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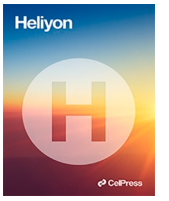

Research article

Green extraction cascade of UV-absorbing compounds, alginate, and fucoidan from *Sargassum* using ethanol and natural deep eutectic solvents

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ABSTRACT

The objective of this study was to evaluate Caribbean Mexican seaweed as a raw material for the production of fucoidans, alginates, and bioactive extracts with antioxidant activity, total flavonoid content, total phenolic content, and UV absorbance. Extractions were first performed using varying ethanol concentrations and maceration times, with the optimal treatment selected based on its superior antioxidant activity, flavonoid and phenolic contents, and UV absorbance. The solid fraction from this treatment was then subjected to extraction using green solvents, specifically natural deep eutectic solvents (NADESs) and ultrasound-assisted extraction (UAE), to isolate alginate and fucoidan. The green extraction cascade enables the recovery of multiple value-added products from each fraction, showcasing both versatility and sustainability. The new DES combination yielded a high amount of crude fucoidan (0.4103 $\pm$ 0.0042 g g^{-1} dry algae), exceeding the yields reported in previous studies. FTIR-ATR analysis confirmed that the extracted fucoidan structure was consistent with that of *Sargassum* spp., although further purification and characterization are needed to determine whether its known bioactive properties are preserved. All treatments exhibited strong UV-B absorbance, highlighting the potential of *Sargassum* extracts as sunscreen filters, with polyphenolic compounds being the primary contributor to UV absorption. Additionally, UV-A absorbance was correlated with flavonoid and carotenoid content, particularly in 50 % ethanol extracts. Future research should explore the potential of *Sargassum* for sunscreen applications and polysaccharide extraction, offering a sustainable solution to the environmental and economic challenges posed by annual *Sargassum* blooms in Mexico.

1. Introduction

In recent years, large numbers of floating species of *Sargassum* algae have washed up on the shores and beaches of the Caribbean and Gulf of Mexico. *Sargassum* flows threaten already fragile coastal ecosystems, such as coral reefs, mangroves, and seagrass beds, and disrupt the livelihoods of communities, especially those associated with the tourism and fishing sectors [1]. Seaweeds offer advantages in relation to their renewable character, wide distribution, and richness and versatility of their valuable bioactive compounds [2]. Recent research suggests that the components found in *Sargassum* can be harnessed for natural sunscreen formulations, providing an eco-friendly alternative to conventional chemical sunscreens.

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Repeated ultraviolet (UV) exposure of human skin can cause severe skin damage, including photo-aging, inflammation, and photocarcinogenesis [3]. Sunscreen has been shown to inhibit the development of keratinocyte cancer and melanoma. Owing to concerns about the environmental effects and health of some organic UV filters, several locations across the world have begun to pass legislation banning the use of these ingredients in sunscreens [3,4]. As a result, there has been an increase in the development of natural products to replace synthetic chemical filters [3]. Studies analyzing different *Sargassum* species have reported effective UVA and UVB protection, with a reduction in reactive oxygen species (ROS) levels and recovery of cell viability [3,5].

Alginate is a polymer composed of different proportions of guluronic and mannuronic acids. Low-grade alginates are extensively used in the industrial sector as thickeners, emulsifiers, and stabilizers. They are commonly employed in applications such as wastewater treatment, textile printing, and paper manufacturing. By contrast, high-grade alginates are specifically tailored to the food industry. They play crucial roles as gelling agents, thickening agents, and encapsulating materials. For instance, high-grade alginates are used to create gel-like textures in foods, such as jams, jellies, and salad dressings. Additionally, they are employed in the production of edible films and coatings for fruits and vegetables, thereby enhancing their shelf life and appearance. High-quality alginates are used for cell immobilization and drug delivery [6]. Fucoidans are sulfated polysaccharides obtained from brown algae (*Phaeophyceae* spp.) that are associated with cell wall components. It contains fucose as its major monosaccharide along with trace amounts of other monomers such as xylose, arabinose, mannose, galactose, glucose, glucuronic acid, and galacturonic acid [6,7]. Fucoidans are important in medicinal and pharmaceutical fields because of their extensive variety of bio-functional properties, such as antioxidant, anticoagulant, antiviral, anti-inflammatory, immunomodulatory, and antitumor properties [6,7].

Ultrasound has been demonstrated to be an economically feasible technology suitable for the extraction of polysaccharides. Ultrasound-assisted extraction (UAE) is faster than hot water extraction and can be easily combined with other technologies, such as supercritical fluid extraction [8] and deep eutectic solvents (DESs). DESs are an advanced class of ionic liquids (ILs), composed of a eutectic mixture of Bronsted-Lewis bases and acids with various types of cationic or anionic groups. Their synthesis is achieved by the combination of two or more substances made up of hydrogen bond donors (HBDs) and hydrogen bond acceptors (HBAs) in a mixed form to create a eutectic structure with a melting point lower than that of a specific compound [6,9]. DESs are advantageous due to their simple and green synthetic processes, cost-effectiveness, having stronger hydrogen bond network, low melting point and volatility, being non-flammable, having high thermal stability and solubility for various substances, for being environmentally friendly and their high efficiency, making them suitable for large-scale production, moreover, lower toxicity and greater biodegradability can be reached when prepared from renewable sources [9]. Green solvents are solvents that are non-volatile, non-toxic, recyclable, and biodegradable that must also be prepared with environmentally friendly reagents, materials and procedures. Among the green solvents, natural deep eutectic solvents (NADESs), a particular type of DESs, have received much attention. NADESs are considered natural because they are prepared from the primary metabolites (e.g. amino acids, sugars, carboxylic acids, vitamins, and natural amino alcohols). NADESs have a significant advantage over DESs and conventional solvents because they meet many criteria as green alternatives: NADESs have simpler constituents that are widely available and can be derived from natural sources (such as agricultural biomass), resulting in lower-costs and lower energy for their synthesis; have greater biocompatibility, are biodegradable, non-toxic and easily decompose; are recyclable due to their non-volatility during the extraction process; and can rival the performance of conventional organic solvents due to their high thermal stability and non-flammability [9,10].

The aim of this study was to exploit pelagic *Sargassum* biomass from the Mexican Caribbean with two primary objectives. First, phytochemical characterization and UV absorption evaluation were conducted using green solvent extractions from *Sargassum* collected from the Mexican Caribbean. The primary emphasis was to assess its potential use as a sunscreen filter. The extracts were analyzed to determine their total antioxidant activity, total flavonoid content, total phenolic content, and photosynthetic pigment content. UHPLC-DAD was used to validate the presence of photosynthetic pigments and to quantify phenolic compounds. Second, the effect of new combinations of NADESs combined with Ultrasound-assisted Extraction (UAE) on the extraction yields of fucoidan and alginate was evaluated. Fourier Transform Infrared Spectroscopy coupled with Attenuated Total Reflection (FTIR-ATR) was used to evaluate the identity, chemical changes and possible physical modifications [11,12] of the extracted fucoidan.

2. Materials and methods

2.1. Materials and standards

Standards astaxanthin (synthetic), lutein, zeaxanthin, canthaxanthin (trans), chlorophyll A, chlorophyll B, β-carotene, 4-hydroxybenzoic acid, chlorogenic acid, gallic acid, ferulic acid, p-coumaric acid, quercetin, and vanillic acid were obtained from Sigma-Aldrich (Darmstadt, Germany). Ethanol (EtOH), 2,2-diphenyl-1-picrylhydrazyl (DPPH), methanol (HPLC grade ≥99.9 %), ascorbic acid (AA), sodium nitrate ($NaNO_3$), aluminum chloride ($AlCl_3$), sodium hydroxide (NaOH), quercetin, Folin-Ciocalteu (F-C) reagent, gallic acid (GA), phosphoric acid, choline chloride, urea, glycerol, glacial acetic acid, and calcium chloride ($CaCl_2$) were purchased from Sigma-Aldrich (Darmstadt, Germany). Acetonitrile (HPLC grade ≥99.9 %) was supplied by J.T. Baker (New Jersey, USA). Na_2CO_3 and ammonium acetate were supplied by *Materiales y Abastos Especializados* (Jalisco, Mexico). Whatman 1 filter paper was supplied by GE Healthcare (Chicago, IL, USA). All reagents listed were A.C.S grade, unless stated otherwise.

2.2. Algal samples

Sargassum samples were collected in July 2023 from Playa del Carmen Beach (20°38′53.0″N 87°03′00.6″W), Quintana Roo, Mexico. *Sargassum* biomass is composed of two species: *Sargassum natans* (90 %) and *Sargassum fluitans* (10 %) [13]. The samples were

dehydrated at 40 °C for 48 h (or until completely dry) and ground until they reached a flour-like consistency. The samples were stored at room temperature (RT) in the dark.

2.3. Sargassum green solvent extract evaluation

A solid-liquid extraction (SLE) was performed at a concentration of 100 g dry algae (DA) per 600 mL of ethanol (ethanol concentration: 25 %, 50 %, and 100 %) [14]. The solutions were stirred at 150 rpm for 40, 80, and 120 h, and then filtered using Whatman 1 filter paper. Liquid samples were stored at 4 °C in the dark until further analysis. An experimental design (Table 1) was used to evaluate the effects of ethanol concentration and maceration time on the antioxidant activity, total flavonoid content, total phenolic content, and UV absorbance. Each treatment was performed in triplicate. The dry weight yields were calculated based on the ratio of dry algae to the solvent used.

2.3.1. Antioxidant activity

Total Antioxidant activity (TAA) was determined using the DPPH scavenging capacity assay [15]. First, 7 μL of the liquid sample (Section 2.2) and 193 μL of a 60 μM DPPH stock solution was placed in a 96-well microplate, mixed, and kept in the dark for 30 min at RT. Methanol was used as the blank (200 μL) and DPPH-methanol was used as the control. Absorbance was measured at 517 nm using a Varioskan LUX microplate reader (Thermo Fisher Scientific, USA). The equation for the standard AA curve was ln(y) = -0.58*ln (x)-0.351 with coefficient $R^2 = 0.993$. The linearity obtained was in the range of 8–210 μg AA mL^{-1}. The results were expressed as AA equivalents per gram of dry *Sargassum* (μg AAE g^{-1} DA).

2.3.2. Total flavonoid content

The total flavonoid content (TFC) of the crude extracts was determined using the aluminum chloride colorimetric method [16] with some modifications. In a glass tube, 500 μL of liquid sample, 2 mL of distilled water (dH_2O), and 100 μL of 5 % (w/w) $NaNO_3$ were added. After 6 min, 100 μL of 10 % (w/w) $AlCl_3$ was added and the mixture was left to settle for 5 min. Subsequently, 1 mL of 10 % (w/w) NaOH and 1.3 mL of methanol were added. The control contained 100 μL of dH_2O. After 30 min, 100 μL of each sample was read using a microplate reader at 425 nm. The equation for the standard quercetin curve was $y = 0.000273x+0.0168$ with a coefficient $R^2 = 0.993$. The linearity obtained was in the range of 10–2000 μg quercetin mL^{-1}. The results are expressed in milligrams of quercetin equivalents per gram of dry *Sargassum* (μg QE g^{-1} DA).

2.3.3. Total phenolic content

Total phenolic content (TPC) was determined using the F-C assay, as described by Sánchez-Rangel et al. (2013) [17]. For each well of a 96-well microplate, 15 μL of liquid sample, 200 μL of dH_2O, and 15 μL of 0.25 N F-C reagent were added and incubated for 3 min. Then, 30 μL of 1N Na_2CO_3 was added and the mixture was incubated for 2h at RT in the dark. Blanks contained dH_2O. Finally, absorbance was measured at 765 nm using a microplate reader. A standard GA was constructed. The equation for the GA standard curve was $y = 0.0472x-0.00513$ with coefficient $R^2 = 0.997$. The linearity obtained was in the range 0.1–2.5 mg GA mL^{-1}. The results are expressed in milligrams of GA equivalents per gram of dry *Sargassum* (mg GAE g^{-1} DA).

2.3.4. Photosynthetic pigment quantification

Total chlorophyll content (TChlC) was calculated as described by Ritchie (2008) [18]. Results were expressed as milligrams of total chlorophyll per gram of dry Sargassum (mg Total Chl g^{-1} DA). Total carotenoid content (TCC) was estimated using reported extinction coefficients for fucoxanthin at 450 nm [19], and the results were expressed as micrograms of fucoxanthin equivalents per gram of dry *Sargassum* (mg FucoE g^{-1} DA).

2.3.5. Analysis of phenolic compounds and photosynthetic pigments with Ultra-high pressure liquid chromatography-diode array detector

Ultra-high-pressure liquid chromatography diode array detection (UHPLC-DAD) was used for the phytochemical characterization of the SLE liquid. The equipment used was an ACQUITY Arc with an Arc Premier 2998 Photodiode Array (PDA) detector and quaternary solvent manager (Waters, Massachusetts, USA). The samples were filtered through syringeless filter vials (0.20 μm PTFE).

Table 1
Full factorial design (3^2) for evaluating the effects of ethanol concentration and maceration time on antioxidant activity, flavonoid and phenolic content, and UV absorbance.

No.	Ethanol concentration (%v/v)	Maceration time (hours)
1	25	40
2	25	80
3	25	120
4	50	40
5	50	80
6	50	120
7	100	40
8	100	80
9	100	120

Photosynthetic pigment separation was performed using a Luna C8 column (3 μm, 100 Å, 4.6 × 100mm) manufactured by Phenomenex (California, USA). A modified version of the application protocol provided by the column manufacturer (Pigment Application ID 1319) was used in this study. The gradient method consisted of a mixture of pure methanol (solvent A) and Methanol/1M Ammonium Acetate (70:30) (solvent B). The concentration gradient was varied as follows: initial 5 % A and 95 % B; b) 5 min 5 % A and 95 % B; c) 10 min 20 % A and 80 % B; d) 30 min 50 % A and 50 % B; e) 40 min 65 % A and 35 % B; f) 50 min 80 % A and 20 % B; g) 57 min 80 % A and 20 % B; h) 60 min 95 % A and 5 % B; and j) 70 min 5 % A and 95 % B. A flow rate of 1 mL min^{-1}, sample temperature of 4 °C, and column temperature of 60 °C were used. The standards and samples were read at 450 nm and an injection volume of 10 μL was used.

Phenolic quantification was performed as described by Mizzi et al. (2020) [20] and Adamcová et al. (2022) [21]. Modifications to the protocol were made progressively with a series of preliminary studies. The separation was carried out using a CORTECS C18 (2.7 μm, 90 Å, 4.6 × 150mm) manufactured by Waters (Massachusetts, USA). The mobile phases consisted of water adjusted to pH 2.4 (with orthophosphoric acid) (phase C), and methanol (phase D). The execution time was 55 min, and the gradient solvent system used was initially 5 % C and 95 % D: a) 15 min 5 % C and 95 % D; b) 35 min 10 % C and 90 % D; c) 50 min 13 % C and 87 % D; and d) 55 min 5 % C and 95 % D. A flow rate of 1 mL min^{-1}, sample temperature of 4 °C, and column temperature of 30 °C were used. The standards and samples were read at 280 nm and an injection volume of 10 μL was used.

Photosynthetic pigment standards retention times were obtained as follows; astaxanthin 20.90 min, β-carotene 54.27 min, canthaxanthin 32.12 min, chlorophyll A 41.71 min, chlorophyll B 47.51 min, lutein 29.74 min and zeaxanthin 29.72 min. Phenolic standards retention time were as follows; 4-hydroxybenzoic acid 8.11 min, chlorogenic acid 9.68 min, ferulic acid 21.89 min, gallic acid 3.12 min, p-coumaric acid 18.54 min, quercetin 25.71 min and vanillic acid 11.49 min. Calibration curves were prepared for the quantification of phenolic compounds. All the calibration-related data are presented in Table 2.

2.3.6. UV absorbance

SLE liquid was analyzed using a Varioskan LUX microplate reader (Thermo Fisher Scientific, USA) to determine the best treatment (highest UV absorbance). Briefly, 40 μL of SLE liquid was diluted 1:8 with 100 % EtOH for readings within the linear range of the spectrophotometer. UV absorbance was measured from 290 to 400 nm.

2.4. Natural deep eutectic solvent-based ultrasound-assisted extraction procedure

2.4.1. Natural deep eutectic solvent preparation

All NADESs were prepared in a 1:2 M ratio of choline chloride with a HBD [6,22] and HBAs: urea (DES-1), glycerol (DES-2), or acetic acid (DES-3). Each NADES was prepared in a reaction bottle and stirred on a heating plate at 80 °C until a clear solution was obtained.

2.4.2. Natural deep eutectic solvent-based ultrasound-assisted extraction of polysaccharides

The substrate for the natural deep eutectic solvent-based ultrasound-assisted extraction (NADES-UAE) procedure, deodorized *Sargassum*, was obtained from the solid fraction described in Section 2.3. The solid fraction with the best UV absorbance was dried at 40 °C for 48 h or until completely dry. Based on the optimal conditions reported by Nie et al. (2020) [22] for *S. horneri*, a temperature of 70 °C, liquid-solid ratio (L/S) of 30 mL g^{-1} DA, and NADES water content of 30 % were used. dH_2O was used as control. SB-5200DTN ultrasound bath (40 kHz, 200W) was used. A combination of three different NADESs and maceration times (30, 45, and 60 min) (Table 3) was used. Each treatment was performed in triplicate.

Alginate and fucoidan were separated as described by Saravana et al. (2018) [6] with minor modifications. After the reaction, the samples were filtered through Whatman 1 filter paper and stored at 4 °C. The supernatant was then mixed 1:1 with a 1 % (w/v) CaCl2 solution and kept at 4 °C overnight to remove alginate. The alginate was separated using Whatman 1 filter paper. It was then washed three times with ethanol to remove the remaining NADES, and dried at 40 °C. The resulting liquid fraction was mixed 1:1 with absolute ethanol and maintained overnight at 4 °C to obtain crude fucoidan. Crude fucoidan was then separated by centrifugation (4 °C, 4000 rpm, 45 min) and dried at 40 °C. Crude alginate/fucoidan yields were estimated as the weight of dried polysaccharides obtained from 1 g of dry algae (DA).

Table 2
Calibration data for phenolic quantification using Ultra-High Pressure Liquid Chromatography diode array detection.

Compound	Equation	R square	Linearity (μg mL^{-1})	LOD[a] (μg mL^{-1})	LOQ[b] (μg mL^{-1})
4-Hydroxybenzoic acid	y = 0.0003071x-0.0002600	0.9995	20–200	5.09	15.45
Chlorogenic acid	y = 0.00029936x-0.001308	0.9987	30–200	8.21	24.90
Ferulic acid	y = 0.0004358x-0.0002672	0.9969	30–200	13.02	39.46
Gallic acid	y = 0.0008582x-0.001565	0.9985	30–200	9.09	27.57
p-Coumaric acid	y = 0.0009028x-0.0009018	0.9986	20–200	8.82	26.75
Quercetin	y = 0.0002171x-0.001251	0.9956	30–200	15.52	47.03
Vanillic acid	y = 0.0001940 + 0.0004474	0.9994	20–200	5.60	16.98

a LOD: Limit of Detection.

b LOQ: Limit of Quantification

Table 3
Full factorial design (3^2) for evaluating the effects of natural deep eutectic solvents (NADESs) and maceration time on alginate and fucoidan extraction using ultrasound-assisted extraction.

No.	Natural Deep eutectic solvent (NADES)	Maceration time (min)
1	DES-1 (urea)	30
2		45
3		60
4	DES-2 (glycerol)	30
5		45
6		60
7	DES-3 (acetic acid)	30
8		45
9		60
10	dH_2O	30
11		45
12		60

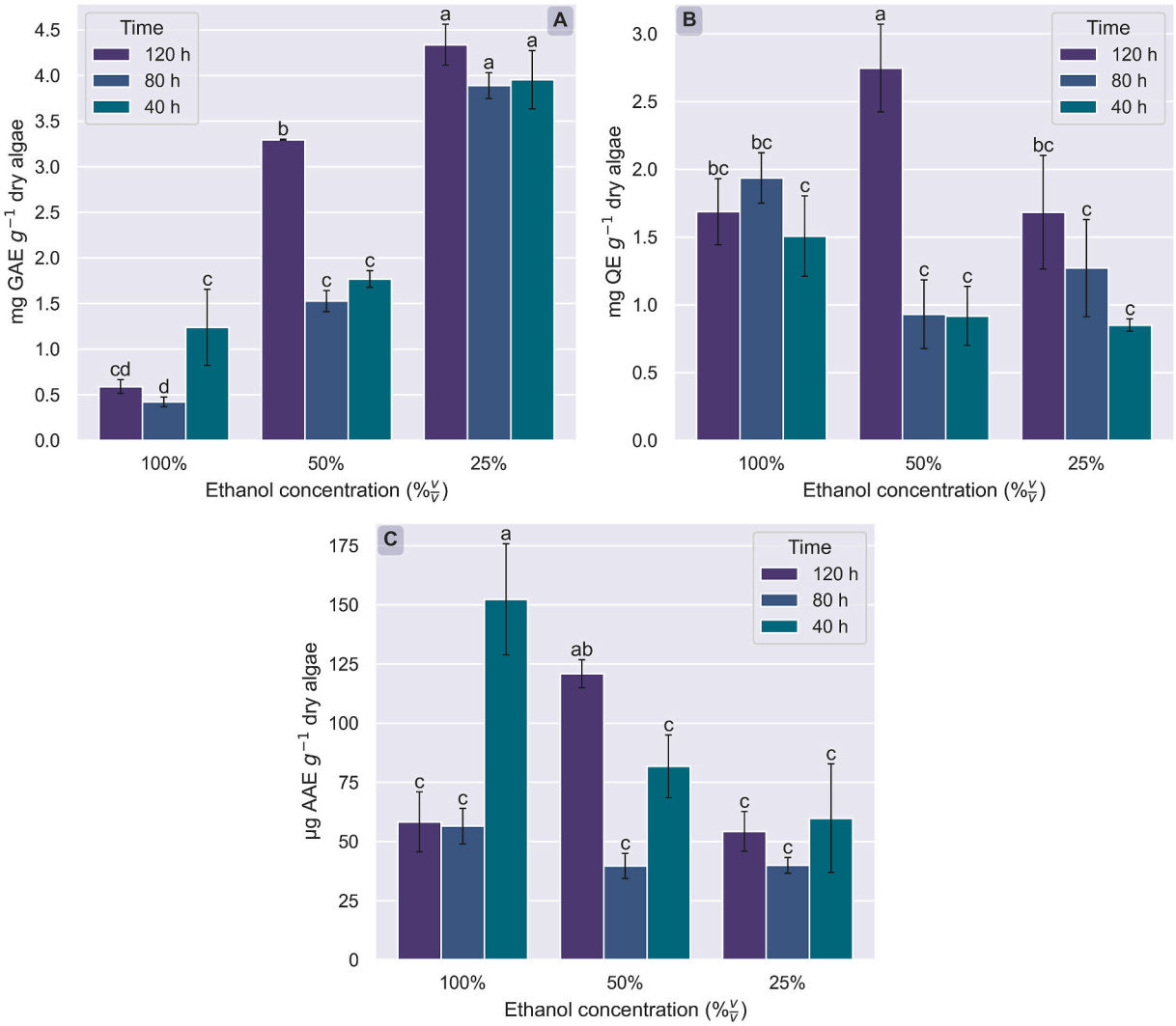

Fig. 1. Effect of extraction conditions on Total Phenolic Content (A), Total Flavonoid Content (B), and Total Antioxidant Activity (C), based on results from the experimental design analysis. The results are expressed in milligrams of quercetin equivalents per gram of dry *Sargassum* (µg QE g^{-1} DA) for TPC, in milligrams of gallic acid equivalents per gram of dry *Sargassum* (mg GAE g^{-1} DA) for TFC, and in ascorbic acid equivalents per gram of dry *Sargassum* (µg AAE g^{-1} DA) for TAA.

2.4.3. Fourier Transform Infrared Spectroscopy coupled with Attenuated Total Reflection analysis

Qualitative analysis of fucoidan structure was performed using Fourier Transform Infrared Spectroscopy coupled with Attenuated Total Reflection (IRAffinity-1S with QATR 10, Shimadzu, Japan). The frequency was set from 400 to 4000 cm^{-1} with a resolution of 2 cm^{-1} and scanning 40 times.

2.5. Statistical analysis and chromatographic data analysis

The chromatographic data were analyzed using Python v3.11.5. The results are presented as the mean ± standard deviation. Significant differences were determined using two-way analysis of variance (ANOVA) followed by Tukey's honest significant difference (HSD) test. Treatments with non-matching letters indicate statistically significant differences. Python v3.11.5 and R v3.6.1 were used for statistical analysis. The complete ANOVA can be seen in the Supplementary Information (Tables S1–S7). The Tukey's tables are provided in an additional Excel file.

3. Results and discussion

3.1. Sargassum green solvent extract evaluation

3.1.1. Antioxidant activity, total phenolic content and total flavonoid content

Fig. 1 shows the effects of ethanol concentration and maceration time on the TPC, TFC, and TAA. For TPC, ANOVA (Supp. Table S3)

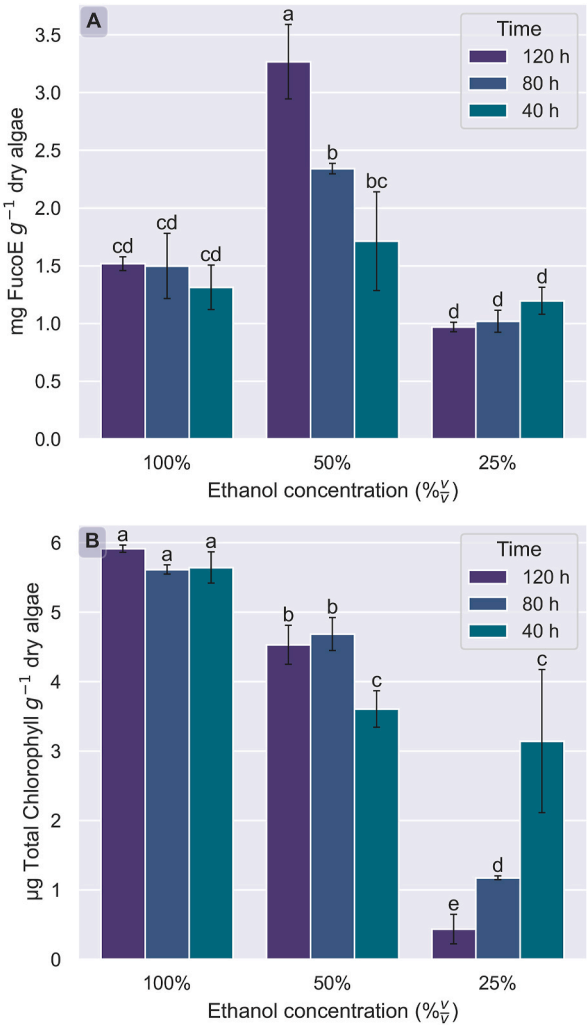

Fig. 2. Effect of Extraction Conditions on Total Carotenoid Content (A) and Total Chlorophyll Content (B), based on results from the experimental design analysis. Total chlorophyll content (TChlC) is expressed as milligrams of total chlorophyll per gram of dry *Sargassum* (mg Total Chl g^{-1} DA). Total carotenoid content (TCC) is expressed as micrograms of fucoxanthin equivalents per gram of dry *Sargassum* (mg FucoE g^{-1} DA).

showed that both variables and their interactions were significant ($p = 1.72 \cdot 10^{-9}$, $p = 0.000306$, and $p = 0.000356$, respectively), with ethanol concentration being the most significant. This was evident as samples treated with 25 % ethanol (3.88 ± 0.14–4.33 ± 0.22 mg GAE g^{-1} DA) (Fig. 1A) had the best results. In the case of TFC, ANOVA (Supp. Table S2) showed that maceration time ($p = 0.000704$) was a statistically significant factor influencing TPC in SLE liquid, followed by the interaction of the two factors ($p = 0.005081$). Ethanol concentration approached statistical significance ($p = 0.063725$), suggesting a potentially meaningful effect. The consequent Tukey test showed that treatments were not statistically significant from each other, nevertheless, the best sample was the one treated with 50 % ethanol for 120 h of maceration time ($2.74 \pm 0.0.32$ mg QE g^{-1} DA) (Fig. 1B).

Our findings align well with previous research that highlights the effectiveness of using a mixture of ethanol and water as the extraction solvent, leveraging the different polarities of both solvents to optimize the extraction process [23]. Specifically, our results corroborate the studies by Hao et al. (2023) [24] and Rashad et al. (2023) [23], which identified ethanol concentrations between 50 and 60 % v/v as optimal for flavonoid extraction. These ethanol concentrations enhance extractability by facilitating solvent penetration into the solid matrix, which is critical for effective extraction [23]. However, it is important to note that high ethanol

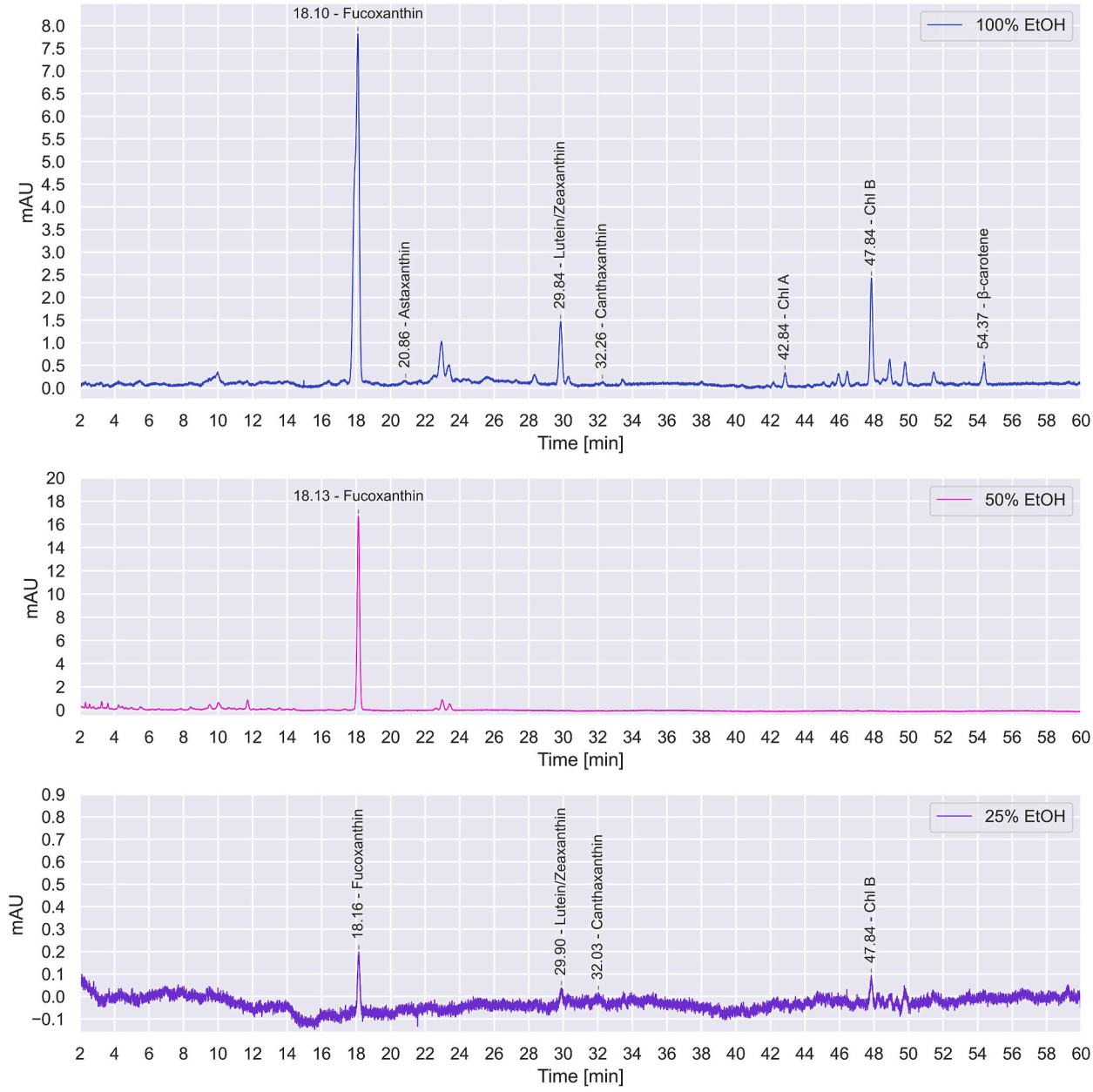

Fig. 3. UHPLC chromatograms of photosynthetic pigment separation from solid–liquid extraction liquid extracts at ethanol concentrations of 100 %, 50 %, and 25 %.

concentrations can lead to increased dissolution of alcohol-soluble pigments and lipophilic components [24], as observed in the coloration of the resulting SLE liquid: reddish-brown with 25 % ethanol, dark green with 50 % ethanol, and light green with 100 % ethanol (Supp. Fig. S1). This phenomenon indicates a shift in the composition of the extracted material, which may affect the purity and selectivity of target compounds. Both TFC and TPC yields obtained in our study are comparable to those reported in other studies using similar solvent extraction methods on *Sargassum* from the Mexican Caribbean [25,26]. This consistency across the studies supports the reliability of our extraction conditions.

In the case of TAA, ANOVA (Supp. Table S1) showed that ethanol concentration ($p = 0.002174$), maceration time ($p = 0.000266$), and their interaction ($p = 0.000551$) were highly statistically significant. The Tukey's test (Fig. 1C) revealed the 100 % EtOH for 40 h of maceration time had the best average result. The high antioxidant activity of the treatment with 50 % ethanol and 120 h maceration time can be attributed to the affinity of this solvent mixture to flavonoids, and to the fact that almost every group of flavonoids has the capacity to act as an antioxidant [27]. This result is directly linked to the high Total Flavonoid Content (TFC) observed under these conditions, as enhanced flavonoid extraction likely contributed to the increased antioxidant activity.

3.1.2. *Photosynthetic pigment quantification*

Fig. 2 shows the effects of ethanol concentration and maceration time on TCC and TChlC. Analysis of variance (ANOVA) (Supp. Table S4) showed that both variables and their interactions were significant for TCC ($p = 4.99{\cdot}10^{-6}$, $p = 0.00933$, and $p = 0.00309$, respectively). This can be seen within each ethanol concentration group, where time caused no differences. Therefore, the interaction of both factors caused the results to be very similar to each other. The samples treated with 50 % ethanol for 120 h exhibited the best results (Fig. 2A). While previous studies have found that the optimal ethanol concentration for extracting carotenoids from algae is typically between 60 and 75 % v/v [28], our best treatment achieved a yield of 3.26 ± 0.32 mg of Fucoxanthin per gram of dry sample

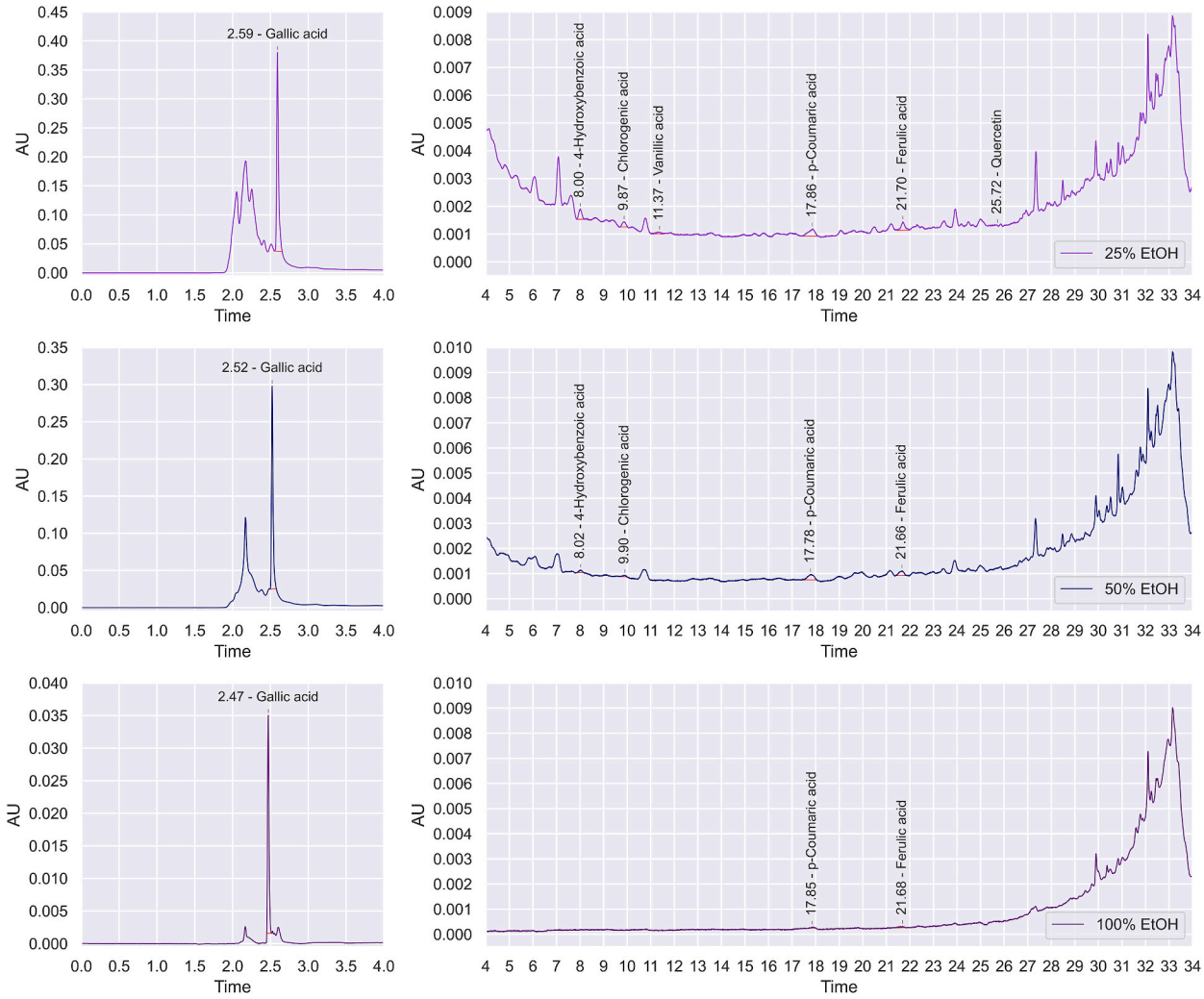

Fig. 4. Chromatograms of phenolic analysis showing the effect of ethanol concentration (25 %, 50 %, and 100 %, all treated for of 120 h maceration time) on compound separation.

using a lower ethanol concentration. Remarkably, carotenoid yield is comparable to the results reported in the literature for various *Sargassum* species [29–31], despite the use of a less concentrated ethanol solution.

For TChlC (Supp. Table S5), the ethanol concentration ($p = 8.98{\cdot}10^{-6}$) was highly statistically significant, whereas maceration time was not significant ($p = 0.32578$). The samples with the highest yield were those treated with 100 % (Fig. 2B) (5.61 ± 0.066–5.91 ± 0.053 mg Total Chl g^{-1} DA), however, those treated with 50 % EtOH yielded similar results. This can be attributed to the fact that chlorophyll is water-insoluble, but can be easily dissolved in organic solvents such as ethanol, acetone, ether, and chloroform [32]. The chlorophyll yield was better than that reported for *S. polycystum* subjected to maceration with methanol [33].

3.1.3. UHPLC-DAD

Total phenolic and pigment contents were determined to establish a baseline understanding of the sample compositions. Subsequently, detailed characterization was conducted using UHPLC-DAD. This step was essential for gaining a more detailed insight into the specific compounds present, allowing for precise breakdown of the phenolic and pigment profiles in the samples. The samples that had a maceration time of 120 h were analyzed because they presented, overall, the best results (higher averages).

The peak that can be observed before astaxanthin is most likely fucoxanthin (Fig. 3). Fucoxanthin is the main carotenoid produced in brown algae [34] and it is the major xanthophyll in *S. polycystum* [35]. The fucoxanthin peak showed a retention time of 18.13 ± 0.028 min. The elution order of the compounds is consistent with those in the literature that use very similar reverse-phase methodologies [35–37]. Fucoxanthin can be seen in all SLE treatments, however, in the 25 % EtOH it is barely noticeable. The remaining photosynthetic pigments are only visible with 100 % EtOH. Sizeable peaks were observed for lutein/zeaxanthin, chlorophyll *a*, chlorophyll *b*, and β-carotene. Diminutive peaks were detected for astaxanthin and canthaxanthin. Fucoxanthin, appreciable amounts of astaxanthin, canthaxanthin, lutein, zeaxanthin, and traces of β-carotene have been detected in *S. polycystum* [35].

As seen in Fig. 4, maceration performed with 25 % EtOH reached a higher TPC, and more compounds could be identified than with 50 % EtOH and 100 % EtOH (Fig. 4). The quantification results are presented in Table 4. All the analyzed compounds have been reported in *S. muticum*, *S. hornschuchii*, *S. fusiforme*, *S. horneri,* and *S. thunbergii* [38–40]. Results presented kg^{-1} DA were obtained with the solid to liquid ratio (solvent/dry algae) used in the SLE procedure (section 2.3). Gallic acid (6.14 ± 0.09 mg kg^{-1} DA) and quercetin (0.36 ± 0.55 mg kg^{-1} DA) content were better than those obtained for *S. muticum* subjected to maceration with water [40], due to a much longer maceration time (100 min vs 120 h). To reinforce our understanding of the compounds in liquid SLE, future studies could employ advanced analytical techniques such as Mass Spectrometry (MS), Nuclear Magnetic Resonance (NMR), Gas Chromatography (GC), and Liquid Chromatography (LC) coupled with MS. These methods would allow for more detailed identification and characterization of the diverse compounds present in dried *Sargassum*, enhancing the scope and precision of our research.

3.1.4. UV absorbance

UV absorbance spectroscopy was employed because of its ability to preliminarily identify and quantify components in extracts based on their characteristic absorption peaks. This method provides a means of estimating concentrations, allowing for comparisons between samples and assessing the efficacy of the extraction methods. Additionally, it aids in monitoring the purity of extracts and offers a cost-effective approach for preliminary analysis, setting the stage for more detailed subsequent investigations. The effects of ethanol concentration can be observed in groups that are separated from each other (Fig. 5). In this regard, treatments with 120 h of maceration time reached the highest overall absorbance, with 25 % ethanol concentration producing the best results. This shows that the phenolic content is the most important factor for UV absorbance. The extracts macerated for 120h maceration time had the highest average UV absorbance. However, in the last part of the UV-A range (seen 380–400 nm) the 50 % ethanol 120 h treatment showed an increased absorbance, which was likely due to the affinity of the mixture for flavonoids and carotenoids. Phenolics, flavonoids, and carotenoids are believed to be the main contributors to the UV absorption. Fucoxanthin absorbs UVB, UVA, and visible light [41]. It protects against oxidative stress induced by UV radiation, both *in vitro* and *in vivo* [42,43]. Fucoxanthin showed acceptable photodegradation (dose 27.5 J/cm^2:5.8 % UVB and 12.5 % UVA absorbance reduction) when added to sunscreen at 0.5 % (w/v), whereas it increased by 72 % of the total sunscreen UV absorption spectra [41]. TCC content suggests that the aqueous extracts of *Sargassum*

Table 4
Quantification of phenolic compounds in Solid-Liquid Extraction samples, all treated for of 120 h maceration time.

	25 % EtOH		50 % EtOH		100 % EtOH	
	μg mL^{-1}	mg kg^{-1} DA	μg mL^{-1}	mg kg^{-1} DA	μg mL^{-1}	mg kg^{-1} DA
4-Hydroxybenzoic acid	1.03 ± 0.056[b]	6.16 ± 0.33	0.89 ± 0.058[b]	5.37 ± 0.35	ND	ND
Chlorogenic acid	4.48 ± 0.096[b]	26.91 ± 0.58	4.39 ± 0.10[b]	26.39 ± 0.60	ND	ND
Ferulic acid	0.74 ± 0.025[b]	4.48 ± 0.15	0.71 ± 0.025[b]	4.30 ± 0.15	0.64 ± 0.025[b]	3.87 ± 0.15
Gallic acid	12.84 ± 0.20[a]	77.03 ± 1.21	9.45 ± 0.29[a]	56.75 ± 1.72	2.68 ± 0.078[b]	16.07 ± 0.47
p-Coumaric acid	1.09 ± 0.027[b]	6.55 ± 0.16	1.07 ± 0.024[b]	6.42 ± 0.14	1.00 ± 0.024[b]	6.02 ± 0.14
Quercetin	2.17 ± 0.087[b]	13.03 ± 0.52	ND	ND	ND	ND
Vanillic acid	BD	BD	ND	ND	ND	ND

a <Less than the Limit of Quantification.

b <Less than the Limit of Detection; BD: below detection; ND: not detected.

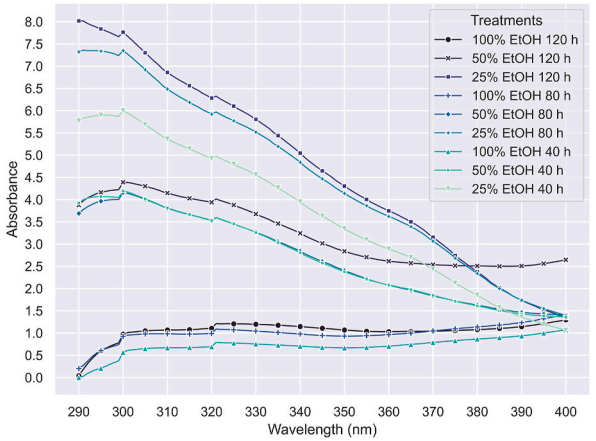

Fig. 5. UV absorbance of treatments from the SLE procedure (markers only for visual purposes). Measurements were multiplied by the dilution factor to obtain the absorbance values for the original solutions.

exhibit similar results. Coumaric acid, ferulic acid, gallic acid, and quercetin exhibit strong UV-B absorption [44,45]. Studies on brown algae have reported high levels of phenolics, characterized by extremely high biological activity, comparatively higher phenolic content, and more active antioxidants than green and red algae.

This suggests that *Sargassum* extracts may be suitable for use in sunscreen formulations, with the potential to also grant them nutraceutical properties, with, for example, antimicrobial, antioxidant, anti-inflammatory and antiproliferative effects, which have all been reported in *Sargassum* spp [46]. Further evaluation is needed to quantify the magnitude of the reported effects of aqueous extracts of *Sargassum* in sunscreen or even other cosmetic formulations. Flavonoids and carotenoids can be extracted or concentrated to increase UV-A absorbance. Sunscreen formulations can be functionally enhanced with *Sargassum,* and the chemical/physical sunscreen

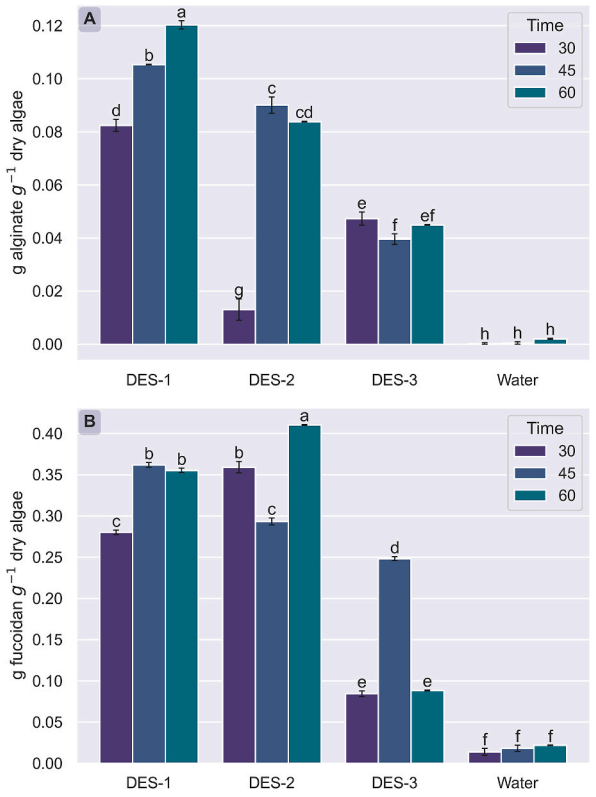

Fig. 6. Effect of extraction conditions on alginate (A) and crude fucoidan (B) yields based on the results of the experimental design analysis. Alginate yield is expressed as grams of alginate per grams of dry algae (g alginate g^{-1} dry algae). Fucoidan yield is expressed as grams of fucoidan per grams of dry algae (g fucoidan g^{-1} dry algae).

filter content can be reduced.

3.2. Natural deep eutectic solvent-based ultrasound-assisted extraction of polysaccharides

3.2.1. Yield of crude polysaccharides

Very few reports have been published regarding the optimal conditions for NADES-UAE of polysaccharides in *Sargassum*. The effects of NADES type and sonication time were evaluated using deodorized *Sargassum* from the solid fraction of samples treated with 25 % ethanol and 120 h of maceration. This solid fraction was selected because the liquid fraction had shown the highest concentration of UV-absorbing compounds, as evaluated in the previous section. The cascade of green solvents enables subsequent purifications and extractions to be more efficient, maximizing yield while utilizing the raw material to its full potential. Since most of the phenolic compounds and photosynthetic pigments were removed from the solid fraction, it was well-suited for the NADES-UAE procedure. To the best of our knowledge, the specific combinations of methods used in this study have not been previously applied to extract alginate and fucoidan from *Sargassum* spp. For both alginate and fucoidan (Supp. Tables S6–S7), the type of NADES, sonication time, and their interactions were highly statistically significant ($p < 0.001$). The physicochemical properties of each NADES will affect the yield of the targeted substances, especially polarity, as it is directly correlated to the solubilizing capacity of NADESs for extracting the target compounds [6].

The best treatment for alginate yield was DES-1 (urea) with a 60 min extraction time (Fig. 6A) with an extraction yield of 0.1203 ± 0.0025 g alginate g^{-1} DA. The yield is fairly similar to that obtained from *S. horneri* with DES-UAE using 1,2-propanediol-choline chloride under the same experimental conditions [22]. The yield was comparable to that obtained in *S. muticum* using the traditional method: pretreatment formaldehyde, acid, and base extraction [47]. However, its performance was lower than that of other sources using the traditional method, but with much longer extraction times (6 or 24 h for each stage) and higher temperatures (90 °C) [48,49]. Nevertheless, the use of NADES in combination with ultrasound produced much higher yields than just UAE alone [8,50] and, in other cases, it was comparable [51] or even superior [52]. This is believed to be due to differences in polysaccharide content among the *Sargassum* species. Additionally, the season of recollection affects the concentration of bioactive compounds and the alginate content [51]. The alginate yield could benefit from the use of alkaline conditions during the NADES-UAE procedure or by increasing the extraction time or temperature; however, this could cause some degradation via depolymerization of the alginates [52].

For crude fucoidan yield, the best treatment was DES-2 (glycerol) with a 60 min extraction time (Fig. 6B) with a yield of 0.4103 ± 0.0042 g fucoidan g^{-1} DA. The higher extraction yield of DES-2 is due to the steric hindrance of the three hydroxyl groups of glycerol,

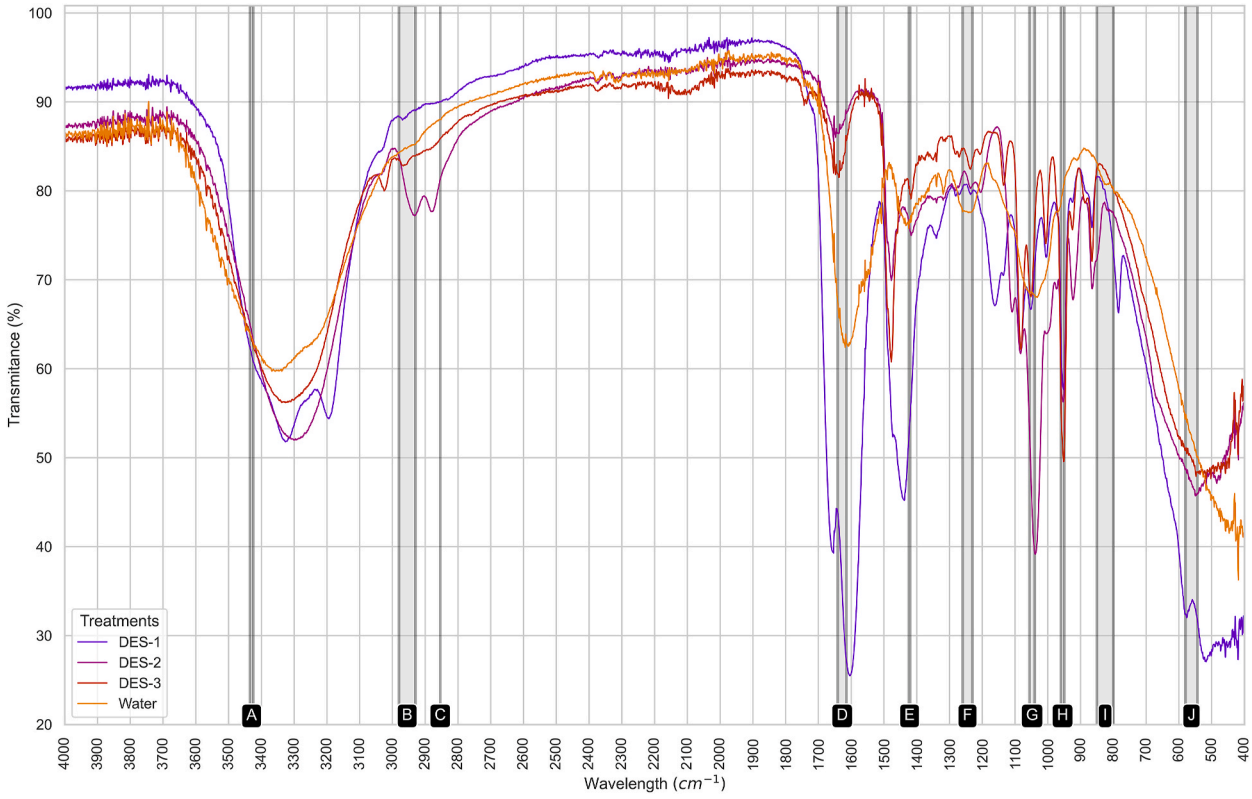

Fig. 7. FTIR-ATR spectrum of fucoidan from the deep eutectic solvent-based ultrasound-assisted extraction procedure. Letters A to I represent the characteristic bonds of fucoidan from *Sargassum* spp [11,58–63].

which can greatly weaken the interactions between the polysaccharide and the chloride anion [6]. The yield of crude fucoidan was higher than that previously reported for several techniques such as DES-UAE with 1,2-propanediol, microwave-assisted extraction (MAE), subcritical water hydrolysis, and enzyme-assisted extraction (EAE), not only for *Sargassum* spp. but also for other genera of brown algae (incl. *Fucus* spp., *Saccharina japonica,* and *Nizamuddinia zanardinii)* [6,8,22,53–57]. Specifically for *Sargassum* spp. crude fucoidan was obtained at 0.0714 g g^{-1} from *S. angustifolium* subjected to UAE [50], at 0.27 g g^{-1} yield from *S. binderi* subjected to UAE [52], at 0.2713 ± 0.0219 g g^{-1} from *S. confusum* subjected to EAE (Celluclast) [58], at 0.0625 g g^{-1} from *S. henslowianum* subjected to UAE [57], at 0.1131 g g^{-1} from *S. horneri* subjected to DES-UAE [22], at 0.135 g g^{-1} from *S. horridum* subjected to SLE [55], at 0.109 g g-1 from *S. muticum* subjected to a form of the traditional method [47] and, at 4.78 ± 0.21 % and 4.73 ± 0.13 % from *S. siliquosum* subject to UAE and MAE, respectively [8]. The combination of NADESs with UAE allowed the highly efficient extraction of fucoidan.

3.2.2. Fourier Transform Infrared Spectroscopy coupled with Attenuated Total Reflection analysis

Samples from treatments with an extraction time of 60 min were analyzed due to their higher yields. Fig. 7 illustrates the characteristic peaks of fucoidan from *Sargassum* spp. found in the literature, meanwhile, Fig. 8 shows the position of the characteristic peaks of fucoidan extracted with the NADES-UAE procedure. Across all NADES-UAE treatments, the extracted fucoidan demonstrated FTIR-ATR spectra consistent with those extracted from *S. aquifolium* [59], *S. binderi* Sonder [60], *S. cristaefolium* [61], *S. ilicifolium* Turner [62], *S. natans* [63] and *S. polycystum* [11]. The intensities of the bands at 3435-3425 cm^{-1} (Fig. 7A) are characteristic of the O-H group of the polysaccharide [11,59,60,62]. However, there are some notable differences. All treatments showed a blue shift (decrease) in the wavelength of the characteristic peak at 3435-3425 cm^{-1}, indicating a change in the conformation of the O-H of the polysaccharide. Nevertheless, the peaks are still in the range for OH bonds for fucoidan in other algae species (3600-3300 cm-1) (Liu et al., 2018). DES-1, DES-2 and DES-3 showed a red shift (increase) for the peak in the range of 1424-1420 cm^{-1}, indicating a possible change in the conformation of the C=O and the CH bonds of the polysaccharide. Peaks centered at 2980-2930 and 2854 cm^{-1} (Fig. 7B and C) confirm the presence of aliphatic C–H stretching and CH stretching pyran ring C-6 group of fucose and galactose [11,59,62,64]. Peaks at 1641-1614 cm^{-1} (Figs. 7D) and 1424-1420 cm^{-1} (Fig. 7E) indicate C=O stretching vibration of O–acetyl group and CH vibration of the polysaccharides composed of fucose, D-glucose, D-mannose, D-xylose, and galacturonic acid. Peaks at 1260-1250 cm^{-1}, 1230 cm^{-1} (Figs. 7F), and 1056-1040 cm^{-1} (Fig. 7G) indicate the S=O stretching vibration, S=O stretching of alkyl sulfoxide, and anomeric region of carbohydrate. Peaks at 950–960 cm^{-1} (Fig. 6H) show the presence of the asymmetrical stretching vibration of the C–O–S bonds [60,65]. Peaks at 850-800 cm^{-1} (Fig. 7I) indicate C–O–S [11,59,60,62]. The peaks at 580-543 cm^{-1} (Fig. 7J) represent the C–O–S secondary axial sulfate group at C-4 of the fructopyranose residue [11,61]. Finally, the peaks at 580-543 cm^{-1} shifted to 519 cm^{-1} in the case of DES-1 and dH2O, suggesting a change in the C-O-S bonds of fructopyranose.

4. Conclusions

4.1. Main conclusions

Samples treated with 25 % ethanol showed the highest total phenolic content, which was strongly correlated with UV absorbance, particularly in the UV-B range, suggesting the potential of *Sargassum* extracts as sunscreen filters. In contrast, samples treated with 50 % ethanol exhibited higher UV-A absorbance, likely due to the solvent's higher affinity for flavonoids and carotenoids. Further evaluation and quantification of the phenolic compounds in *Sargassum*, particularly for sunscreen formulations, is recommended. The Deep Eutectic Solvent (DES) method achieved a significantly higher extraction yield of crude fucoidan compared to previous reports. Although the FTIR-ATR spectra of the extracted fucoidan aligned with those of *Sargassum* spp., further purification and analysis are required to confirm the preservation of its bioactive properties. Alginate extraction using DES-Ultrasound Assisted Extraction yielded similar results to traditional methods, demonstrating the efficiency of this green approach. The potential to utilize *Sargassum* as both a sunscreen filter and a source of high-value polysaccharides could greatly benefit Mexico's coastal regions, which are impacted by annual *Sargassum* blooms, by transforming the biomass into valuable resources.

4.2. Further research

Due to the limited data found in literature, only assumptions could be made in regards to the optimal ratio of water to ethanol for the extraction of carotenoids and flavonoids; more research is needed to stablish an optimal mixture ratio. Further studies will aim to identify and characterize more compounds in Sargassum extracts, enhancing our understanding of their role and efficacy in dermatologic and photoprotective applications.

CRediT authorship contribution statement

Santiago Guerrero-Higareda: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Conceptualization. **Danay Carrillo-Nieves:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used Paperpal (https://paperpal.com/) to improve the clarity and linguistic

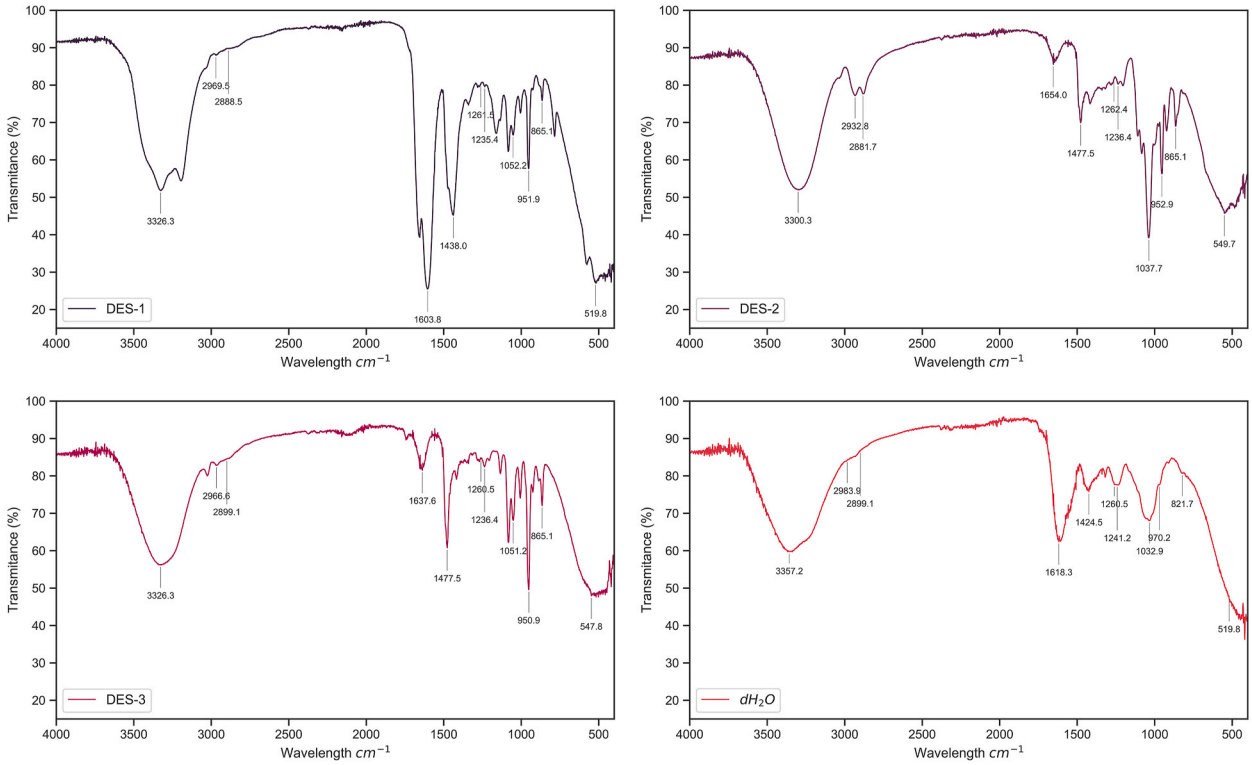

Fig. 8. FTIR-ATR spectrum of fucoidan obtained from NADES-UAE procedure with characteristic peaks.

excellence. After using this tool, the authors reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2025.e41810.

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