PREGLEDNI RAD / REVIEW

CONTENT OF PIGMENTS AND ANTIOXIDANT ACTIVITY OF ADRIATIC SEA MACROALGAE AS AFFECTED BY ULTRASOUND ASSISTED EXTRACTION

Zrinka Čošić[1], Erika Dobroslavić[2*], Sandra Pedisić[1], Zoran Zorić[3]

[1] Faculty of Food Technology and Biotechnology, Centre for Food Technology and Biotechnology, Petra Kasandrića 3, 23 000 Zadar, Croatia

[2] University of Dubrovnik, Department of Applied Ecology, Ćira Carića 4, 20 000 Dubrovnik, Croatia

[3] University of Zadar, Department of Ecology, Agronomy and Aquaculture, Trg kneza Višeslava 9, 23 000 Zadar, Croatia

Corresponding author: erika.dobroslavic@unidu.hr

Abstract

The aim of the research was to examine the influence of ultrasound-assisted extraction (UAE) conditions (solvent: 96 % ethanol and 80 % acetone; temperature 30 and 50 °C; time 10, 20, 30 min) on the yield of pigments and the antioxidant activity of the brown algae Halopteris scoparia and Sargassum hornschuchii and the red alga Corallina elongata from the Adriatic Sea. The highest total pigment content was achieved using either solvent in Halopteris scoparia (at 50 °C) and Sargassum hornschuchii (at 30 °C), regardless of the solvent used, while 96 % EtOH was more efficient for Corallina elongata (at 50 °C). The highest antioxidant activity deter-mined by DPPH method in all extracts was achieved at 50 °C in all three algal species using 96% ethanol as a solvent for Sargassum hornschuchii and using 80% acetone for Halopteris scoparia (at any extraction time) and Corallina elongata (30 min). At optimal parameters, the highest content of pigments was determined in the extract of the brown alga Halopteris scoparia which also showed the highest radical scavenging ability. Thirteen pigments were identified and quantified by high performance liquid chromatography (HPLC), six of which were identified as chlorophylls and seven as carotenoids. A high correlation was found between antioxidant activity and the presence of total carotenoids and chlorophyll a and b.

Keywords: *Halopteris scoparia, Sargassum hornschuchii, Corallina elongata, carotenoids, chlorophylls, identification, antioxidant activity*

Sažetak

Cilj istraživanja bio je ispitati utjecaj uvjeta ekstrakcije potpomognute ultrazvukom (otapalo: 96 % etanol i 80 % aceton; temperatura 30 i 50 °C; vrijeme 10, 20, 30 minuta) na prinos pigmenata i antioksidacijsku aktivnost smeđih algi Halopteris scoparia i Sargassum hornschuchii te crvene alge Corallina elongata iz Jadranskog mora. Najveći sadržaj ukupnih pigmenata postignut je u vrstama Halopteris scoparia (pri 50 °C) i Sargassum hornschuchii (pri 30 °C), neovisno o upotrijebljenom otapalu, dok je 96% etanol bio učinkovitiji za Corallina elongata (pri 50 ° C).Najveća antioksidacijska aktivnost, određena DPPH metodom, u svim ekstraktima postignuta je pri 50 °C kod svih triju vrsta algi, uz primjenu 96 % etanola kao otapala za Sargassum hornschuchii i 80 % acetona za Halopteris scoparia (neovisno o vremenu ekstrakcije) i Corallina elongata (30 minuta). Pri optimalnim parametrima, najveći sadržaj pigmenata utvrđen je u ekstraktu smeđe alge Halopteris scoparia, koja je također pokazala najveću sposobnost hvatanja slobodnih radikala. Trinaest pigmenata identificirano je i kvantificirano visokoučinkovitom tekućinskom kromatografijom (HPLC), od čega je šest identificirano kao klorofili, a sedam kao karotenoidi. Utvrđena je visoka korelacija između antioksidacijske aktivnosti i prisutnosti ukupnih karotenoida te klorofila a i b.

Ključne riječi: *Halopteris scoparia, Sargassum hornschuchii, Corallina elongata, karotenoidi, klorofili, identifikacija, antioksidacijska aktivnost*

Introduction

Recently, the demand for natural resources rich in bioactive compounds has risen sharply due to their potential health benefits and their use in pharmaceuticals, dietary supplements and cos-metics. This growing interest is driven by the desire for sustainable, environmentally friendly alternatives to synthetic compounds and coincides with consumer preferences for natural and organic products. Macroalgae have emerged as a valuable source of bioactive molecules as they can biosynthesize a variety of natural organic compounds, including volatile organic compounds, polyunsaturated fatty acids, polyphenols, vitamins, minerals, polysaccharides and pigments (Ganesan et al., 2008). Macroalgae are rich in natural pigments, mainly carotenoids, chlorophylls and phycobiliproteins, and are divided into green algae (Chlorophyta, ~1200 species), red algae (Rhodophyta, ~6000 species) and brown algae (Ochrophyta, ~1750 species. Brown macroalgae are characterized by their high fucoxanthin content, red macroalgae are characterized by phycobiliproteins and green macroalgae have the highest chlorophyll content (Silva et al., 2020. Chlorophyll a, the main pigment in macroalgae, captures the energy of sunlight, while chlorophylls b, c and d which was found in red algae serve as accessory pigments (Pangestuti & Kim, 2011)Carotenoids, which include carotenes (α- and β-carotene, lycopene)and xanthophylls (fucoxanthin, violaxanthin, antheraxanthin, zeaxanthin, lutein, neoxanthin, help transfer energy by covering light spectra that are not absorbed by chlorophylls, thus ex-tending the range of wavelengths for photosynthesis (Silva et al., 2020. In addition, carote-noids protect tissues from photooxidative damage and prevent harmful oxygen-induced reac-tions under intense light conditions (Pereira et al., 2021). Phycobiliproteins are water-soluble fluorescent pigments characteristic of red algae, including

phycocyanins (blue), phycoerythrins (red), and allophycocyanins (light blue). Among these, phycoerythrins are especially abundant in red algae (Silva et al., 2020). Algal pigments not only play a specific ecological role, but also offer numerous health benefits for humans and animals. They have antioxidant, anti-carcinogenic, anti-inflammatory, anti-obesity, anti-angiogenic and neuroprotective properties (Christaki et al., 2013; Gammone & D'Orazio, 2015; Holdt & Kraan, 2011; Hosokawa et al., 1999; Patel et al., 2022; Wang et al., 2015). Various natural colorants derived from algal pigments are environmentally friendly alternatives to synthetic food colorants and are widely used in the food industry (Cikoš et al., 2022). Algal pigments are sensitive to high temperatures and light, which can lead to degradation or isomerization (Quitério et al., 2022). Therefore, optimization of the extraction process is critical to maximize yield and purity while reducing solvent use and energy consumption. Conventional extraction methods require large amounts of hazardous solvents and multiple concentration and purification steps (Zia et al., 2022). To solve these problems, non-conventional methods are currently being developed among which ultrasound-assisted extraction (UAE) is widely applied. Ultrasound-assisted extraction (UAE) uses ultrasonic waves to break down cell walls and enhance mass transfer between the sample and solvent, thereby increasing extraction efficiency. Its main advantages are reduced extraction time and solvent consumption, as well as suitability for extracting thermolabile compounds. Compared to other novel extraction techniques, ultrasonic devices are more affordable, easier to use and compatible with a wide range of solvents (Dey & Rathod, 2013). Solvents such as ethanol, distilled water and methanol are used in small quantities and with different solid-to-solvent ratios, and extraction time is shorter, making UAE a fast and cost-effective method compared to conventional techniques (Quitério et al., 2022). Two brown macroalgae species *Halopteris scoparia* and *Sargassum hornschuchii*, as well the red macroalgae *Corallina elongata* from the Adriatic Sea were previously shown to contain various pigments in different concentrations (El Din & El-Sherif, 2012; Esteban et al., 2009; Hadjkacem et al., 2024), proving that they possess potential for application in various purposes including pharmaceuticals, dietary supplements and cosmetics. However, the extraction of pigments from these algae has not been sufficiently explored, especially regarding complete pigment profiles determined by HPLC, making a hurdle to their efficient utilization. To the best of authors' knowledge, UAE has not been applied or optimized for the extraction of pigments on neither of the three Adriatic macroalgae. Therefore, the aim of this research was to examine the effects of UAE parameters (extraction solvent, temperature and extraction time) on the total and individual content of pigments in the *Halopteris scoparia*, *Sargassum hornschuchii* and *Corallina elongata* extracts and to evaluate their antioxidant activity by DPPH method.

Materials and methods

Sample preparation

Samples of the brown algae *Halopteris scoparia* were collected from the sea in the Zadar area, Punta Bajlo, in November 2021, at a depth of 1 m (44° 05' 50" N; 14° 14' 43" E). Samples of the brown algae *Sargassum horschuchii* were collected from the sea near Mandre, Pag Island, in December 2021, at a depth of 20 m (44° 28' 37" N; 14° 54' 58" E), and samples of the red algae *Corallina elongata* were collected from the sea one kilometre northwest of Brbišćica Bay, on the offshore side of Dugi Otok, in November 2021, at a depth of 0.5 m (44° 03' 16" N; 14° 59' 14" E). The algae were washed, frozen at -60 °C, and freeze-dried for 48 hours at -55 °C (Alpha 1–4 LSCPlus, Osterode am Harz, Germany). The freeze-dried samples were ground into a powder and stored at -20 °C until further analysis.

Chemicals and reagents

Ethanol (96%) and acetone (99%) used for the extraction were purchased from Gram-Mol Ltd. (Zagreb, Croatia). HPLC quality methanol was purchased from Honeywell Riedel-de-Haën (Bucharest, Romania). Methyl tert-butyl ether of HPLC quality, DPPH (2,2-diphenyl-1-picrylhydrazyl), Trolox (6-hydroxy-2,5,7, 8- tetramethylchroman-2-carboxylic acid) and commercial standards of chlorophyll a and b, fucoxanthin, lutein and β-carotene were purchased from Sigma-Aldrich (Steinheim, Germany). Distilled water was of Milli-Q quality (Millipore Corp., Bedford, NY, USA).

Ultrasound-assisted extraction

The extraction of pigments from the freeze-dried algae was carried out using an ultrasonic bath on an Elmasonic® S 40H device (Elma, Germany). For ultrasonic-assisted extraction (UAE), 96% ethanol, water and 80% acetone were used as solvents; temperatures of 30 and 50 °C and extraction times of 10, 20 and 30 min were applied. 0.5 g of the freeze-dried algae powder was weighed into a test tube and 3 mL of the chosen extraction solvent was added. After extraction, the extract was filtered through filter paper into a 5 mL volumetric flask, which was then filled up to the mark with the appropriate extraction solvent. The extract was stored at 4 °C until further analysis. Prior to high-performance liquid chromatography (HPLC) and antioxidant capacity analysis, the extract was pre-filtered through a 0.45 µm syringe filter.

Determination of chlorophylls and carotenoids using HPLC/UV-VIS with PDA detection

The determination of individual carotenoids and chlorophylls in algae extracts was performed using high-performance liquid chromatography (HPLC, 1260 Infinity II LC System, Agilent, Santa Clara, CA, USA) according to the method previously described by Castro-Puyana et al. (Castro-Puyana et al., 2017). A UV/VIS PDA detector was used. Mobile phase A consisted of a mixture of methanol/methyl tert-butyl ether/water (90:7:3, v/v/v), and mobile phase B was methanol/methyl tert-butyl ether (10:90, v/v). Separation of components was achieved using a Develosil RP-Aqueous reverse-phase column (C30) (250 mm × 4.6 mm i.d., particle size 5 µm) (Phenomenex, Torrance, CA, USA) and a DevelosilGuard pre-column. The column equilibra-tion time was 2 min, with a flow rate of 0.8 mL/min for the entire cycle, and an injection volume of 10 µL. The mobile phase gradient elution was as follows: 0 min, 0% B; 20 min, 30% B; 35 min, 50% B; 45 min, 80% B; 50 min, 100% B; 52 min, 0% B. The temperature was maintained at 35 °C, and the total run time was 52 min. The principle of determining carotenoids and chlorophylls using the described method is based on gradient elution, where the extracted carotenoids and chlorophylls elute in a decreasing polarity sequence. Carotenoids were de-tected at 450 nm, and chlorophylls at 660 nm. Quantification of carotenoids and chlorophylls was calculated using the external standard method, with calibration curves for individual pigments. The calibration curves were as follows: Chlorophyll a, $y = 35.932\ x$, $R^2 = 0.9715$; Chlorophyll b, $y = 13.326\ x$, $R^2 = 0.9195$; Xanthophyll lutein, $y = 11.86\ x$, $R^2 = 0.9498$; Fu-coxanthin, $y = 51.483\ x$, $R^2 = 0.9988$. Diadinoxanthin and neoxanthin were calculated using the lutein calibration curve. The calibration curve for β-carotene was $y = 374.11\ x$, $R^2 = 0.9817$.

Determination of antioxidant activity using DPPH method

The antioxidant capacity of the extracts was determined by their reaction with the free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) according to a previously described method (Brand-Williams et al., 1995), with adjustments. Methanol and Trolox (TE) are used as control and standard. In brief, 1 mL of the extract, 1 mL of methanol and 0.5 mL of a 0.5 mM DPPH solution were placed into a glass test tube. After 20 min of incubation at room temperature in the dark, the absorbance was measured at 517 nm. The results were expressed as µmol TE 100 g^{-1} dw.

Experimental design and statistical analysis

Statistical analysis was performed using Statistica ver. 14.0 (TIBCO Software Inc, Palo Alto, CA, USA. A mixed 2- and 3-stage full factorial design consisting of 12 experimental trials was used to evaluate UAE of algal pigments for each of the three algal species. The factors analysed included solvent, temperature and extraction time, while the dependent variables were pigment content and antioxidant activity. All extractions and analyses were performed in duplicate. Descriptive statistics were used to summarize the basic information of the experimental data set, with results presented as means ± SE. Normality and homoscedasticity of the data were tested using the Shapiro–Wilk test and Levene test, respectively. Analysis was performed using ANOVA with posthoc Tukey's HSD test or the non-parametric Kruskal–Wallis test with multiple comparisons of mean ranks. Differences in pigment composition and antioxidant capacity between the algae species from the optimized UAE extracts were tested using one-way ANOVA followed by Tukey's HSD test. Pearson's correlation coefficients were calculated to examine the relationships between pigments and antioxidant capacity. All tests were considered significant at $p \leq 0.05$.

Results and discussion

In the present research, the content and identification of pigments (using HPLC UV/VIS PDA and antioxidant activity (DPPH method were analyzed in the algae extracts obtained at various UAE conditions from three different species collected in the Adriatic sea.

Content of pigments in the obtained algal extracts

According to the results for chlorophyll content in the tested algae, six types of chlorophyll were identified and quantified: chlorophyll b, two derivatives of chlorophyll b, chlorophyll a, and two derivatives of chlorophyll a (Table 1. Notably higher fractions of chlorophyll a and its derivatives were found in most extracts compared to chlorophyll b and its derivatives, with the exception of ethanolic extracts of *Halopteris scoparia* and *Corallina elongata* which were rich in chlorophyll b derivatives. Halopteris scoparia also had significantly higher content of total chlorophylls compared to other two species with the highest total yield of 272.18 mg per 100 g dry weight (dw) (ethanol, 50 °C, 30 min), among which the mass fraction of chlorophyll a was 63.47 mg per 100 g dw which is slightly lower than the value of 72.55 detected in a hexane-isopropanol-water (10:80:10) extract of Halopteris scoparia samples from Spain (Rubiño et al., 2022).

The highest total yield of chlorophylls in *Sargassum hornschuchii* was 38.11 mg per 100 g dw (ethanol, 30 °C, 20 min), all of each was due to the content of chlorophyll a and its derivatives. The mass fractions of chlorophyll a in the ethanolic extracts were higher than 9.30 mg per 100 g dw reported previously in *Sargassum hornschuchii* samples from Egypt (El Din & El-Sherif, 2012), while the values obtained with acetone were lower. The highest yield of chlorophylls in *Corallina elongata* was 21.43 mg per 100 g dw (ethanol, 50 °C, 30 min), where chlorophyll b derivative 2 was the major contributor. Most of the *Corallina elongata* extracts did not contain chlorophyll b which is in allignment with previous data where chlorophyll a was the main chlorophyll in this algal specie with smaller portions of other chlorophylls (Ismail & Osman, 2016) or none (Ismail et al., 2017).
In the extracts of the algae, six xanthophylls (fucoxanthin, lutein, a derivative of lutein, two derivatives of neoxanthin, and diadinoxanthin) and one carotene (β-carotene) were identified (Table 2).

The highest total yield of carotenoids was recorded in *Halopteris scoparia* at 226.72 mg per 100 g dw using ethanol as the solvent, extraction temperature of 50 °C, and an extraction time of 30 min. This value is similar to the total content of carotenoids (219.5 mg per 100 g dw) detected in brown algae *Cystoseira barbata* (Ak & Turker, 2018), higher than 55 or 101 mg per 100 g dw detected in *Turbinaria conoides* and *Sargassum crassifolium*, respectively, but also notably lower than 356 or 406 per 100 g dw detected in *Padina australis* and *Dictyota dentata* which also contained higher conents of fucoxanthin (Heriyanto et al., 2017). *Halopteris scoparia* extracts contained all of the detected compounds with lutein and neoxanthin derivatives as major contributors to the total yield. Fucoxanthin was identified only in *Halopteris scoparia*, with the highest yield (21.33 mg per 100 g dw) obtained using ethanol at a temperature of 50 °C and an extraction time of 30 min. This value is lower than 95.43 mg per 100 g dw determined in a *Halopteris scoparia* extract obtained previously using hexane-isopropanol-water (10:80:10) as a solvent (Rubiño et al., 2022). The highest total yield in *Sargassum hornschuchii* was also relatively high at 194.46 mg per 100 g dw obtained using 80% acetone as the solvent, extraction temperature of 30 °C, and an extraction time of 20 min. The only xanthophylls detected in *Sargassum hornschuchii* were lutein and neoxanthin derivatives, which is different than other species belogning to the Sargassum genera where fucoxanthin was found to be the main representative (Heriyanto et al., 2017; Lourenço-Lopes et al., 2022; Shukor et al., 2022; Tabakaeva & Tabakaev, 2019), with *Sargassum polycystum* containing up to 2740 mg of fucoxanthin per 100 g of dw (Balasubramaniam et al., 2020). The content of β-carotene in both *Halopteris scoparia* and *Sargassum hornschuchii* was relatively low compared to the content of xanthophylls, which is consistent with literature data where xanthophylls were the predominant carotenoids in brown algae (Generalić Mekinić et al., 2023). The total carotenoid yield in *Corallina elongata* achieved by the presence of lutein and neoxanthin derivatives and diadinoxanthin was notably lower than in the two brown algal species with a value 6.87 mg per 100 g dw using 80 % acetone as the solvent, extraction temperature of 50 °C, and an extraction time of 30 min, which was expected since red algae generally contain less carotenoids compared to brown algae (Balasubramaniam et al., 2020). The obtained carotenoid yield was lower than 13 mg or 9 mg per 100 g dw detected in red algae *Asparagopsis taxiformis* and *Nemalion elminthoides* (Nunes et al., 2017), respectively, but also higher than 5.86 mg per 100 g of dw detected in *Pyropia orbiculans* (Uribe et al., 2018). The content of chlorophylls in *Corallina elongata* was higher than the content of total carotenoids, which is consistent with previous research on this alga (Ismail et al., 2017; Ismail & Osman, 2016).

Antioxidant activity of the obtained algae extracts

One of the key aspects of photosynthetic organisms like algae for human applications is their antioxidant potential, derived from free radical extracts generated through cellular physiological and biochemical processes. In order to determine the antioxidant activity of the obtained algae extracts, DPPH analysis was carried out and the results are shown in Table 3. As it can be observed, all three algal species exhibited antioxidant activity with range of values from 1434.86–3021.86, 1741.11–2447.32 and 752.37–1365.07 μmol TE per 100 g dw for *Halopteris scoparia, Sargassum hornschuchi* and *Corallina elongata*, respectively. *Halopteris scoparia* exhibited the highest values of antioxidant activity, while *Corallina elongata* exhibited the lowest values. Literature data is in alignment with the observed results since it was previously shown that brown algae exhibited higher antioxidant activity than the red alga (Balboa et al., 2013; Gupta & Abu-Ghannam, 2011; Vega et al., 2020). The values detected in the brown algae are similar to the antioxidant activity determined by DPPH in 50% ethanolic *Fucus vesiculosus* (2672 μmol TE per 100 g dw) extract obtained by UAE, but also lower than the antioxidant activity of *Ascophyllum nodosum* (5069 μmol TE per 100 g dw) and *Bifurcaria bifurcata* (14304 μmol TE per 100 g dw) extracts obtained in the same research (Agregán et al., 2018).

Table 1. Mass fractions of chlorophylls (mg 100 g^{-1} dw) in the analyzed algae extracts

Tablica 1. Maseni udjeli klorofila (mg 100 g^{-1} s.t.) u analiziranim ekstraktima algi

Algal specie / Vrsta alge	Solvent / Otapalo	T (°C)	t (min)	Chlorophylls / Klorofili						
				C1	C2	C3	C4	C5	C6	Total
Halopteris scoparia	96% ethanol	30	10	nd	19.65	91.97	26.99	4.37	nd	142.98
			20	nd	12.07	127.49	23.65	3.72	nd	166.93
			30	nd	14.69	169.04	22.06	3.61	nd	209.4
		50	10	7.76	10.41	41.75	53.65	6.67	nd	112.48
			20	2.99	3.25	3.6	1.39	1.48	nd	90.72
			30	nd	42.92	151.29	63.47	14.5	nd	272.18
	80 % acetone	30	10	43.79	nd	nd	68.73	1.55	1.53	71.81
			20	43.69	nd	nd	65.89	1.11	0.16	67.16
			30	27.41	nd	nd	65.58	1.52	0.17	67.27
		50	10	87.81	3.44	nd	140.34	3.41	0.39	147.58
			20	87.44	4	nd	143.16	4.24	0.33	151.73
			30	90.14	5.09	nd	147.97	4.86	0.39	158.31
Sargassum hornschuchii	96 % ethanol	30	10	nd	nd	nd	21.81	nd	nd	21.81
			20	nd	nd	nd	35.01	2.87	0.23	38.11
			30	nd	nd	nd	25.3	2.41	0.18	27.89
		50	10	nd	nd	nd	19.34	1.28	0.11	20.73
			20	nd	nd	nd	25.43	2.17	0.14	27.74
			30	nd	nd	nd	26.99	3.43	0.1	30.52
	80 % acetone	30	10	nd	nd	nd	3.01	nd	2.24	5.25
			20	nd	nd	nd	4.76	nd	2.87	7.63
			30	nd	nd	nd	3.4	nd	2.26	5.66
		50	10	nd	nd	nd	1.92	nd	1.06	2.98
			20	nd	nd	nd	7.95	nd	1.88	9.83
			30	nd	nd	nd	7.12	nd	1.74	8.86
Corallina elongata	96 % ethanol	30	10	nd	nd	nd	4.04	nd	nd	4.04
			20	nd	nd	nd	3.8	nd	nd	3.8
			30	nd	nd	nd	4.21	nd	nd	4.21
		50	10	nd	nd	12.1	nd	0.57	nd	12.67
			20	nd	nd	17.12	nd	0.86	nd	17.98
			30	nd	nd	20.25	nd	1.18	nd	21.43
	80 % acetone	30	10	nd	nd	nd	1.58	nd	nd	1.58
			20	nd	nd	nd	1.97	nd	0.08	2.05
			30	nd	nd	nd	5.46	nd	nd	5.46
		50	10	nd	nd	nd	7.51	nd	nd	7.51
			20	nd	nd	nd	7.04	0.58	nd	7.62
			30	nd	nd	nd	7.24	0.68	nd	7.92

T–temperature; t–time; C1–chlorophyll b; C2–chlorophyll b derivative 1; C3–chlorophyll b derivative 2; C4–chlorophyll a; C5–chlorophyll a derivative 1; C6–chlorophyll a derivative 2; nd–not detected a derivative 1; C6–chlorophyll a derivative 2; nd–not detected.

Influence of the UAE parameters on the total pigments' yield and antioxidant activity

Table 4 shows the results of statistical analysis of the influence of the UAE parameters (solvent, temperature and extraction time) on the yield of pigments and the antioxidant activity of the obtained extracts determined by DPPH method. The choice of solvent depends on the solubility and polarity of the extract, and the best choice of solvent depends largely on the type of algae. In most studies, ethanol and acetone have proven to be very effective for the extraction of pigments from algae. In terms of safety and environmental protection, these two solvents are also the best choice for food applications (Cheng et al., 2020; Duddela et al., 2019; Regal et al., 2020). In the present research, the effect of solvent on *Halopteris scoparia* and *Sargassum hornschuchii* had no statistically significant effect, which can be explained by the fact that both chlorophylls and carotenoids contributed largely to the content of overall pigments. In the case of *Corallina elongata* higher pigment yields were obtained with ethanol which can be attributed to

Table 2. Mass fractions of carotenoids (mg 100 g^{-1} dw) in the analyzed algae extracts

Tablica 2. Maseni udjeli karotenoida (mg 100 g^{-1} s.t.) u analiziranim ekstraktima algi

Algae	Solvent	T (°C)	t (min)	Xanthophylls							Caroten	Total
				X1	X2	X3	X4	X5	X6	Total	B1	
Halopteris scoparia	96 % ethanol	30	10	10.81	49.10	16.85	12.82	4.16	8.06	101.8	0.62	102.42
			20	7.89	37.33	18.37	8.45	3.56	6.11	81.71	0.54	82.25
			30	10.60	45.75	24.45	12.64	3.39	8.72	105.55	0.75	106.3
		50	10	10.76	45.92	28.84	11.86	11.65	11.1	120.13	0.85	120.98
			20	17.39	64.83	37.96	19.45	16.81	16.99	173.43	1.2	174.63
			30	21.33	89.19	46.87	26.07	19.33	22.22	225.01	1.71	226.72
	80 % acetone	30	10	10.02	46.65	19.91	13.67	11.93	7.44	109.62	0.4	110.02
			20	9.73	30.64	20.19	11.74	11.12	7.23	90.65	0.4	91.05
			30	0.84	45.9	20.6	12.22	11.40	7.71	98.67	0.41	99.08
		50	10	19.64	82.76	36.77	28.38	22.57	18.2	208.32	0.42	208.74
			20	19.82	84.77	42.12	26.56	23.84	18.78	215.89	1.2	217.09
			30	20.87	87.77	40.22	28.28	23.99	19.39	220.52	0.37	220.89
Sargassum hornschuchii	96 % ethanol	30	10	nd	147.69	18.41	nd	nd	nd	166.1	0.18	166.28
			20	nd	115.42	29.96	nd	nd	nd	145.38	0.34	145.72
			30	nd	84.03	22.72	nd	nd	nd	106.75	0.25	107
		50	10	nd	58.49	15.77	nd	nd	nd	74.26	0.18	74.44
			20	nd	81.35	21.31	nd	nd	nd	102.66	0.25	102.91
			30	nd	86.36	21.75	nd	nd	nd	108.11	0.27	108.38
	80 % acetone	30	10	nd	121.39	40.86	nd	nd	nd	162.25	0.28	162.53
			20	nd	145.88	48.13	nd	nd	nd	194.01	0.45	194.46
			30	nd	117.46	39.2	nd	nd	nd	156.66	0.31	156.97
		50	10	nd	69.49	23.83	nd	nd	nd	93.32	0.23	93.55
			20	nd	99.76	32.53	nd	nd	nd	132.29	0.31	132.6
			30	nd	100.87	32.81	nd	nd	nd	133.68	0.31	133.99
Corallina elongata	96 % ethanol	30	10	nd	nd	nd	2.81	nd	nd	2.81	0.12	2.93
			20	nd	nd	nd	nd	2.92	nd	2.92	0.12	3.04
			30	nd	nd	nd	nd	3.06	nd	3.06	0.13	3.19
		50	10	nd	nd	nd	3.7	nd	nd	3.7	0.15	3.85
			20	nd	nd	nd	5.33	nd	nd	5.33	0.19	5.52
			30	nd	nd	nd	6.64	nd	nd	6.64	0.23	6.87
	80 % acetone	30	10	nd	nd	nd	nd	nd	nd	nd	nd	nd
			20	nd	nd	nd	nd	nd	nd	nd	nd	nd
			30	nd	nd	nd	nd	nd	nd	nd	nd	nd
		50	10	nd	1.65	nd	nd	5.02	nd	6.67	nd	6.67
			20	nd	1.43	nd	nd	5.16	nd	6.59	nd	6.59
			30	nd	1.54	nd	nd	5.32	nd	6.86	nd	6.86

T–temperature; t–ime; X1–fucoxanthin; X2–lutein derivative X3–neoxanthin derivative 1; X4–neoxanthin derivative 2; X5–diadinoxanthin; X6–lutein; B1–ß-caroten; nd–not detected.

the chlorophyll b content which was a major contributor to total pigments in this alga and was only present in the ethanolic *Corallina elongata* extract. In case of antioxidant activity, 80% acetone was more efficient for *Halopteris scoparia* and *Corallina elongata,* while there was no sifnigicant effect of solvent in the case of *Sargassum hornschuchii*. Since differences were observed between the effect of solvent on the content of pigments and the antioxidant activity, it is likely that other compounds or specific pigments that were present in the 80% acetone extracts contributed to the antioxidant activity more. Defining optimal temperature is a crucial step in an extraction process as elevated temperature can enhance the extraction process by increasing the solubility of the sample, reducing the viscosity and surface tension of the solvent, thereby improving the extraction rate (Kadam et al., 2013), but can also cause degradation, hydrolysis, or oxidation of sensitive compounds, such as pigments. The optimal temperature range for UAE is typically set between 40 and 60 °C (Shen et al., 2023). Our results show that temperature had a different effect on pigment content depending on the type of algae. For *Halopteris scoparia* and *Corallina elongata*, significantly higher pigment yields were higher pigment yields were

Table 3. Antioxidant activity in the analyzed algae extracts

Tablica 3. Antioksidacijska aktivnost u analiziranim ekstraktima algi

Solvent	T (°C)	t (min)	DPPH (μmol TE 100 g^{-1} dw)		
			Halopteris scoparia	*Sargassum hornschuchii*	*Corallina elongata*
96 % ethanol	30	10	1434.86±2.07	1915.11±6.38	754.44±10.23
		20	1445.40±5.50	1957.38±3.96	767.90±10.79
		30	1445.44±10.35	2008.21±0.54	786.49±8.62
	50	10	1846.15±11.78	1929.83±13.19	752.37±0.44
		20	1846.22±22.66	1946.34±4.33	786.37±0.51
		30	1865.23±18.08	2447.32±4.92	874.71±1.59
80 % acetone	30	10	2455.62±26.27	1746.11±6.00	912.21±1.43
		20	2475.43±12.46	1989.73±0.47	933.72±1.66
		30	2543.42±4.72	2055.58±2.37	1054.24±1.52
	50	10	2825.39±12.08	1801.28±0.54	1082.63±6.84
		20	2962.17±42.44	2044.28±1.04	1146.53±3.45
		30	3021.86±25.16	2203.67±2.26	1365.07±0.61

T–temperature; t–time; dw–dry weight

Table 4. Influence of UAE parameters on the mass fraction of pigments (mg 100 g^{-1} dw) and the antioxidant activity (μmol TE 100 g^{-1} dw) in the analysed algae extracts

Tablica 4. Utjecaj parametara UAE na masene udjele pigmenata (mg 100 g^{-1} s.t.) i antioksidacijsku aktivnost (μmol TE 100 g^{-1} s.t.)

Source of variation	N	*Halopteris scoparia*		*Sargassum hornschuchii*		*Corallina elongata*	
		TP	AA	TP	AA	TP	AA
Solvent		p=0.358	p≤0.001*	p=0.597	p=0.054	p=0.007*	p≤0.001*
96 % ethanol	12	285.12±34.66^{a}	1647.22±44.36^{a}	145.06±9.51^{a}	2034.03±35.32^{a}	17.89±2.17^{b}	787.88±25.65^{a}
80 % acetone	12	331.15±34.66^{a}	2713.98±44.36^{b}	152.27±9.51^{a}	1973.40±35.32^{a}	8.71±2.17^{a}	1082.40±25.65^{b}
Temperature (ºC)		p≤0.001*	p=0.029*	p≤0.001*	p=0.025	p≤0.001*	p≤0.002*
30	12	234.00±27.38^{a}	2146.18±93.46^{a}	172.49±6.33^{b}	1918.76±29.27^{a}	8.04±2.02^{a}	833.24±29.61^{a}
50	12	382.27±27.38^{b}	2443.67±93.46^{b}	124.84±6.33^{a}	2014.58±29.27^{b}	18.57±2.02^{b}	973.05±29.61^{b}
Time (min)		p=0.194	p=0.861	p=0.227	p≤0.001*	p=0.821	p=0.047*
10	8	287.80±40.98^{a}	2248.88±120.73^{a}	136.69±11.18^{a}	1839.32±27.19^{a}	14.28±3.20^{a}	849.57±38.01^{a}
20	8	266.61±40.98^{a}	2293.64±120.73^{a}	164.22±11.18^{a}	1953.96±27.19^{b}	11.67±3.20^{a}	879.63±38.01^{a}
30	8	367.00±40.98^{a}	2342.26±120.73^{a}	145.08±11.18^{a}	2106.73±27.19^{c}	13.96±3.20^{a}	980.25±38.01^{b}

Values within a parameter marked with different letters are statistically different at p<0.05. N–number of trials. TP–total pigments. AA–antioxidant activity. *statistically significant at p<0.05. dw–dry weight.

obtained at higher temperatures (50 °C), while for *Sargassum hornschuchii*, higher pigment yields were obtained at lower temperatures (30 °C). Since *Halopteris scoparia* and *Corallina elongata* contained more chlorophylls in relatively larger fraction than *Sargassum hornschuchii,* and chlorophylls were previously shown to be more efficiently extracted at 50 °C compared to 30 °C (Fu et al., 2017; Jinasena et al., 2016), the observed effect may be attributed to the content of chlorophylls. In case of antioxidant activity, 50 °C resulted in higher values in all of the three algal species which is contrary to the results observed in the brown algae *Hormosira banksii* 70 % ethanolic UAE extracts where an increase of temperature from 30 °C to 50 °C decreased the antioxidant activity (Dang et al., 2017). In the case of *Halopteris scoparia* and *Corallina elongata* the temperature effect was the same for both pigment yield and antioxidant activity, while for *Sargassum hornschuchii* it differed, indicating that either a presence of other antioxidant compounds or specific pigments had a higher influence on the antioxidant activity which is supported by the correlation coefficients discussed further in text.

Effective control of UAE time is critical to optimizing both yield and extract quality since too short or too long a time can compromise the efficiency and cost-effectiveness of the process. Initially, the ultrasonic waves cause cavitation, causing the cell walls of the material to swell, hydrate and form pores. This process facilitates the penetration of the solvent and promotes the release of bioactive compounds into the solvent. However, prolonged exposure to ultrasound can lead to structural damage due to excessive heating, which can ultimately reduce extraction yields (Kumar et al., 2021). Our results showed no statistically significant difference at all three tested extraction times (10, 20, and 30 min) for all three tested algae. This confirms that high yields of bioactive components can be achieved in a short time, which is a significant advantage of UAE over conventional extractions. On the other hand, time was a significant factor influencing the antioxidant activity of the *Sargassum hornschuchi* and *Corallina elongata* extracts, indicating that other antioxidant compounds such as polyphenols and polysaccharides were likely extracted during longer time which contributed to the

antioxidant activity (Duddela et al., 2019).

In order to assess the effect of pigments in the obtained extracts on the antioxidant activity, Pearson correlation coefficients were calculated for each compound and are presented in Table 5. The correlation coefficients show that total pigments had high correlation with the antioxidant activity determined by DPPH, among which total chlorophylls had low correlation, while total carotenoids had very high correlation indicating that they contribute to the antioxidant activity more. Amrani-Allalou et al. 2020. observed opposite results where carotenoids had lower correlation than chlorophylls with the antioxidant activity of *Pallenis spinosa* extracts determined by DPPH. In the same research, however, only chlorophyll a and b were detected in the extracts, which in the present research, also had significant correlation with the antioxidant activity which was very high in the case of chlorophyll b and high in case of chlorophyll a. Chlorophyll a was also previously shown to have higher antioxidant activity than carotenoids (Mitić et al., 2013), likely due to the the presence of porphyrin ring in the structure, which reduces free radicals such as DPPH through electron donation by antioxidants (Amrani-Allalou et al., 2020).

Table 5. Pearson's correlation coefficient for the antioxidant activity and the content of pigments

Tablica 5. Pearsonovi koeficijenti korelacije za antioksidacijsku aktivnost i sadržaj pigmenata

Pigment	Pearson coefficient
Total pigments	0.71*
Chlorophylls	0.39*
C1	0.95*
C2	-0.46
C3	0.42
C4	0.75*
C5	0.22
C6	-0.17
Carotenoids	0.83*
X1	0.52*
X2	0.70*
X3	0.78*
X4	0.53*
X5	0.58*
X6	0.54*
Caroten	0.40*

C1–chlorophyll b; C2–chlorophyll b derivative 1; C3–chlorophyll b derivative 2; C4–chlorophyll a; C5–chlorophyll a derivative 1; C6–chlorophyll a derivative; 2X1–fucoxanthin; X2–lutein derivative; X3–neoxanthin derivative 1; X4–neoxanthin derivative 2; X5–diadinoxanthin; X6–lutein. *statistically significant at $p<0.05$.

Among individual carotenoids, lutein derivative and neoxanthin derivative 1 which were the main carotenoid representatives in *Halopteris scoparia* and *Sargassum hornuschii* had high correlation with the antioxidant activity, while others such as diadinoxanthin which was relevant in the *Corallina elongata* extract had low to moderate correlation indicating that in the case of this alga chlorophylls contributed more to the antioxidant activity. Lutein was previously found to contribute to antioxidant activity more than o ther carotenoids (Wang et al., 2006), and the structural variation of its derivative found in the present study might have contributed to more efficient radical scavenging ability compared to lutein. Neoxanthin was also found to be a major contributor to antioxidant activity among other carotenoids in microalgae *Nephroselmis sp.*, which supports the findings in the present study (Coulombier et al., 2020). In addition, both lutein and neoxanthin from microalga *Chlamydomonas reinhardtii* were shown to effectively reduce oxidative damage (Şahin et al., 2020).

Conclusions

This study presents a comprehensive analysis of the influence of different ultrasound-assisted extraction (UAE) conditions on pigment yield and antioxidant activity in the brown algae *Halopteris scoparia* and *Sargassum hornschuchii* and the red alga *Corallina elongata* from the Adriatic Sea. This study is ground-breaking in the application of UAE for pigment extraction from these particular algae species. Statistical analysis showed that the extraction time had no significant influence on the yield of total pigments, while the choice of solvent and temperature had significant influence. In *Halopteris scoparia* (at 50 °C) and *Sargassum hornschuchii* (at 30 °C) the solvent did not affect the total concentration of pigments, while 96% ethanol was more efficient for *Corallina elongata* (at 50 °C). The highest antioxidant activity was achieved at 50 °C in all three algal species using either solvent for *Sargassum hornschuchii* and using 80% acetone for *Halopteris scoparia* (at any extraction time) and *Corallina elongata* (30 min). Among the algae studied, *Halopteris scoparia* exhibited the highest pigment content and free radical scavenging ability under optimal extraction conditions. A total of thirteen pigments were identified and quantified in the extracts, including six chlorophylls and seven carotenoids. Only *Halopteris scoparia* contained all of the detected compounds, including fucoxanthin which was not detected in the other two algal species. Chlorophyll a was more abundant than chlorophyll b whose mass fraction was notably high in the ethanolic extracts of *Halopteris scoparia* and *Corallina elongata*. The main carotenoids in all of the algal species were lutein and neoxanthin derivatives, with diadinoxanthin also being a major contributor in *Corallina elongata* ethanolic extracts. A correlation was found between antioxidant activity and the presence of carotenoids and chlorophyll a and b, suggesting that these pigments contribute significantly to the antioxidant properties of the algal extracts. These results highlight the potential of UAE in optimizing the extraction of valuable bioactive compounds from marine algae, which offer promising applications in food and pharmaceutic industries.

Declarations

Funding: This research was funded by the Croatian Government and the European Union through the European Regional Development Fund - the Competitiveness and Cohesion Op-erational Programme (KK.01.1.1.01) for funding The Scientific Centre of Excellence for Marine Bioprospecting - BioProCro. Conflicts of interest/Competing interests: The authors declare no conflicts or competing interests. Authors' contributions: Conceptualization, Z.Č., Z.Z.; methodology, Z.Č. and S.P.; formal analysis, Z.Č.; investigation, Z.Č. and E.D.; data curation, Z.Č. and E.D.; writing - original draft preparation, Z.Č. and E.D.; writing—review and editing, S.P. and Z.Z..; supervision, S.P. All authors have read and agreed to the published version of the manuscript.

References

Agregán R., Munekata P., Franco D., Carballo J., Barba F., Lorenzo J. (2018) Antioxidant potential of extracts obtained from macro- and micro-algae assisted by ultrasound. *Medicines*, 5 (2), 33.

Ak I., Turker G. (2018) Antioxidant activity of five seaweed extracts. *New Knowledge Journal of Science*, 7 (2), 149–155.

Amrani-Allalou H., Boulekbache-Makhlouf L., Mapelli-Brahm P., Sait S., Tenore G.C., Benmeziane A., Kadri N., Madani K., Meléndez Martínez A.J. (2020) Antioxidant activity, carotenoids, chlorophylls and mineral composition from leaves of Pallenis spinosa. *Journal of Complementary and Integrative Medicine*, 17 (1), 1–9.

Balasubramaniam V., June Chelyn L., Vimala S., Mohd Fairulnizal M.N., Brownlee I.A., Amin I. (2020) Carotenoid composition and antioxidant potential of Eucheuma denticulatum, Sargassum polycystum and Caulerpa lentillifera. *Heliyon*, 6 (8), e04654.

Balboa E.M., Conde E., Moure A., Falqué E., Domínguez H. (2013) In vitro antioxidant properties of crude extracts and compounds from brown algae. *Food Chemistry*, 138 (2–3), 1764–1785.

Brand-Williams W., Cuvelier M.E., Berset C. (1995) Use of a free radical method to evaluate antioxidant activity. *LWT – Food Science and Technology*, 28 (1), 25–30.

Castro-Puyana M., Pérez-Sánchez A., Valdés A., Ibrahim O.H.M., Suarez-Álvarez S., Ferragut J.A., Micol V., Cifuentes A., Ibáñez E., García-Cañas V. (2017) Pressurized liquid extraction of Neochloris oleoabundans for the recovery of bioactive carotenoids with anti-proliferative activity against human colon cancer cells. *Food Research International*, 99, 1048–1055.

Cheng S.-H., Khoo H.E., Kong K.W., Prasad K.N., Galanakis C.M. (2020) Extraction of carotenoids and applications. U: Carotenoids: Properties, Processing and Applications, str. 259–288. Elsevier.

Christaki E., Bonos E., Giannenas I., Florou-Paneri P. (2013) Functional properties of carotenoids originating from algae. *Journal of the Science of Food and Agriculture*, 93 (1), 5–11.

Cikoš A.-M., Šubarić D., Roje M., Babić J., Jerković I., Jokić S. (2022) Recent advances on macroalgal pigments and their biological activities (2016–2021). *Algal Research*, 65, 102748.

Coulombier N., Nicolau E., Le Déan L., Barthelemy V., Schreiber N., Brun P., Lebouvier N., Jauffrais T. (2020) Effects of nitrogen availability on antioxidant activity and carotenoid content of the microalgae Nephroselmis sp. *Marine Drugs*, 18 (9), 453.

Dang T.T., Van Vuong Q., Schreider M.J., Bowyer M.C., Van Altena I.A., Scarlett C.J. (2017) Optimisation of ultrasound-assisted extraction conditions for phenolic content and antioxidant activities of the alga Hormosira banksii using response surface methodology. *Journal of Applied Phycology*, 29 (6), 3161–3173.

Dey S., Rathod V.K. (2013) Ultrasound assisted extraction of β-carotene from Spirulina platensis. *Ultrasonics Sonochemistry*, 20 (1), 271–276.

Duddela V.P., Narravula R.S., Chandrashekhar T., Parveen N. (2019) Effect of various solvents on chlorophyll and carotenoid extraction in green algae: Chlamydomonas reinhardtii and Chlorella vulgaris. *Annals of Plant and Soil Research*, 21 (4), 341–345.

El Din N.G.S., El-Sherif Z.M. (2012) Nutritional value of some algae from the north-western Mediterranean coast of Egypt. *Journal of Applied Phycology*, 24 (3), 613–626.

Esteban R., Martínez B., Fernández-Marín B., Becerril J.M., García-Plazaola J.I. (2009) Carotenoid composition in Rhodophyta: insights into xanthophyll regulation in Corallina elongata. *European Journal of Phycology*, 44 (2), 221–230.

Fu J.-J., Shen S., Liu W., Wang H.-B., Gao W.-D. (2017) An optimal thermal condition for maximal chlorophyll extraction. *Thermal Science*, 21 (4), 1857–1860.

Gammone M., D'Orazio N. (2015) Anti-obesity activity of the marine carotenoid fucoxanthin. *Marine Drugs*, 13 (4), 2196–2214.

Ganesan P., Kumar C.S., Bhaskar N. (2008) Antioxidant properties of methanol extract and its solvent fractions obtained from selected Indian red seaweeds. *Bioresource Technology*, 99 (8), 2717–2723.

Generalić Mekinić I., Šimat V., Rathod N.B., Hamed I., Čagalj M. (2023) Algal carotenoids: chemistry, sources, and application. *Foods*, 12 (14), 2768.

Gupta S., Abu-Ghannam N. (2011) Bioactive potential and possible health effects of edible brown seaweeds. *Trends in Food Science and Technology*, 22 (6), 315–326.

Hadjkacem F., Elleuch J., Pierre G., Fendri I., Michaud P., Abdelkafi S. (2024) Production and purification of fucoxanthins and β-carotenes from Halopteris scoparia and their effects on digestive enzymes and harmful bacteria. *Environmental Technology*, 45 (15), 2923–2934.

Heriyanto, Juliadiningtyas A.D., Shioi Y., Limantara L., Brotosudarmo T.H.P. (2017) Analysis of pigment composition of brown seaweeds collected from Panjang Island, Central Java, Indonesia. *Philippine Journal of Science*, 146 (3), 323–330.

Holdt S.L., Kraan S. (2011) Bioactive compounds in seaweed: functional food applications and legislation. *Journal of Applied Phycology*, 23 (3), 543–597.

Hosokawa M., Wanezaki S., Miyauchi K., Kurihara H., Kohno H., Kawabata J., Odashima S., Takahashi K. (1999) Apoptosis-inducing effect of fucoxanthin on human leukemia cell line HL-60. *Food Science and Technology Research*, 5 (3), 243–246.

Ismail M.M., El Zokm G.M., El-Sayed A.A.M. (2017) Variation in biochemical constituents and master elements in common seaweeds from Alexandria coast, Egypt. *Environmental Monitoring and Assessment*, 189 (12).

Ismail M.M., Osman M.E.H. (2016) Seasonal fluctuation of photosynthetic pigments of common red seaweeds species collected from Abu Qir, Alexandria, Egypt. *Revista de Biologia Marina y Oceanografia*, 51 (3), 515–525.

Jinasena M., Amarasinghe A., Amarasinghe B., Prashantha M. (2016) Extraction and degradation of chlorophyll a and b from Alternanthera sessilis. *Journal of the National Science Foundation of Sri Lanka*, 44 (1), 11.

Kadam S.U., Tiwari B.K., O'Donnell C.P. (2013) Application of novel extraction technologies for bioactives from marine algae. *Journal of Agricultural and Food Chemistry*, 61 (20), 4667–4675.

Kumar K., Srivastav S., Sharanagat V.S. (2021) Ultrasound assisted extraction (UAE) of bioactive compounds from fruit and vegetable processing by-products: a review. *Ultrasonics Sonochemistry*, 70, 105325.

Lourenço-Lopes C., Fraga-Corral M., Garcia-Perez P., Carreira-Casais A., Silva A., Simal-Gandara J., Prieto M.A. (2022) A HPLC-DAD method for identifying and estimating the content of fucoxanthin, β-carotene and chlorophyll a in brown algal extracts. *Food Chemistry Advances*, 1, 100095.

Mitić V., Stankov Jovanović V., Dimitrijević M., Cvetković J., Petrović G., Stojanović G. (2013) Chemometric analysis of chlorophyll a, b and carotenoid content in green leafy vegetables. *Biologica Nyssana*, 4 (1–2), 49–55.

Nunes N., Ferraz S., Valente S., Barreto M.C., Pinheiro de Carvalho M.A.A. (2017) Biochemical composition, nutritional value, and antioxidant properties of seven seaweed species from the Madeira Archipelago. *Journal of Applied Phycology*, 29 (5), 2427–2437.

Pangestuti R., Kim S.-K. (2011) Biological activities and health benefit effects of natural pigments derived from marine algae. *Journal of Functional Foods*, 3 (4), 255–266.

Patel A.K., Albarico F.P.J.B., Perumal P.K., Vadrale A.P., Nian C.T., Chau H.T.B., Anwar C., Wani H.M. ud din, Pal A., Saini R., Ha L.H., Senthilkumar B., Tsang Y.-S., Chen C.-W., Dong C.-D., Singhania R.R. (2022) Algae as an emerging source of bioactive pigments. *Bioresource Technology*, 351, 126910.

Pereira A.G., Otero P., Echave J., Carreira-Casais A., Chamorro F., Collazo N., Jaboui A., Lourenço-Lopes C., Simal-Gandara J., Prieto M.A. (2021) Xanthophylls from the sea: algae as source of bioactive carotenoids. *Marine Drugs*, 19 (4), 188.

Quitério E., Grosso C., Ferraz R., Delerue-Matos C., Soares C. (2022) A critical comparison of the advanced extraction techniques applied to obtain health-promoting compounds from seaweeds. *Marine Drugs*, 20 (11), 677.

Regal P., Lamas A., Fente C.A., Franco C.M., Cepeda A. (2020) Analysis and metabolomics of carotenoids. U: Carotenoids: Properties, Processing and Applications, str. 189–222. Elsevier.

Rubiño S., Peteiro C., Aymerich T., Hortós M. (2022) Major lipophilic pigments in Atlantic seaweeds as valuable food ingredients. *Food Research International*, 159, 111609.

Şahin S., Aybastıer Ö., Dawbaa S., Karkar B., Çakmak T. (2020) Study of the ability of lutein and neoxanthin to prevent oxidatively induced DNA base damage. *Chromatographia*, 83 (8), 919–926.

Shen L., Pang S., Zhong M., Sun Y., Qayum A., Liu Y., Rashid A., Xu B., Liang Q., Ma H., Ren X. (2023) A comprehensive review of ultrasonic assisted extraction (UAE) for bioactive components. *Ultrasonics Sonochemistry*, 101, 106646.

Shukor M.I., Susanti D., Sabarudin N.S., Nor N.M., Taher M. (2022) Effects of solvent extraction and drying methods of Malaysian seaweed Sargassum polycystum on fucoxanthin content. *AIP Conference Proceedings*, 030025.

Silva A., Silva S.A., Carpena M., Garcia-Oliveira P., Gullón P., Barroso M.F., Prieto M.A., Simal-Gandara J. (2020) Macroalgae as a source of valuable antimicrobial compounds. *Antibiotics*, 9 (10), 642.

Tabakaeva O.V., Tabakaev A.V. (2019) Carotenoid profile and antiradical properties of brown seaweed Sargassum miyabei extracts. *Chemistry of Natural Compounds*, 55 (2), 364–366.

Uribe E., Vega-Gálvez A., Heredia V., Pastén A., Di Scala K. (2018) An edible red seaweed Pyropia orbicularis: influence of vacuum drying on physicochemical composition and pigments. *Journal of Applied Phycology*, 30 (1), 673–683.

Vega J., Álvarez-Gómez F., Güenaga L., Figueroa F.L., Gómez-Pinchetti J.L. (2020) Antioxidant activity of extracts from marine macroalgae. *Aquaculture*, 522, 735088.

Wang H.-M.D., Chen C.-C., Huynh P., Chang J.-S. (2015) Exploring the potential of using algae in cosmetics. *Bioresource Technology*, 184, 355–362.

Wang M., Tsao R., Zhang S., Dong Z., Yang R., Gong J., Pei Y. (2006) Antioxidant activity and mutagenicity of lutein from marigold flowers. *Food and Chemical Toxicology*, 44 (9), 1522–1529.

Zia S., Khan M.R., Shabbir M.A., Aslam Maan A., Khan M.K.I., Nadeem M., Khalil A.A., Din A., Aadil R.M. (2022) Advanced thermal and nonthermal extraction techniques for bioactive compounds. *Food Reviews International*, 38 (6), 1166–1196.