

Extraction of Bioactive Compounds from *Ulva lactuca*

Sofia Pappou ^{1,2}, Maria Myrto Dardavila ^{1,*} , Maria G. Savvidou ¹ , Vasiliki Louli ¹ , Kostis Magoulas ¹
and Epaminondas Voutsas ¹

¹ Laboratory of Thermodynamics and Transport Phenomena, Zografou Campus, School of Chemical Engineering, National Technical University of Athens, 9 Iroon Polytechniou Str., 15780 Athens, Greece; mard18005@marine.aegean.gr (S.P.); msavvid@central.ntua.gr (M.G.S.); svlouli@chemeng.ntua.gr (V.L.); mag@chemeng.ntua.gr (K.M.); evoutsas@chemeng.ntua.gr (E.V.)

² Department of Marine Sciences, University of Aegean, University Hill, Lesvos Island, 81100 Mytilene, Greece

* Correspondence: mirtodar@mail.ntua.gr

Abstract: Macroalgae *Ulva lactuca*, has been employed as a natural source for the production of extracts with potent bioactivity. The biochemical characterization showed that the macroalgae biomass contains a remarkable amount of the polysaccharide Ulvan (49.9 wt%) which is a valuable chemical compound well known for its benefits in human health. Four nontoxic solvents, water, ethyl acetate, ethanol, and an ethanol/water mixture (70:30 v/v) were examined for their recovery efficiency of total carotenoid and phenolic contents. Experimental results showed that the aqueous mixture of ethanol was the most efficient solvent in the recovery of bioactive compounds with extraction yield of 10–15% dw. The effect of extraction parameters, namely time, temperature, and the ratio of biomass to solvent, on the carotenoid and phenolic compounds' content, antioxidant activity, and extraction yield, was investigated, using the ethanol/water mixture as a solvent. The extract obtained under 60 °C, 3 h of extraction time and 1:10 biomass to solvent mass ratio showed the highest antioxidant activity. This extract maintained its antioxidant capacity almost stable for five days of storage under cool and dark conditions. Finally, specific phenolic and carotenoid compounds in the *U. lactuca* extracts were identified using the High-Performance Liquid Chromatography (HPLC) technique.

Keywords: *U. lactuca*; extraction; non-toxic solvents; carotenoids; phenolics; antioxidant activity



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1. Introduction

Macroalgae (seaweeds), are macroscopic algae with high growth rate that can be cultivated with minimal use of freshwater at infrastructures installed on non-arable land. However, their cultivation cost is considerably lower compared to microalgae [1]. Macroalgae have great potential for several industrial applications [2] and therefore the interest in algal biotechnology has been increasing in the past few decades, particularly, on the exploitation of various macroalgae species for the production of a vast variety of products in the food, cosmetic and pharmaceutical industry. Consequently, during the last few decades, world macroalgae production increased at a rate of 8.7% per year [2].

Ulva lactuca is a widespread macroalgae growing along the Mediterranean coast which belongs to the phylum *Chlorophyta*, commonly known as “Sea lettuce” [3]. *U. lactuca*, contains commercially valuable chemical compounds that can be exploited in cosmetic, pharmaceutical, chemical, food, and energy applications. It has been reported that up to 60% carbohydrates, 10–47% proteins, 1–3% lipids, and 7–38% mineral ash are contained in *U. lactuca* [4]. Bioactive compounds of major industrial importance found in *U. lactuca* are phenolics, pigments (chlorophylls and carotenoids), and polysaccharides [5].

Phenolic compounds of macroalgae origin have been proven to confer anti-allergic, skin anti-aging, and whitening properties to cosmetic products [6]. Moreover, the consumption of an adequate level of polyphenols, such as myricetin, morin, and quercetin found in

U. lactuca [5], could result in the prevention of diseases such as obesity, metabolic syndrome, Alzheimer's, or cancer [7].

Carotenoids present in *U. lactuca*, such as astaxanthin, β -carotene, fucoxanthin, and lutein, have been proven to have anti-inflammatory, antiaging, antioxidant, and other activities. They are frequently added in vitamin supplements and cosmetics, while they are used as natural food dyes and fish and poultry feed additives [6,8]. Carotenoids possess anti-inflammatory properties due to their ability to neutralize free radicals, creating a chemical protection against the proliferation of cancer cells [7].

Ulva spp. contain a significant amount of polysaccharides, varying from 15 to 65% of the total dry mass. These polysaccharides include ulvans, sulphated polysaccharides with rhamnose, uronic acids, and xylose as major components, as well as glucans including starch [4]. Ulvan polysaccharide and its oligosaccharides have anti-viral, antioxidant, anti-tumour, anti-coagulant, antihyperlipidemic, hepato protective, immuno-stimulating, anti-depressant, and anti-anxiolytic activities [4]. Consequently, Ulvan has industrial applications in the chemical, biomedical, and agricultural sectors [4].

Regarding the outcome of the bioactive compounds' extraction process, different variables are important, such as solvent type, solvent to solid ratio, temperature, and extraction time. These operational conditions require individual or combined study in order to maximize yields, the extraction rate, and the bioactive potency of the extracts [9,10].

The extraction yield of the targeted bioactive compounds is solvent dependent and different solvents can be used according to the polarity and location of those compounds. An aqueous solvent is suitable for the separation of polysaccharides, while phenolics and carotenoids are usually extracted with organic solvents [10,11]. In most cases, the extracts obtained with organic solvents appear more bioactive, while it has been confirmed that extracts derived from polar solvents have higher antibacterial activity [11].

When the bioactive extracts are targeted for cosmetics, pharmaceutical, and food industries, the range of the most convenient solvents is limited. The employed solvents, apart from being efficient in separating different compounds from their natural sources, must also be non-toxic [12]. Ethanol, water, and their mixtures are ideal solvents for the production of extracts with high antioxidant capacity, while they are generally recognized as safe (GRAS) and have a lower environmental impact in comparison to other solvents [13].

Regarding *U. lactuca*, experimental studies have been focused on the extraction and characterization of polysaccharides (ulvan) and proteins [3,14–21], while some others have been focused on the study of *U. lactuca* extracts' content in antioxidant chemical compounds (phenolics, carotenoids, tocopherols, etc.) and of their antioxidant capacity [8,22,23].

In this study, a complete biomass characterization of *U. lactuca* (total lipids, proteins, polysaccharides, and mineral ash contents) was performed. Moreover, the effectiveness of four nontoxic solvents (water, ethanol, ethyl acetate, and ethanol/water mixture 70:30 v/v) on the total carotenoids and total phenolics content, was studied. The ethanol/water mixture was determined as the most efficient among the four for extracting the targeted bioactive compounds from *U. lactuca*, thus it was employed for further investigation. The aim of the present study is the acquisition of *U. lactuca* extracts rich in carotenoid and phenolic compounds that exhibit potent antioxidant activity, appropriate for use in a variety of cosmetics, pharmaceutical, and food products. To that end, using the ethanolic solvent, the effects of extraction parameters, namely time, temperature, and the ratio of biomass to solvent on the carotenoid and phenolic compounds content, antioxidant activity, and extraction yield, were investigated in order to identify the optimum extraction conditions. To date, there has not been documented a study for *U. lactuca* targeting the investigation of the individual and combined effects of important extraction parameters regarding the carotenoid and phenolic content, as well as the antioxidant activity.

2. Materials and Methods

2.1. Chemicals and Reagents

The chemical reagents used in the present study were all of analytical grade and are presented in the Appendix A.

2.2. Macroalgal Strain

U. lactuca seaweed was purchased in July 2020, from Lusalgae Ltd., Figueira da Foz, Portugal. According to the supplier, the cultivated biomass was washed several times with both filtered and tap water, and after the excess water removal, it was dried on a ventilated drying oven at 60 °C for 48 h. It was then milled on a commercial mill and vacuum packed. The biomass was stored in our laboratory facilities under dry and dark conditions, avoiding direct contact with sunlight.

2.3. Methods

2.3.1. Biochemical Characterization

Lipid Content

Lipid extraction was conducted based on the Folch method [24]. Particularly, 0.5 g of seaweed were extracted with 15 mL of chloroform/methanol (1:2) mixture, in an ultrasonic bath (Elma TRANSONIC DIGITAL) for 4 min and left overnight at room temperature in the dark. Then, the sample was centrifuged (HERMLE Z 206 A) at 3000 rpm for 10 min. The supernatant was transferred into a separating funnel where the chloroform phase was collected in a pre-weighed flask. The chloroform phase was then concentrated in a rotary evaporator (Hei-VAP Advantage HL G3, Heidolph, Germany) at 45 °C and 150 mbar to recover the lipids. Total lipids were gravimetrically determined as wt% of the biomass.

Protein Content

For the determination of the protein content, the extraction procedure described by Kazir et al., 2019 [25] was followed. *U. lactuca* biomass was stirred overnight at 4 °C with distilled water 5% *w/v*. The supernatant was separated using a centrifuge at 5000 rpm for 15 min. Mercaptoethanol (0.5% *v/v*) was added to the pellet, pH was adjusted to 12.0 with the addition of 1 M NaOH solution and left for 2 h under stirring at room temperature. The supernatant was separated again via centrifugation. The supernatants from both centrifugation cycles were mixed and the pH was adjusted to 7.0 using 1 M HCl solution. Solid ammonium sulfate was added to the supernatant up to 85% saturation. The solution was kept for 1 h at room temperature and then centrifuged in order to separate the precipitate. The sediment was then washed several times with deionized water, oven-dried (at 100 °C overnight) and measured in order to calculate the protein content gravimetrically as wt% of the biomass.

Polysaccharide Content

The polysaccharide content was determined based on the method of Mao et al., 2006 [26]. According to this method, 100 mL of distilled water was added to five grams of previously defatted *U. lactuca* biomass and the extraction was carried out at 80–90 °C under continuous stirring for 2 h. The supernatant was collected after centrifugation at 3000 rpm for 10 min in a pre-weighed flask. The supernatant was then concentrated using a rotary evaporator at 50 °C and 74 mbar and precipitated with 4 volumes of ethanol. Centrifugation and concentration were repeated. The extracted polysaccharide was washed with distilled water, freeze-dried, and weighed.

U. lactuca's polysaccharide content was gravimetrically determined as wt% of the biomass.

Determination of Mineral Ash

Ash content was determined gravimetrically. Specifically, 2.5 g of *U. lactuca* biomass were weighed in a crucible and combusted for 3 h at 550 °C using a muffle furnace (Ther-

molyne 47900). Water and other volatile materials are vaporized, and organic substances are burned in the presence of oxygen to CO₂, H₂O, and N₂. After the combustion completion, the crucible was placed in a desiccator and weighed soon after cooling.

The mineral ash content of *U. lactuca* macroalgae was gravimetrically determined as wt% of the biomass.

2.3.2. Solvent Extractions

Pure ethanol, water, and ethyl acetate, as well as a mixture of ethanol/water 70:30 (*v/v*) were investigated for their efficiency in the extraction of carotenoids and phenolics from *U. lactuca* biomass.

For the solvent screening, preliminary extractions were conducted. Specifically, 100 mL of each solvent was added to 10 g biomass and the extractions were carried out under continuous magnetic stirring in double-walled glass vessels for 12 h. A thermostat was employed to control the extraction temperature which was set at 25 °C.

Using the same experimental setup, the effect of some of the most influential extraction parameters, namely time, temperature, and the ratio of biomass to solvent, was investigated using the ethanol/water mixture as a solvent. The extraction conditions are presented in detail in Table 1. After the completion of each extraction, the extract was collected via centrifugation at 4000 rpm for 15 min. Subsequently, total phenolics, total carotenoids, antioxidant activity of the extracts, and the extraction yield were measured.

Table 1. Extraction conditions using ethanol/water (70:30 *v/v*) as solvent.

Extract Abbreviation	Extraction Duration (Hours)	Temperature (°C)	Biomass to Solvent Ratio (<i>w/v</i>)
E1	6	25	1:10
E2	10	25	1:10
E3	12	25	1:10
E4	12	25	1:20
E5	12	25	1:40
E6	16	25	1:10
E7	16	25	1:20
E8	16	25	1:40
E9	24	25	1:10
E10	12	40	1:10
E11	12	40	1:40
E12	3	60	1:10
E13	6	60	1:10
E14	12	60	1:10

Determination of Total Phenolics Content (TPC)

Total phenolics content in *U. lactuca* extracts was estimated spectrophotometrically using Folin-Ciocalteu reagent as described by Singleton et al., 1965 [27]. Specifically, 7.9 mL of distilled water and 0.1 mL of extract were transferred in glass vials and the mixture was homogenized by vortexing. Afterwards, the addition of 0.5 mL of Folin-Ciocalteu reagent and homogenization were realized, followed by the addition of 1.5 mL (20% *w/v*) Na₂CO₃ solution. The final mixture was incubated for 30 min in a water bath at 40 °C and its absorbance was subsequently measured at 765 nm using a SHIMADZU UV-1900, UV-VIS spectrophotometer and compared to a gallic acid calibration curve. The measurement was conducted in triplicate.

Determination of Total Carotenoids Content (TCC)

The estimation of total carotenoids content was carried out spectrophotometrically according to Association of Official Analytical Collaboration (AOAC) methods [28]. After each extraction, the absorbance of 3 mL of extract was measured at 450 nm and total

carotenoids content was calculated according to Equation (1) acquired by the standard β -carotene calibration curve:

$$\text{TCC} = (6.9691\text{Abs } 450 \text{ nm} - 0.1286) \quad (1)$$

Determination of Extraction Yield

After each extraction, the collected extract was employed in order to determine the extraction yield achieved. Particularly, the extracts were weighed, placed in a round bottom flask and the ethanol/water 70:30 *v/v* solvent was evaporated using a rotary evaporator at 45 °C and 100 mbar. Consequently, the remaining residue was dried at 100 °C for 1 h and the obtained dry extract was weighed. The extraction yield was gravimetrically determined as wt% of the biomass.

Determination of Antioxidant Activity (IC₅₀)

Antioxidant activity was assessed using the 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) assay [29], which is a simple, well-established method, and among the most frequently used in the literature. Briefly, 100 μL of *U. lactuca* extracts were added to a 3 mL methanolic solution of DPPH (0.03% *w/v*). Following on, the absorbance of the mixture was recorded at 515 nm after incubation for 20 min at room temperature. The IC₅₀ values reported in the present study denote the concentration of sample that is required to scavenge 50% of DPPH free radicals (Sharma and Bhat, 2009). All the measurements were performed in triplicate.

HPLC Analysis

Carotenoid and phenolic compounds were identified using an HPLC system composed of a Jasco LG 1580-04 gradient unit equipped with a Jasco PU 1580 Intelligent HPLC pump and a Rheodyne 20 μL loop injection valve, connected in series with an SPD M20A diode array detector. The detection of the carotenoid compounds was realized using a YMC C30 reversed-phase column (5 μm , 250 mm \times 4.6 mm i.d.), while a ThermoFisher C18 reversed-phase column (5 μm , 250 mm \times 4.6 mm i.d.) was employed for the detection of the phenolic compounds. The columns' temperature was maintained at 30 °C and the flow rate of the eluents was adjusted at 1 mL min⁻¹. Prior to injection, the extracts were filtered using a syringe filter PTFE/L 25 mm, 0.45 μm (Membrane Solutions).

For the carotenoids profile study, the mobile phase consisted of methanol (solvent A) and MTBE (solvent B) mixture. An A:B linear gradient was applied starting from 95:5, changing to 70:30 in 30 min, then to 50:50 in 20 min [30]. The detection of carotenoids was performed at a wavelength of 480 nm. The identification of the carotenoids peaks was realized using external standards of all-trans neoxanthin, all-trans violaxanthin, all-trans astaxanthin, all-trans lutein, and 9-*cis* astaxanthin. Comparison with HPLC literature data was performed when no external standards were available.

In the case of the phenolics profile study, water containing 0.1% acetic acid (solvent A) and acetonitrile (solvent B) were used as the mobile phase [31]. For the first 5 min, an isocratic elution of 90% of A and 10% of B was applied, followed by an A:B linear gradient changing to 84:16 in 18 min, then to 82:18 in 26 min, then to 72:28 in 31 min, then to 60:40 in 32 min. Subsequently, an isocratic elution of 60% A and 40% B until 40 min and a linear gradient of 90:10 in 43 min were applied [31]. External standards of gallic acid, caffeic acid, and catechin were used for the identification of the chromatograph's phenolics peaks, and their detection was recorded at 320 nm.

3. Results and Discussion

3.1. Biochemical Composition of *U. lactuca*

The biochemical composition of macroalgae *U. lactuca* tested in this work is shown in Table 2. Polysaccharides were the most abundant components of the biomass (49.9 wt%) and they were obtained in the form of a dark yellow colored gel. Also, *U. lactuca* exhibited

a high ash content (27.7 wt%). Total lipids and total extracted proteins were calculated as 3.5% and 8.4% of the biomass, respectively.

Table 2. Biochemical composition of macroalgae *U. lactuca*.

Chemical Component	Content g g ⁻¹ Biomass
Total lipids	0.035
Polysaccharides	0.499
Mineral ash	0.277
Total proteins	0.084
Other *	0.105

* Fibers and other carbohydrates.

The estimated contents of polysaccharides, lipids, and mineral ash are found close to the ones reported in the literature for *U. lactuca*. Particularly, values up to 60%, 3%, and 38% have been reported for these compounds respectively [4]. The protein content of the *U. lactuca* employed in the present work (8.4%), is considered among the lowest when compared to the corresponding values found in the literature (10–47%) which refer to several macroalgae [4]. Nevertheless, it should be mentioned that the biochemical composition of marine macroalgae varies significantly between species, growth conditions, and growth phase. Therefore, even when the biomass of the same species is examined, differences may occur because of the different environmental conditions of growth such as salinity, water temperature, depth, and pollution [32].

3.2. Extraction of Phenolics and Carotenoids—Selection of Solvent

Studies have confirmed that alcoholic solutions and/or hydrophilic solvent mixtures provide extracts with better antioxidant activity [32,33]. This can be attributed to the selective extraction of polar chemical substances, such as phenolic compounds, that exhibit significant antioxidant capacity [11]. In this study, four different solvents (water, ethanol, ethyl acetate, and a mixture of ethanol/water 70:30 *v/v*) were tested in order to evaluate their ability to recover the bioactive compounds of interest.

According to Figure 1, solvent selectivity was encountered both for carotenoids and phenolics. Water was the best solvent among the four for phenolic compounds and the ethanolic mixture for carotenoids extraction. Pure ethanol resulted in a lower solvation of phenolics, while carotenoids exhibited an extraction ability in between that of water and the ethanol/water mixture. Ethyl acetate exhibited the worst results in all bioactive compounds' extraction.

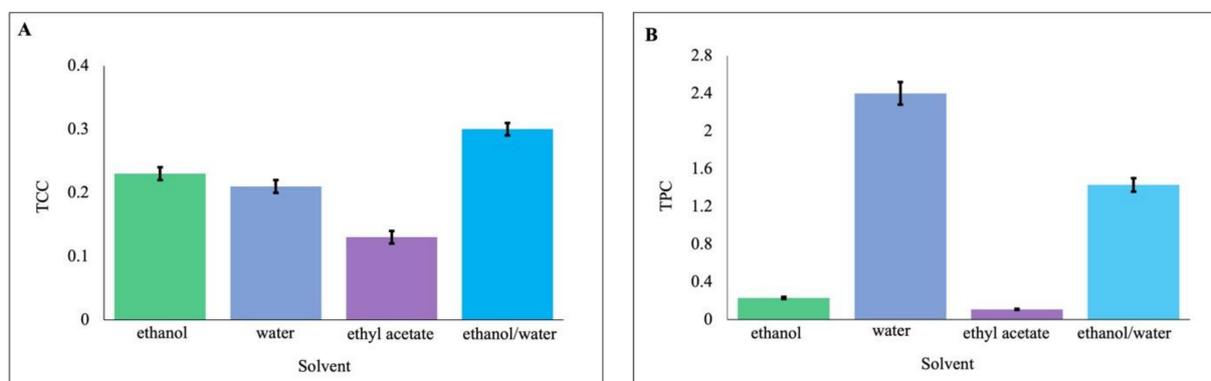


Figure 1. Total carotenoids (A) and phenolics (B) content of the *U. lactuca* extracts (mg g⁻¹) using different solvents (ethanol, water, ethyl acetate, and ethanol/water 70:30 *v/v*). Presented data are mean values of three replications \pm standard deviation ($n = 3 \pm$ s.d.).

According to the above results, it can be concluded that among the four tested solvents the most suitable ones, for the purpose of the present work, are water and the ethanol/water mixture. Nevertheless, the required energy for the recovery of the solvent used, and the acquisition of the dry extract is of great importance, and it should be accounted for the design of a feasible extraction process. Among the two most effective solvents, the ethanol/water 70:30 *v/v* mixture exhibits a lower enthalpy of vaporization (ΔH_v) [34]. Therefore, considering the most efficient recovery of all targeted bioactive compounds, environmental impact, GRAS characteristics of solvents, and extraction process feasibility, the ethanol: water 70:30 *v/v* mixture, would be the recommended solvent for the study of the extraction parameters.

3.3. Effect of Extraction Parameters on Carotenoid and Phenolic Content, Antioxidant Capacity and Extraction Yield

The effect of the operational conditions, i.e., time, temperature, and biomass to solvent ratio, using ethanol/water as solvent was examined. The obtained extracts are evaluated according to the total carotenoids content, total phenolic content, antioxidant activity, and extraction yield as shown below (Table 3).

Table 3. Experimental results from the extraction of *U. lactuca* using ethanol/water (70:30 *v/v*) as solvent.

Extract Abbreviation	TCC ^a (mg g biomass ⁻¹)	TPC ^b (mg GAE g biomass ⁻¹)	Extraction Yield ^c (%)	IC50 ^d (g biomass mL solvent ⁻¹)
E1	0.047	0.979	11.8	0.720
E2	0.044	0.954	12.9	0.390
E3	0.074	1.079	11.5	0.164
E4	0.058	0.979	12.8	0.350
E5	0.062	1.205	15.6	0.480
E6	0.061	1.004	11.7	0.500
E7	0.072	1.104	15.4	0.900
E8	0.082	1.330	15.0	1.200
E9	0.063	0.979	10.9	0.840
E10	0.122	1.230	10.8	0.308
E11	0.137	1.807	15.1	0.227
E12	0.086	1.506	9.8	0.128
E13	0.105	1.757	10.4	0.263
E14	0.118	1.857	12.0	0.291

^a $\sigma_{TCC} = \pm 0.004$, ^b $\sigma_{TPC} = \pm 0.062$, ^c $\sigma_{\text{extraction yield}} = 0.629$, ^d $\sigma_{IC50} = 0.036$.

3.3.1. Effect of Extraction Time

The effect of extraction time (h) on total carotenoids and phenolics content, antioxidant activity, and extraction yield is presented in Figure 2 and Table 3. For the evaluation of the extraction time effect on each dependent variable (TCC, TPC, IC50, and Yield), two different series of extractions were performed, at 25 °C (6, 10, 12, 16 and 24 h) and 60 °C (3, 6, and 12 h), while solvent to biomass ratio was set at 1:10 *w/v*. The increase in temperature from 25 °C to 60 °C that led to the better recovery of bioactive compounds, resulted in a decreased extraction time of a maximum of 12 h at 60 °C, in order to avoid the use of extra energy. As shown in Figure 2, the most and least affected dependent variable by time is the antioxidant activity (IC50) of the extracts and the extraction yield, respectively. At 25 °C, the best antioxidant activity was measured at the extract obtained after 12 h (E3), while at 60 °C, 3 h were sufficient in order to obtain the best extract as far as antioxidant activity is concerned (E15). The extraction yield exhibits small variations with time at 25 °C, while at 60 °C showed an incremental trend. Total carotenoid and phenolic content at 60 °C exhibited an increase with time, while at 25 °C the variations of the recovered bioactive compounds over time are negligible.

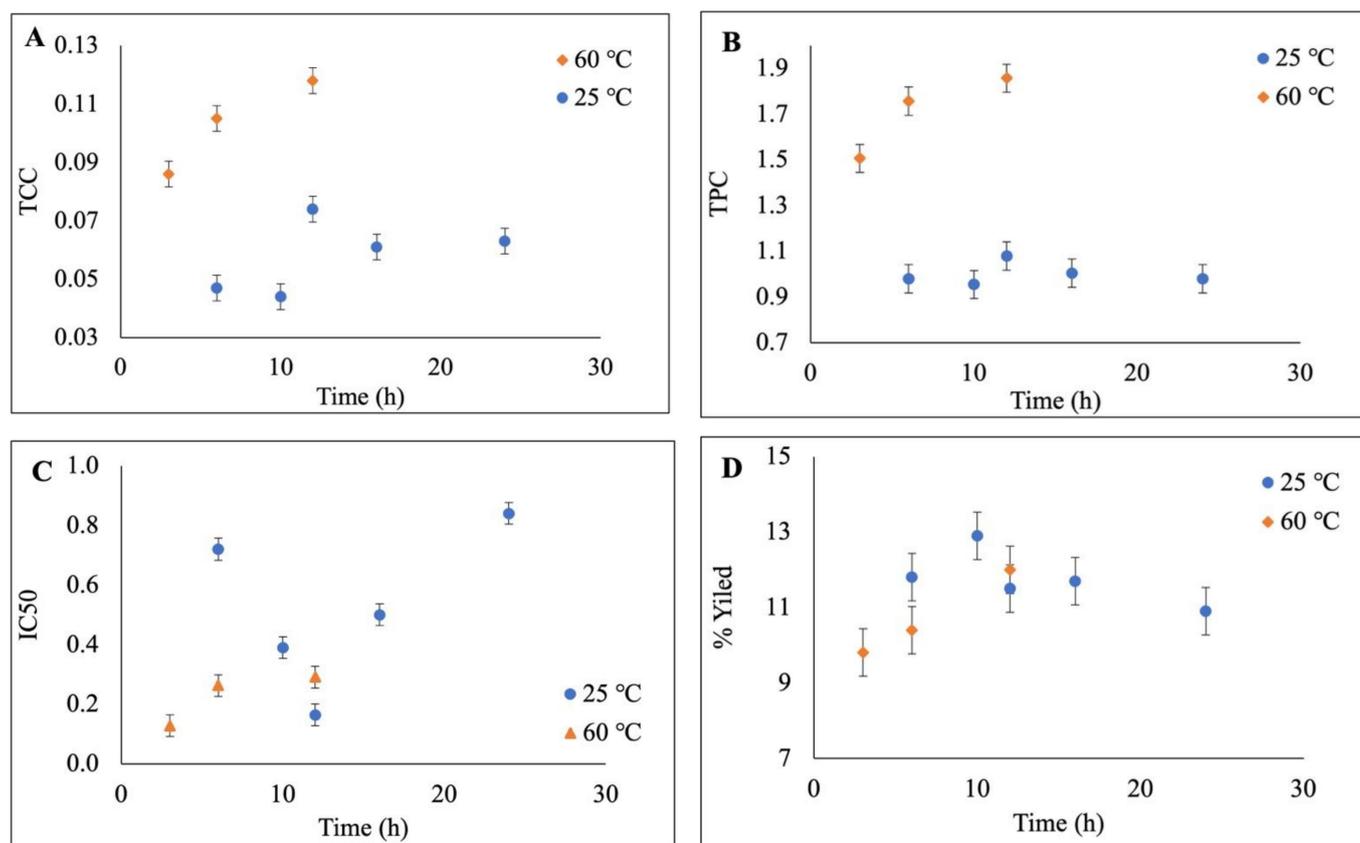


Figure 2. Effect of extraction duration (h) on carotenoids content (A), total phenolic content (B), antioxidant activity (C) and extraction yield (D) of *U. lactuca* extracted with ethanol/water 70:30 *v/v* at a biomass to solvent ratio 1:10 *w/v*, under 25 °C and 60 °C. Experimental data are the mean values of three replications.

According to Teramukai et al., 2020 [35], carotenoids' content increased at all temperatures that were examined for extraction during the first 12 h, a finding that agrees with the results of this study. Because of the presence of many conjugated double bonds, carotenoids are easily degraded at higher temperatures and long incubation time, although a higher temperature and longer extraction time can increase the extraction rate of carotenoids [35]. Therefore, an optimal temperature and extraction time should exist in order to achieve the most effective extract as far as antioxidant activity is concerned. Sachindra et al., 2005 [36] reported that the optimized temperature and time for astaxanthin extraction from shrimp waste were 70 °C and 2.5 h, respectively, while the astaxanthin content decreased after 2.5 h incubation at temperatures higher than 70 °C, supporting the findings of this work.

3.3.2. Effect of Biomass to Solvent Ratio

Figure 3 provides the results obtained for total carotenoids and phenolics, antioxidant activity, and extraction yield at different biomass to solvent ratios ($r = 1:10, 1:20$ and $1:40$). Two different series of extractions were performed, for 12 and 16 h, while the temperature was set at 25 °C. Antioxidant activity was significantly affected by the biomass to solvent ratio, while the 1:10 *w/v* ratio appeared to be the optimum one among the three ratios tested for both sets of extraction time. The carotenoid recovery did not seem to be affected significantly by the alteration of the biomass to solvent ratio for the 12 h extraction, while it slightly increased for the 16 h one. Finally, the extracts' phenolic content and the extraction yield increase by increasing the biomass to solvent ratio. Topuz et al., 2016 [37] reported that the most affected dependent variable from the biomass to solvent ratio parameter was the total phenolic compounds content.

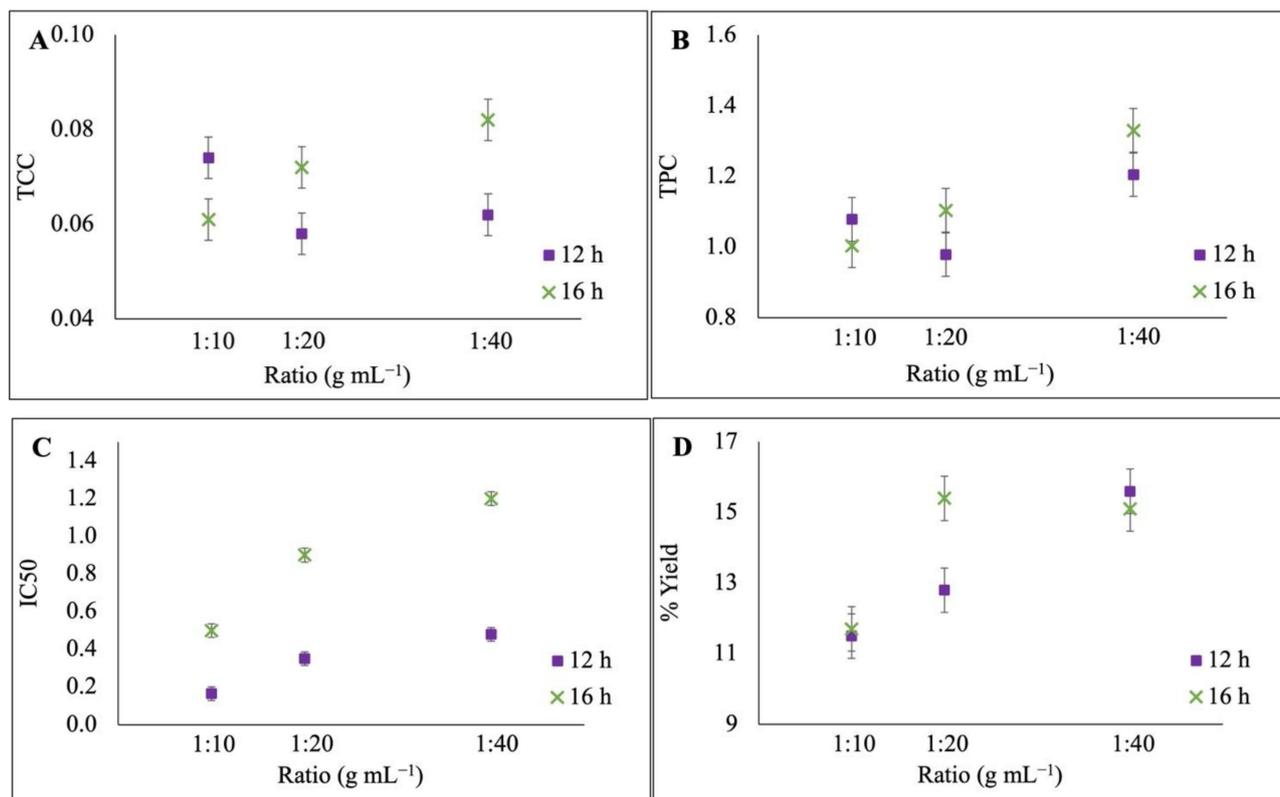


Figure 3. Effect of biomass to solvent ratio on carotenoids content (A), total phenolic content (B), antioxidant activity (C) and total extraction yield (D) of *U. lactuca* extracted with ethanol/water 70:30 v/v at 25 °C for 12 and 16 h extraction time. Experimental data are the mean values of three replications.

3.3.3. Effect of Temperature

The results obtained regarding total carotenoids and phenolics, antioxidant activity, and extraction yield at different temperatures (25, 40 and 60 °C), for 12 h extraction and biomass to solvent ratio 1:10 w/v, are presented in Figure 4. Total carotenoids were increased from 25 to 40 °C and then remained practically constant (Figure 4A), while total phenolic content showed a significant increase with temperature (Figure 4B). Results showed that temperature increase led to a negative effect on the antioxidant activity for the solvent ratio 1:10 w/v and for 12 h extraction time (Figure 4C). However, this negative effect of temperature on the antioxidant activity is not observed for other ratios and extraction times, e.g., E1–E13 and E5–E11. Furthermore, the increase in temperature has a negligible effect on the overall extraction yield (Figure 4D). Generally, plant and algal cells are surrounded by a rigid cell wall that acts as a mechanical barrier to extract carotenoids, while the strong interaction between carotenoids and other macromolecules prevents their mass transfer during the extraction [37]. Therefore, in order to break down the cell wall, the extraction occasionally involves treatments, such as heating [37]. According to Strati et al., 2010 [38], the improved extractability of carotenoids as temperature increases, is possibly related to the destruction of the cellular structure. On the other hand, as the extraction temperature increases, several factors can affect the extracted carotenoids, such as degradation and isomerization from all-trans to *cis* isomers [38]. Thus, the extraction conditions of carotenoids should be optimized, since some are more unstable than others, depending on their chemical structure. Mäki-Arvela et al., 2014 [39] suggested carotenoid extraction temperatures lower than 70 °C, in order to minimize their degradation. Moreover, under higher extraction temperatures (40–50 °C), a significant increase in the total phenolic content was observed [37]. In like manner, it can be supported by the results presented in the current study (Figures 3 and 4), that under higher temperature and a higher biomass to

solvent ratio, the solubility of the seaweed's phenolic compounds is enhanced. Interestingly, some studies have shown that an increase in temperature favors the extraction process by enhancing both the solubility and the diffusion coefficient of the solutes; nevertheless, the phenolic compounds can be denatured beyond a certain temperature value ($>50\text{ }^{\circ}\text{C}$) [40]. Consequently, the elevated carotenoid and phenolic recovery observed with the increase in the extraction temperature in the present study, does not necessarily result in a respective enhancement of antioxidant activity.

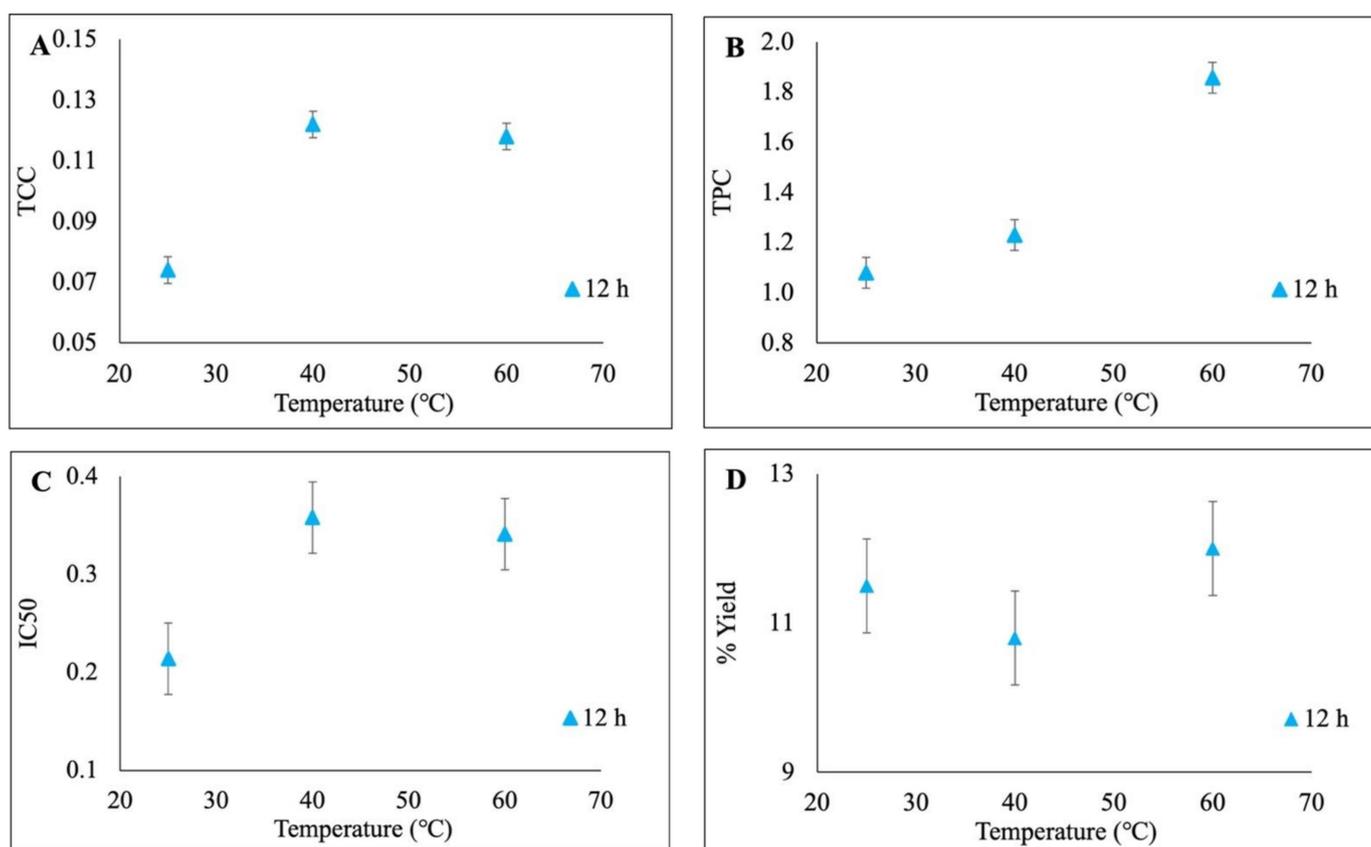


Figure 4. Effect of different extraction temperatures on carotenoids content (A), total phenolic content (B), antioxidant activity (C) and total extraction yield (D) of *U. lactuca* extracted with ethanol/water 70:30 *v/v* at a biomass to solvent ratio 1:10 *w/v* and for 12 h extraction time. Experimental data are the mean values of three replications.

3.3.4. Overall Evaluation of the Extraction Parameters Effect

The total carotenoids' content did not fluctuate significantly for the different biomass to solvent ratios applied, while extraction time and temperature showed a slightly higher impact. Particularly, the increase in temperature to $60\text{ }^{\circ}\text{C}$ favored the carotenoids recovery, and the highest carotenoid content was obtained from the 12 h extraction under $60\text{ }^{\circ}\text{C}$. The total phenolic content increased under increased temperature and biomass to solvent ratio. The effect of extraction time was less significant at $25\text{ }^{\circ}\text{C}$ and more intense at $60\text{ }^{\circ}\text{C}$, whereas the 12 h extraction was the optimum one for both temperatures. A straight comparison regarding the antioxidant capacity of seaweed extracts between different studies is difficult due to biological variation of the raw material and extraction methods; however, previous studies have shown that phenolic compounds are the main contributors to the antioxidant activity of various seaweeds [37]. In this study, the antioxidant activity was the most influenced factor by all the studied parameters, as depicted in Figures 2–4. Therefore, with regards to the total bioactive potency of the extracts, the best biomass to solvent ratio was the 1:10 *w/v*, while the extraction times of 12 h and 3 h were the two most favorable under

25 °C and 60 °C, respectively. The stability of carotenoids is one of the crucial parameters in the consideration of developing commercial products from algae extract. Some carotenoids are thermally and chemically labile; therefore, the process parameters should be optimized in order to produce high-quality products, stable against exposure to light and heat for a prolonged time [39].

The extraction efficiency expressed as the extraction yield showed a slight increase with the biomass to solvent ratio increase, while no particular effect was found from the parameters of extraction time and extraction temperature. Such findings were also mentioned by the work of Ummat et al., 2020 [41], where no statistical differences were observed within each extraction parameter on the extraction yield (%), while the recovery of the individual bioactive compounds was altered according to the parameter extractions such as duration and temperature. In the same study, the yields obtained from conventional extraction of several species of seaweed were in the range of 10.5–19.3%, while in the current study *U. lactuca* extraction yield ranged between 10% and 15%, depending on the extraction parameters.

With the main objective of this work being the optimum antioxidant activity, the extracts E3 and E12 are proposed as the optimum ones. The antioxidant activity of these two extracts was equal; nevertheless, the extract obtained under 60 °C, at 3 h (E12), exhibited higher recovery of total carotenoid and phenolic compounds, and thus it is evaluated as the best one.

3.3.5. Study of the Extracts' Antioxidant Activity over Time

The evaluation of the antioxidant activity of the best *U. lactuca* extract (E12) over time was studied at two levels. First, the extract's scavenging activity was examined versus time when in contact with free radicals. Second, the period during which the extract preserves its antioxidant activity when kept in storage was determined.

The antioxidant activity of the E12 extract was measured exactly after 20 min, 30 min, 60 min, and 90 min of DPPH incubation to determine how rapidly the antioxidant compounds act. As shown in Figure 5A, the scavenging activity of the extract exhibited a linear decrease throughout the 90 min of study. The highest rate of DPPH decay occurred within the first 20 min of incubation, denoting a rapid reaction of the mixture of antioxidant species present in the extract with the free radicals. According to Savatovic et al., 2012 [42], the expression of the results in terms of a kinetic approach not only considers the activity of the antioxidants but also provides information on how quickly they act, which is probably the result of the different kinetic behavior that the various antioxidants species present in the extract exhibit.

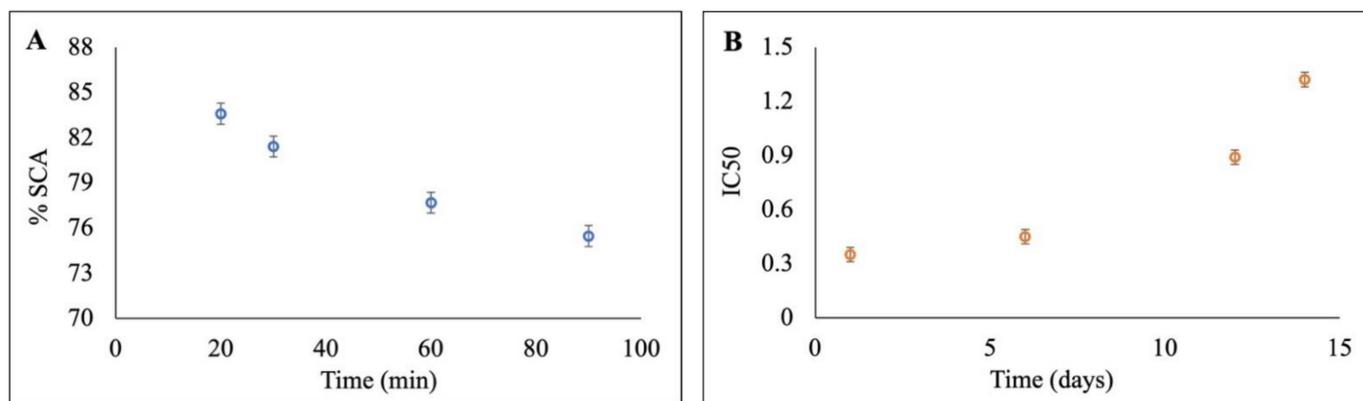


Figure 5. Percent scavenging activity (% SCA) of *U. lactuca* extract E12 versus time (min) (A) and antioxidant activity (IC50) of *U. lactuca* extract E12 when stored at 4 °C in the dark for 15 days (B). Experimental data are mean values of three replications.

Additionally, in order to test the effect of the storage time on the antioxidant activity, the optimum extract's IC₅₀ was measured in frequent intervals for 15 days. When stored at 4 °C in the dark to minimize the degradation of carotenoids [39], only a slight decrease in the extract's antioxidant capacity was evident for the first 5 days of storage, followed by a drastic decrease thereon, as depicted in Figure 5B.

3.4. Carotenoids Profile

Figure 6 presents the HPLC chromatogram of *U. lactuca* extract E12, in which seven carotenoids were identified. According to the data presented by Fernandes et al., 2020 [30] whose HPLC protocol for carotenoids separation and detection was followed by the present work, peaks 2 and 7 correspond to 9-*cis* neoxanthin and 9-*cis* lutein, respectively. All the other peaks were identified by using external standards. According to these standards, peak 1 corresponds to *all-trans*-neoxanthin, peak 3 to *all-trans*-violaxanthin, peak 4 to *all-trans*-astaxanthin, peak 5 to *all-trans*-lutein, and peak 6 to 9-*cis*-astaxanthin. In general, the carotenoids reported for *Ulva* spp. in the published literature are lutein, violaxanthin, fucoxanthin, zeaxanthin, neoxanthin, astaxanthin, α - and β -carotene and their isomers, which are in accordance with the carotenoids commonly found in Chlorophyta [21,43].

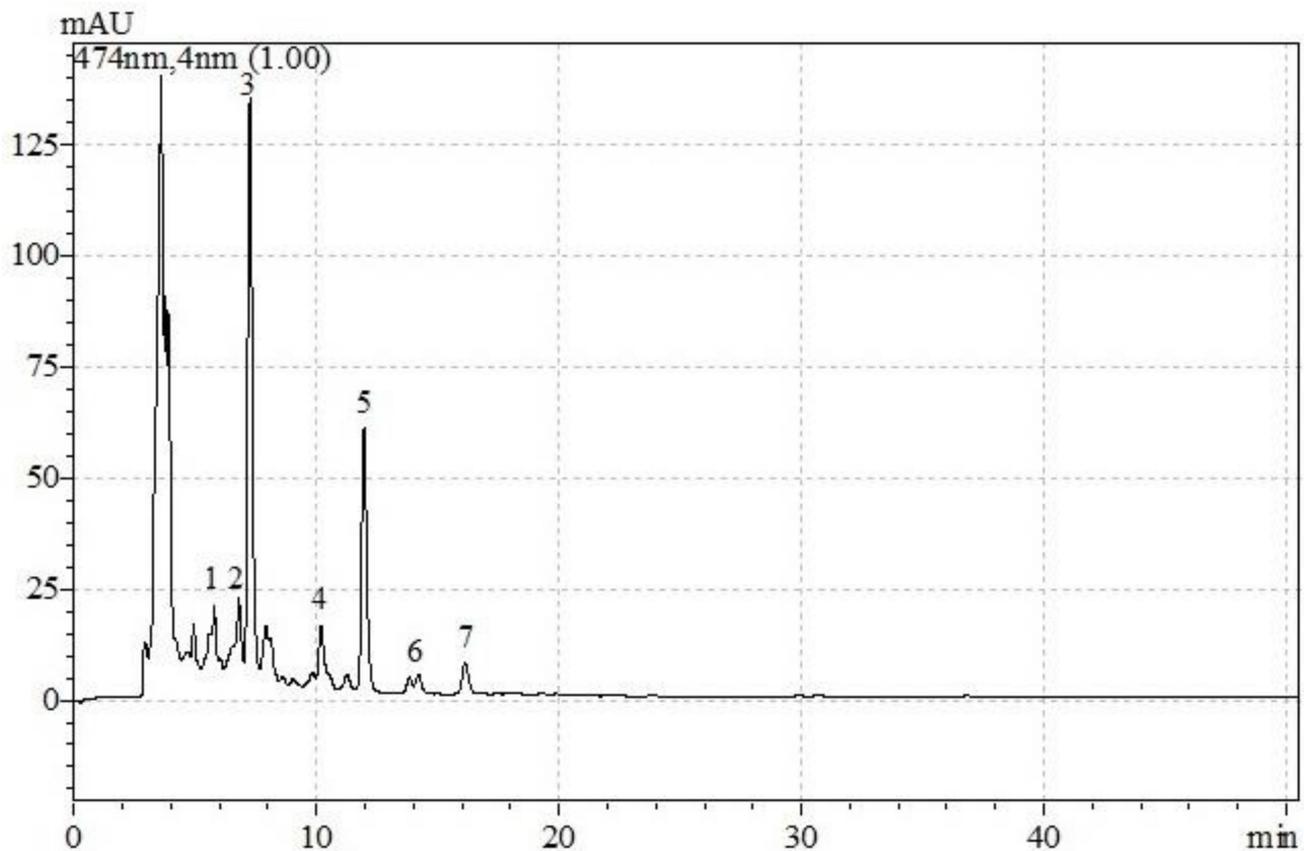


Figure 6. HPLC chromatogram of the E12 *U. lactuca* extract. Peak 1: *all-trans*-neoxanthin; Peak 2: 9-*cis*-neoxanthin; Peak 3: *all-trans*-violaxanthin; Peak 4: *all-trans*-astaxanthin; Peak 5: *all-trans*-lutein; Peak 6: 9-*cis* astaxanthin; Peak 7: 9-*cis*-lutein.

In conclusion, the extract that exhibited the most potent antioxidant activity was found to contain important carotenoid compounds [43]. Particularly, *all-trans*-violaxanthin possesses anti-inflammatory effects and *all-trans*-lutein can help in the prevention of cataracts, coronary syndromes, strokes, and retinitis, as well as in the treatment of macular disintegration [44].

3.5. Phenolics Profile

Several different phenolic compounds are present in most macroalgae species. As shown in Figure 7, the HPLC analysis of the *U. lactuca* E12 extract resulted in the identification of four phenolic compounds: gallic acid (Peak 1), caffeic acid (Peak 2), catechin (Peak 3), and rutin (Peak 4).

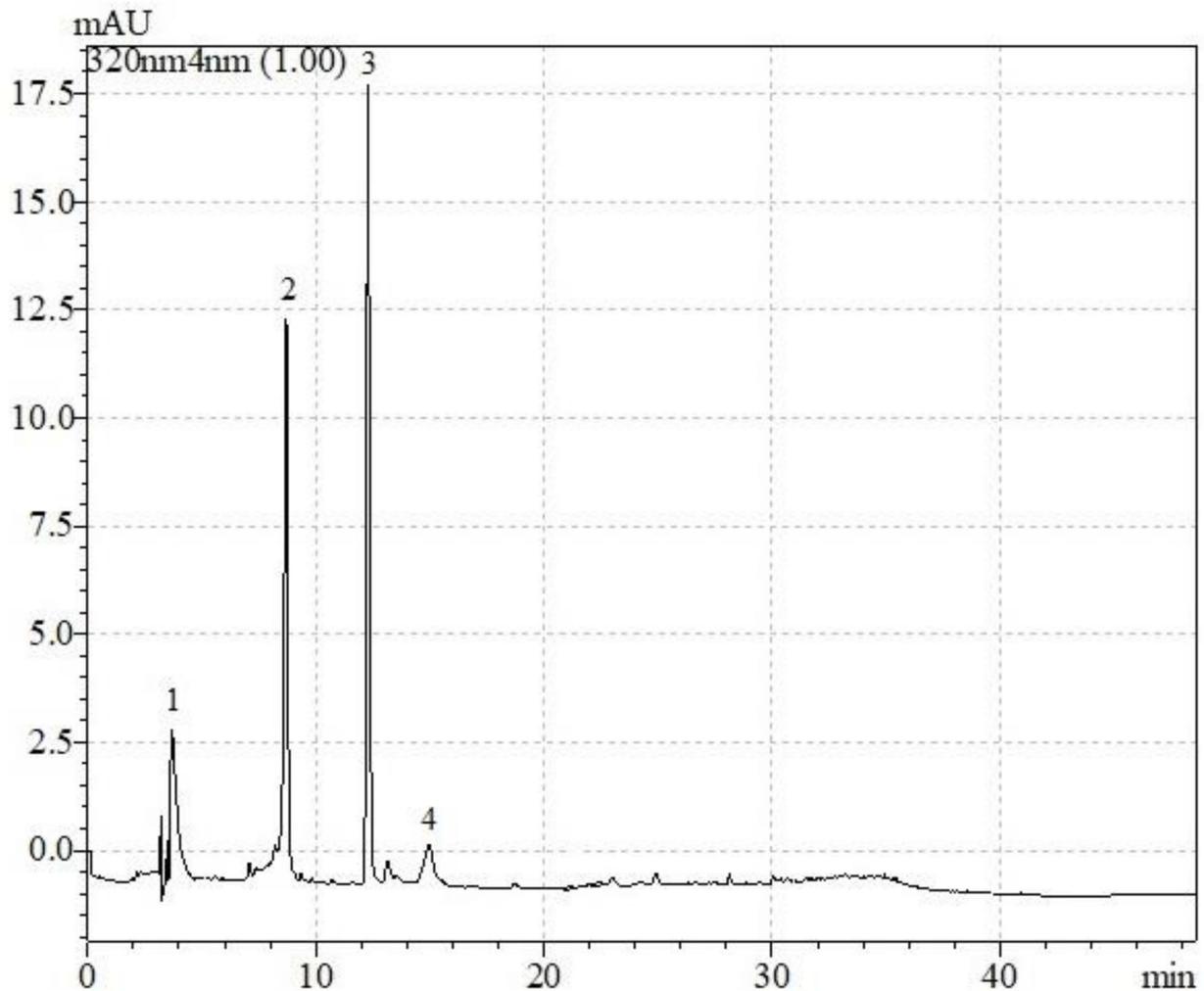


Figure 7. HPLC chromatogram of the E12 *U. lactuca* extract. Peaks: 1 gallic acid, 2 caffeic acid, 3 catechin, 4 rutin.

Gallic acid is one of the most abundant phenolic acids and is frequently used as a standard in the quantification of extracts' total phenolic content, as in the present work. The presence of gallic acid has been previously reported in methanol, ethanol, and aqueous *U. lactuca* extracts [45,46]. Caffeic acid is among the hydroxycinnamic acids that have been found in *U. lactuca* extract obtained using water as a solvent [46]. Catechin is a well-known flavonoid abundant in brown algae [47], and also present in methanolic extracts of macroalgae *U. fasciata* and *U. lactuca* [46,48] according to the literature. Rutin is another flavonoid whose presence in *U. lactuca* extracts obtained from different methanolic solvent systems has been reported [47].

These compounds, being powerful antioxidants, exhibit further diverse bioactivities such as antiviral, antimicrobial, antitumoral, anti-inflammatory, etc., which are important for human well-being [7].

4. Conclusions

The *U. lactuca* biochemical characterization showed that the predominant component of the employed macroalgae was polysaccharide (ulvan) which accounts for 49.9 wt% of the total dry algal biomass. The corresponding values of mineral ash, total proteins, and total lipids were 27.4 wt%, 8.4 wt% and 3.5 wt%, respectively. The screening of four non-toxic solvents namely, water, ethyl acetate, ethanol, and ethanol/water (70:30 *v/v*), showed that the aqueous ethanolic mixture was the most efficient in the recovery of carotenoids and phenolics from *U. lactuca* via the classic solid–liquid extraction method. The study of the effect of the extraction parameters, i.e., temperature, time, and biomass to solvent ratio, on the carotenoid and phenolic compounds recovery, antioxidant activity, and extraction yield, resulted in the determination of the most efficient parameter values. Particularly, the *U. lactuca* extract that was obtained under 60 °C, 3 h extraction time and using a biomass to solvent ratio equal to 1:10, exhibited the highest antioxidant activity and recovery of the targeted bioactive compounds. Carotenoid and phenolic compounds were identified via HPLC analysis. According to the findings of the present work, the antioxidant activity was the most influenced factor by all the studied parameters. The best extract maintained its antioxidant capacity almost stable for five days under storage in cool and dark conditions. Considering the ease and effectiveness of the extraction process, the nontoxic nature of the ethanol/water solvent, the good antioxidant activity of the *U. lactuca* extracts, and their content of high added value compounds, it can be suggested that the obtained extracts could be exploited as possible additives/constituents in cosmetic, pharmaceutical and food products. In that context, the present study serves as a useful steppingstone for further investigations on the optimization and the upscaling of the whole process.

5. Highlights

- *U. lactuca* is a rich source of bioactive compounds and dietary supplements
- High polysaccharides content (49.9 wt%) was found
- Ethanol/water 70:30 *v/v* was the most efficient solvent. The optimum extraction parameters were 60 °C, biomass to solvent ratio 1:10 and 3 h extraction time
- The best extract maintained its high antioxidant capacity for 5 days

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Appendix A

Table A1. List of chemical reagents.

Chemical Reagents	Supplier	Purity/Concentration
2,2-Diphenyl-1-picrylhydrazyl	Alfa Aesar	95%
Folin-Ciocalteu reagent	Carlo Erba reagents	Special grade
Methanol	Fisher Scientific	≥99.8%
Chloroform	Fisher Scientific	Analytical reagent grade
Ethanol	Fisher Scientific	≥99.8%
Ethyl acetate	Merck	HPLC grade
MTBE	Fisher Scientific	≥99.5%
2-Mercaptoethanol	Sigma-Aldrich	≥98%
Water	Fisher Scientific	HPLC grade
β-carotene	Alfa Aesar	99%
<i>all-trans</i> Astaxanthin	Acros Organics	≥98%
<i>all-trans</i> Lutein	Extrasynthese	≥95%
<i>all-trans</i> Neoxanthin	Supelco	Analytical standard
<i>all-trans</i> Violaxanthin	Sigma-Aldrich	≥90.0%
<i>9-cis</i> Astaxanthin	Alga Technologies	Natural CO ₂ extract of Haematococcus Pluvialis microalgae
Gallic acid	Acros Organics	98%
Caffeic acid	Acros Organics	99%+
Catechin	Sigma-Aldrich	≥98%

References

- Chemodanov, A.; Robin, A.; Jinjikhavily, G.; Yitzhak, D.; Liberzon, A.; Israel, A.; Golberg, A. Feasibility study of *Ulva* sp. (Chlorophyta) intensive cultivation in a coastal area of the Eastern Mediterranean Sea. *Biofuel. Bioprod. Biorefin.* **2019**, *13*, 864–877. [[CrossRef](#)]
- White, W.L.; Wilson, P. World seaweed utilization: A summary. *J. Aquac. Res. Dev.* **2015**, *6*, 7–25. [[CrossRef](#)]
- Postma, P.R.; Cerezo-Chinarro, O.; Akkerman, R.J.; Olivieri, G.; Wijffels, R.H.; Brandenburg, W.A.; Eppink, M.H.M. Biorefinery of the macroalgae *Ulva lactuca*: Extraction of proteins and carbohydrates by mild disintegration. *J. Appl. Phycol.* **2018**, *30*, 1281–1293. [[CrossRef](#)] [[PubMed](#)]
- Dominguez, H.; Loret, E. *Ulva lactuca*, a source of troubles and potential riches. *Mar. Drugs* **2019**, *17*, 357. [[CrossRef](#)]
- Caf, F.; Yilmaz, Ö.; Durucan, F.; Özdemir, N.Ş. Biochemical components of three marine macroalgae (*Padina pavonica*, *Ulva lactuca* and *Taonia atomaria*) from the Levantine Sea coast of Antalya, Turkey. *J. Biodivers. Environ. Sci.* **2015**, *6*, 401–411.
- Morais, T.; Cotas, J.; Pacheco, D.; Pereira, L. Seaweeds compounds: An Eco sustainable source of cosmetic ingredients? *Cosmetics* **2021**, *8*, 8. [[CrossRef](#)]
- Jimenez-Lopez, C.; Pereira, A.G.; Lourenço-Lopes, C.; Garcia-Oliveira, P.; Cassani, L.; Fraga-Corral, M.; Prieto, M.A.; Simal-Gandara, J. Main bioactive phenolic compounds in marine algae and their mechanisms of action supporting potential health benefits. *Food Chem.* **2021**, *341*, 128262. [[CrossRef](#)]
- Spolaore, P.; Joannis-Cassan, C.; Duran, E.; Isambert, A. Commercial applications of microalgae. *J. Biosci. Bioeng.* **2006**, *101*, 87–96. [[CrossRef](#)]
- Domínguez, R.; Zhang, L.; Rocchetti, G.; Lucini, L.; Pateiro, M.; Munekata, P.; Lorenzo, J. Elderberry as potential source of antioxidants. Characterization, optimization of extraction parameters and bioactive properties. *Food Chem.* **2020**, *330*, 127266. [[CrossRef](#)]
- Strati, I.F.; Oreopoulou, V. Process optimization for recovery of carotenoids from tomato waste. *Food Chem.* **2011**, *129*, 747–752. [[CrossRef](#)]
- Suchinina, T.; Shestakova, T.; Petrichenko, V.; Novikova, V. Solvent polarity effect on the composition of biologically active substances, UV spectral characteristics, and antibacterial activity of *Euphrasia brevipila* herb extracts. *Pharm. Chem. J.* **2011**, *44*, 683–686. [[CrossRef](#)]
- Torres-Valenzuela, L.S.; Ballesteros-Gómez, A.; Rubio, S. Green solvents for the extraction of high added-value compounds from agri-food waste. *Food Eng. Rev.* **2020**, *12*, 83–100. [[CrossRef](#)]
- Monteiro, M.; Santos, R.A.; Iglesias, P.; Couto, A.; Serra, C.R.; Gouvinhas, I.; Barros, A.; Oliva-Teles, A.P.; Enes, P.; Díaz-Rosales, P. Effect of extraction method and solvent system on the phenolic content and antioxidant activity of selected macro- and microalgae extracts. *J. Appl. Psychol.* **2020**, *32*, 349–362. [[CrossRef](#)]
- He, J.; Xu, Y.; Chen, H.; Sun, P. Extraction, structural characterization, and potential antioxidant activity of the polysaccharides from four seaweeds. *Int. J. Mol. Sci.* **2016**, *17*, 1988. [[CrossRef](#)] [[PubMed](#)]

15. Costa, C.; Alves Paula, A.; Pinto, R.; Sousa, R.A.; Borges da Silva, E.A.; Reis, R.L.; Rodrigues, A.E. Characterization of ulvan extracts to assess the effect of different steps in the extraction procedure. *Carbohydr. Polym.* **2012**, *88*, 537–546. [[CrossRef](#)]
16. Ortiz, J.; Romero, N.; Robert, P.; Araya, J.; Lopez-Hernandez, J.; Bozzo, C.; Navarrete, E.; Osorio, A.; Rios, A. Dietary fiber, amino acid, fatty acid and tocopherol contents of the edible seaweeds *Ulva lactuca* and *Durvillaea antarctica*. *Food Chem.* **2006**, *99*, 98–104. [[CrossRef](#)]
17. Yaich, H.; Garna, H.; Besbes, S.; Paquot, M.; Blecker, C.; Attia, H. Effect of extraction conditions on the yield and purity of ulvan extracted from *Ulva lactuca*. *Food Hydrocoll.* **2013**, *3*, 375–382. [[CrossRef](#)]
18. Yaich, H.; Garna, H.; Besbes, S.; Barthélemy, J.-P.; Paquot, M.; Blecker, C.; Attia, H. Impact of extraction procedures on the chemical, rheological and textural properties of ulvan from *Ulva lactuca* of Tunisia coast. *Food Hydrocoll.* **2014**, *40*, 53–63. [[CrossRef](#)]
19. Yaich, H.; Amir, A.B.; Abbes, F.; Bouaziz, M.; Besbes, S.; Richel, A.; Blecker, C.; Attia, H.; Garna, H. Effect of extraction procedures on structural, thermal and antioxidant properties of ulvan from *Ulva lactuca* collected in Monastir coast. *Int. J. Biol. Macromol.* **2017**, *105*, 1430–1439. [[CrossRef](#)]
20. Tian, H.; Yin, X.; Zeng, Q.; Zhu, L.; Chen, J. Isolation, structure, and surfactant properties of polysaccharides from *Ulva lactuca* L. from South China Sea. *Int. J. Biol. Macromol.* **2015**, *79*, 577–582. [[CrossRef](#)] [[PubMed](#)]
21. El-Baky, H.H.A.; El-Baz, F.K.; El-Baroty, G.S. Natural preservative ingredient from marine alga. *Int. J. Food Sci. Tech.* **2009**, *44*, 1688–1695. [[CrossRef](#)]
22. Abirami, R.G.; Kowsalya, S. Nutrient and Nutraceutical Potentials of Seaweed Biomass *Ulva lactuca* and *Kappaphycus alvarezii*. *J. Agric. Sci. Technol.* **2011**, *5*, 109–115.
23. Wang, T.; Jónsdóttir, R.; Ólafsdóttir, G. Total phenolic compounds, radical scavenging and metal chelation of extracts from Icelandic seaweeds. *Food Chem.* **2009**, *116*, 240–248. [[CrossRef](#)]
24. Folch, J.; Lees, M.; Stanley, G. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **1957**, *226*, 497–509. [[CrossRef](#)]
25. Kazir, M.; Abuhassira, Y.; Robin, A.; Nahor, O.; Luo, J.; Israel, A.; Golberg, A.; Livney, Y. Extraction of proteins from two marine macroalgae, *Ulva* sp. and *Gracilaria* sp., for food application, and evaluating digestibility, amino acid composition and antioxidant properties of the protein concentrates. *Food Hydrocoll.* **2019**, *87*, 194–203. [[CrossRef](#)]
26. Mao, W.; Zang, X.; Li, Y.; Zhang, H. Sulfated polysaccharides from marine green algae *Ulva conglobata* and their anticoagulant activity. *J. Appl. Phycol.* **2006**, *18*, 9–14. [[CrossRef](#)]
27. Singleton, V.; Rossi, J. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144–1588.
28. Association of Official Analytical Chemists (AOAC). *Official Methods of Analysis of the Association of Official Analytical Chemists International*, 16th ed.; The Association of Official Analytical Chemists: Washington, DC, USA, 1995.
29. Brand-Williams, W.; Cuvelier, M.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *LWT* **1995**, *28*, 25–30. [[CrossRef](#)]
30. Fernandes, A.; Petry, F.; Mercadante, A.; Jacob-Lopes, E.; Zepka, L. HPLC-PDA-MS/MS as a strategy to characterize and quantify natural pigments from microalgae. *Curr. Res. Nutr. Food Sci.* **2020**, *3*, 100–112. [[CrossRef](#)]
31. Benavente-García, O.; Castillo, J.; Lorente, J.; Ortuño, A.; Del Rio, J. Antioxidant activity of phenolics extracted from *Olea europaea* L. leaves. *Food Chem.* **2000**, *68*, 457–462. [[CrossRef](#)]
32. Bikker, P.; Van Krimpen, M.; Van Wikselaar, P.; Houweling-Tan, B.; Scaccia, N.; Van Hal, J.; Huijgen, W.; Cone, J.; López-Contreras, A. Biorefinery of the green seaweed *Ulva lactuca* to produce animal feed, chemicals and biofuels. *J. Appl. Phycol.* **2016**, *28*, 3511–3525. [[CrossRef](#)] [[PubMed](#)]
33. Altemimi, A.; Lakhssassi, N.; Baharlouei, A.; Watson, D.G.; Lightfoot, D.A. Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants* **2017**, *6*, 42. [[CrossRef](#)]
34. Newsham, D.M.T.; Mendez-Lecanda, E.J. Isobaric enthalpies of vaporization of water, methanol, ethanol, propan-2-ol and their mixtures. *J. Chem. Thermodyn.* **1982**, *14*, 291–301. [[CrossRef](#)]
35. Teramukai, K.; Kakui, S.; Beppu, F.; Hosokawa, M.; Miyashita, K. Effective extraction of carotenoids from brown seaweeds and vegetable leaves with edible oils. *Innov. Food Sci. Emerg. Technol.* **2020**, *60*, 102302. [[CrossRef](#)]
36. Sachindra, N.; Mahendrakar, N. Process optimization for extraction of carotenoids from shrimp waste with vegetable oils. *Bioresour. Technol.* **2005**, *96*, 1195–1200. [[CrossRef](#)]
37. Topuz, O.K.; Gokoglu, N.; Yerlikaya, P.; Ucak, I.; Gumus, B. Optimization of antioxidant activity and phenolic compound extraction conditions from red seaweed (*Laurencia obtuse*). *J. Aquat. Food Prod. Technol.* **2016**, *25*, 414–422. [[CrossRef](#)]
38. Strati, I.; Oreopoulou, V. Effect of extraction parameters on the carotenoid recovery from tomato waste. *Int. J. Food Sci. Technol.* **2010**, *46*, 23–29. [[CrossRef](#)]
39. Mäki-Arvela, P.; Hachemi, I.; Murzin, D. Comparative study of the extraction methods for recovery of carotenoids from algae: Extraction kinetics and effect of different extraction parameters. *J. Chem. Technol. Biotechnol.* **2014**, *89*, 1607–1626. [[CrossRef](#)]
40. Spigno, G.; Tramelli, L.; De Faveri, D. Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. *J. Food Eng.* **2007**, *81*, 200–208. [[CrossRef](#)]
41. Ummat, V.; Tiwari, B.; Jaiswal, A.; Condon, K.; Garcia-Vaquero, M.; O'Doherty, J.; O'Donnell, C.; Rajauria, G. Optimisation of Ultrasound Frequency, Extraction Time and Solvent for the Recovery of Polyphenols, Phlorotannins and Associated Antioxidant Activity from Brown Seaweeds. *Mar. Drugs* **2020**, *18*, 250. [[CrossRef](#)]

42. Savatovic, S.; Cetkovic, G.; Canadanovic-Brunet, J.; Djilas, S. Kinetic behavior of DPPH radical scavenging activity of tomato waste extracts. *J. Serbian Chem. Soc.* **2012**, *77*, 1381–1389. [[CrossRef](#)]
43. Yalçın, S.; Karakaş, Ö.; Okudan, E.; Başkan, K.; Çekiç, S.; Apak, R. HPLC detection and antioxidant capacity determination of brown, red and green algal pigments in seaweed extracts. *J. Chromatogr. Sci.* **2020**, *59*, 325–337. [[CrossRef](#)]
44. Torregrosa-Crespo, J.; Montero, Z.; Fuentes, J.L.; García-Galbis, M.R.; Garbayo, I.; Vilchez, C.; Martínez-Espinosa, R.M. Exploring the Valuable Carotenoids for the Large-Scale Production by Marine Microorganisms. *Mar. Drugs* **2018**, *16*, 203. [[CrossRef](#)] [[PubMed](#)]
45. Aslan, E.; Aksu, A.; Korkmaz, N.E.; Taskin, O.S.; Caglar, N.B. Monitoring the antioxidant activities by extracting the polyphenolic contents of algae collected from the Bosphorus. *Mar. Pollut. Bull.* **2019**, *141*, 313–317. [[CrossRef](#)] [[PubMed](#)]
46. Farvin, K.H.S.; Jacobsen, C. Phenolic compounds and antioxidant activities of selected species of seaweeds from Danish coast. *Food Chem.* **2013**, *138*, 1670–1681. [[CrossRef](#)]
47. Mekinić, I.G.; Skroza, D.; Šimat, V.; Hamed, I.; Čagalj, M.; Perković, Z.P. Phenolic content of brown algae (Pheophyceae) species: Extraction, identification, and quantification. *Biomolecules* **2019**, *9*, 44–69.
48. Ibrahim, R.; Saber, A.; Hammad, H. The possible role of the seaweed *Ulva fasciata* on ameliorating hyperthyroidism-associated heart inflammations in a rat model. *Environ. Sci. Pollut. Res.* **2020**, *28*, 6830–6842. [[CrossRef](#)]