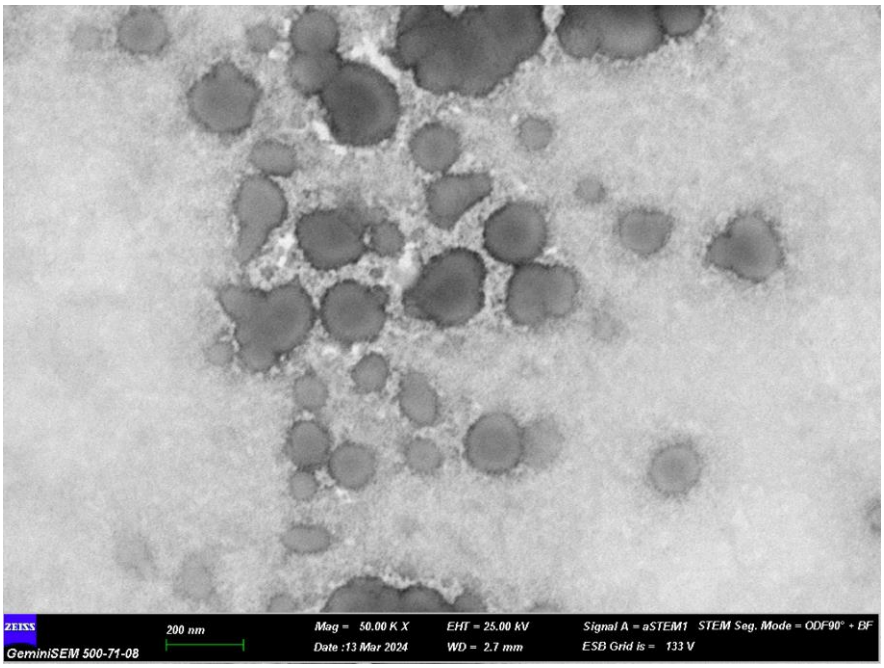

Figure 3. STEM image of extract-loaded nanoliposome, scale bar 200 µm and magnification x 50.000.

3.2. Total Phenolic Content

The total amount of polyphenols in the algal extract was measured using the Folin-Ciocalteu method and is reported as mg GAE/g of the dried seaweed biomass (see Table 2). The standard graph was prepared using gallic acid to calculate the total phenolic content. According to this, the total TPC of *C. barbata* from the Marmara Sea was determined as 2.66 ± 0.19 (mg GAE/g).

3.3. Antioxidant Capacity

The DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical scavenging activity method is based on the ability of antioxidants to neutralize the DPPH radical. DPPH is a stable free radical that absorbs light at a wavelength of 517 nm. When an antioxidant donates an electron to the DPPH radical, the absorption at 517 nm decreases. This radical remains stable at room temperature, which allows for the effective interaction between antioxidants and DPPH molecules. As a result of this interaction, electrons are transferred to the DPPH, leading to a reduction in absorption at 517 nm. A lower absorbance at this wavelength signifies higher DPPH free radical scavenging activity (Bozkurt *et al.*, 2020).

In this study, antioxidant capacities were determined for *C. barbata* extracts from the Marmara Sea and nanoliposomal extract. Table 2 displays the inhibition values for both extracts and liposomal extracts. A UV-vis spectrophotometer measured solutions at 517 nm wavelength with three replicates. Based on this data, it can be inferred that the nanoliposomal extract is more efficient at eliminating free radicals than the free extract (see Table 2).

Table 2. DPPH capacity values of free algal extract and encapsulated algal extract.

Cystoseira barbata	DPPH capacity %
Free algal extract	19.41 ± 1.57 [b]
Nanoliposomal extract	31.33 ± 1.96 [a]

[*a-b] Means superscripts with different letters differ significantly (p<0.05).

4. DISCUSSION and CONCLUSION

Phenolic compounds and flavonoids are present in seaweeds and are responsible for their antioxidant activity. Antioxidants are essential for the protection against reactive oxygen species such as hydroxyl, superoxide, and peroxyl radicals, which can cause illnesses such as cancer and aging when present in excessive amounts (Anjali *et al*., 2019). These molecules present in macroalgae have the potential to reduce oxidative stress by inhibiting the formation and chain reactions of free radicals (Mashjoor *et al*., 2016). While the beneficial properties of these extracts are well-established, there is a notable absence of research focused on enhancing their antioxidant activity through liposomal formulations (Kelman *et al.*, 2012; Maghraby *et al.*, 2022). Addressing this research gap is essential for increasing their use and activity range. In the current study, a nanoliposomal formulation was developed to encapsulate *C. barbata* extracts.

Encapsulation efficiency (EE) was determined using the formula outlined in Equation 1. The encapsulation efficiency of the *C. barbata* extract was found to be 46.53%. EE values for nanoliposomes can range from 0% to 95%, influenced by factors such as the wall component, the ratio of phospholipid to core or polymer, the method of encapsulation, and physical and chemical properties like viscosity and particle size (Tavakoli *et al*., 2018). Similar results regarding encapsulation efficiency for brown algae extracts within nanoliposomes have been observed, aligning with current findings. The highest recorded encapsulation efficiency was achieved using lecithin-derived nanoliposomes containing *Padina distromatica*, reaching an efficiency of 52.8% (Shirani *et al*., 2024). Moreover, a study on *Sargassum boveanum* algae extract incorporated into nanoliposomes reported an encapsulation efficiency of 45.5% (Savaghebi *et al*., 2019).

The encapsulation formulation developed in this study ensures that half of the extract utilized is actively tested, allowing for the generation of valuable results in subsequent analyses and enabling cost savings through the use of a minimal amount of extract in future investigations.

The average size of the extract-loaded nanoliposomes was 170.9 nm, with a polydispersity index (PDI) of 0.224 (Figure 1). A literature review indicates that the size of the extract-loaded liposomes in this study is smaller than the ideal size, while the PDI value aligns with findings from other studies. For instance, in experiments using *Phaeodactylum tricornutum* extract, an average particle size of 246.1 nm and a PDI of 0.18 were reported following encapsulation (Koo *et al*., 2023). In the liposome formulation of the red alga *Jania rubens*, the particle size measured 161 nm with a PDI of 0.211 (Maghraby *et al.*, 2022). Conversely, in studies involving *Gracilaria gracilis* algae, attempts to reduce the particle size with chitosan resulted in a smallest particle size of 336.9 nm and a PDI of 0.26 (Haghdoost *et al*., 2023). The zeta potential of the extract-loaded liposomes in this study was calculated to be -51.3 mV (Figure 2), which falls outside the range of -30 to +30 mV, indicating that the particles maintain stability without coalescing. Compared to the literature, zeta potential values for extract-loaded liposomes typically range from -37.3 to -50.7 mV (Savagheb *et al*., 2020). Thus, the nanoliposome formulation of *C. barbata* extract in this study demonstrates greater stability in a liquid medium than other studies reported in the literature. The STEM images presented in Figure 3 demonstrated the encapsulation of extracts with nanoliposomal vesicles, obtained through the homogenization method.

In most cases, alcoholic solvents have been the most preferred for the extraction of bioactive compounds from marine algae (Zou *et al*., 2008; Custódio *et al.*, 2016). The existing literature highlights that the choice of solvent significantly influences the selectivity of phenolic compounds (Farvin and Jacobsen, 2013). Ethanol was used because of its lower toxicity, high extraction yield, and polarity, as well as because of the convenience of processing and safety for human consumption (Sultana *et al.*, 2007). A study conducted by Wang *et al*. (2009) demonstrates that water is less effective than polar organic solvents in the extraction of

polyphenolic compounds. This evidence substantiates our decision to employ ethanol as the solvent in our processes.

Literature comparisons show that our *C. barbata* extract's TPC is comparable to that of other brown algae species such as *Saccharina latissima* (2.44 mg GAE/g) and *Laminaria ochroleuca* (2.81 ± 0.25 mg GAE/g) (Silva *et al*., 2021), affirming its potential as a valuable antioxidant source. The amphipathic structure of nanoliposomes facilitates the effective encapsulation of antioxidant agents within these lipid carriers. This encapsulation plays a vital role in preventing the initiation of oxidation processes at the water/oil interface in various products (Gorjian *et al.*, 2022). The DPPH radical scavenging activity of the free extract and extract-loaded nanoliposomes was measured to determine whether the liposome formulation negatively affected the antioxidant effect.

In this study, we assessed the antioxidant activity of both free extracts and those encapsulated in nanoliposomes. Table 2 presents the antioxidant capacities for DPPH measurements of the free extract compared to the nanoliposomal extract. The results indicate that the nanoliposomal extract exhibited superior antioxidant activity relative to the free extract. These findings reveal a differing trend in antioxidant activity when compared to existing literature. This observation contradicts the work of Zou, *et al*. (2014a), who found that nanoliposomes containing tea polyphenols exhibited lower antioxidant activity than free phenolic compounds. Additionally, Zou, *et al*. (2014b) reported a decrease in the antioxidant activity of epigallocatechin gallate following its encapsulation in nanoliposomes. These discrepancies highlight a divergence from the results of the current study.

The role of bioactive compounds in lipid vesicles is influenced by their location and interactions with the lipid layers. The positioning of phenolic compounds within nanoliposomes varies based on their polarity and chemical structure. Some phenolic compounds may adhere to the membrane surface, while others can penetrate the lipid layers or the core of the nanoliposomes (Savaghebi *et al.,* 2020). Consequently, the remarkable antioxidant activities of the *C. barbata* nanoliposome formulation, as demonstrated by the DPPH assay, can be attributed to their rapid release and the interaction of phenolic compounds with the lipid layers. In addition, the variations in findings are likely attributable to the differing components of the nanoliposomes used in each study, as each employed unique nanoliposomal formulations. This study exclusively utilized lecithin for the preparation of nanoliposomes.

This could be due to the self-organization of lecithin around the extracts, forming nanoliposomes that facilitate the release of the extracts from the nano vesicular systems. Ultimately, this process significantly enhances the antioxidant activity of the extracts. This study highlights that lipid nanocarriers are innovative and effective systems for the nanoencapsulation of bioactive compounds derived from seaweeds. The findings indicate high encapsulation efficiency, a narrow particle size distribution, and a favorable zeta potential of the nanoliposomes, demonstrating the formulation's exceptional stability.

After an in-depth analysis of the data, it was determined that the nanoliposomal encapsulation of ethanol extracts from *C. barbata* enhances antioxidant capacity. Thus, this encapsulation technique emerges as a promising strategy to boost the efficacy of *C. barbata* extract as an antioxidant. Future studies should incorporate additional antioxidant assays (e.g., ABTS and FRAP) to complement the DPPH results and provide a more comprehensive evaluation of antioxidant potential. Furthermore, characterization of the encapsulated extract revealed a particle size of 170 nm, as confirmed by dynamic light scattering (DLS) results. This finding underscores the potential for using lecithin-based structures in the effective nano-encapsulation of algal extracts.

This research demonstrates that the use of nanoliposomes containing seaweed-derived bioactive compounds is a highly effective natural preservative that should be adopted in the development of functional foods. This strategy will significantly enhance the nutritional quality available to consumers. Furthermore, these findings establish a foundation for future investigations into the

ecological functions of these compounds, as well as their potential applications within the fields of biotechnology and pharmaceuticals.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Melih Cafer: Project administration, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **İnci Tüney:** Project administration, Writing- review & editing, Visualization, Supervision, Investigation, Formal analysis, Conceptualization. **Münevver Müge Çağal:** Writing- review & editing, Writing- original draft, Visualization, Supervision, Investigation, Project administration, Funding acquisition, Formal analysis, Conceptualization.

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