



Selective extraction of high-value metabolites from the red macroalga *Cryptopleura ramosa*: The influence of using bio-solvents[☆]

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ABSTRACT

Despite its abundance in coastal environments, the red macroalga *Cryptopleura ramosa* (*C. ramosa*) remains largely underexplored. Consequently, detailed information on its chemical composition is still scarce. In this work, a comprehensive solvent-dependent extraction approach was performed to evaluate the potential of *C. ramosa* as a source of bioactive compounds within sustainable blue biorefinery frameworks. A multi-solvent extraction strategy employing bio-solvents, namely water, ethanol, and the ammonium lactate was applied to selectively recover metabolites across a wide polarity range. The resulting extracts were characterized by UV-Vis spectrophotometry, UHPLC-DAD-ESI/MS and GC-FID (monosaccharide composition determination), but also for their antioxidant activity through ABTS radical scavenging assay. Clear solvent selectivity was observed. Water preferentially extracted highly polar glycosylated compounds, among which syringetin-3-O-galactoside was the predominant metabolite (relative abundance (RA) = 16.97%). Ethanol extract enriched moderately polar and lipophilic compounds, including polymethoxylated flavonoids, notably casticin (RA = 37.61%) and exhibited the highest antioxidant activity (379.42 $\mu\text{M TEAC}\cdot\text{mL}^{-1}$). Ammonium lactate proved to be efficient on the selective recovery of polar and UV-protective metabolites, including mycosporine-like amino acids, flavonoid glycosides (RA = 57.51%) and violaxanthin (RA = 76.31%), highlighting its potential as bio-based extraction solvent. Monosaccharide analysis confirmed that the structural matrix of *C. ramosa* is dominated by sulfated galactans, as evidenced by its high galactose content (> 80%). Thus, the present study provides the first comprehensive chemical insight into *C. ramosa*, highlighting the potential of solvent-dependent strategies to selectively recover valuable metabolites from *C. ramosa*.

1. Introduction

Marine macroalgae serve as both structural and biological reservoirs of valuable bioactive compounds, which have attracted increasing interest in their commercial applications, not only in cosmetics, health, and food industries, but also in the production of clean energy [1,2]. Their ability to synthesize diverse metabolites at a rapid growth rate

across freshwater, terrestrial, and marine environments has established them as important natural sources of carbohydrates, pigments, lipids, phenolic compounds, bioactive peptides, proteins and other high-value compounds [1,3]. Among seaweeds, red algae (*Rhodophyta*) are particularly rich in phycobiliproteins, sulfated polysaccharides [4] and a wide range of secondary metabolites such as phenolics, terpenoids, glycosides and mycosporine-like amino acids (MAAs). All these compounds exhibit

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a wide spectrum of biological activities including antioxidant, antimicrobial, anti-inflammatory, anti-aging and UV-protective effects [4–6]. In recent years, the growing demand for sustainable biomaterials and marine-derived bioactive molecules has intensified efforts to diversify exploitable marine resources and to develop scalable and eco-efficient production systems [7]. This development aligns with broader strategies promoting a circular and blue bioeconomy, which emphasizes the use of renewable and regenerative marine biomass [8]. Within this context, macroalgae are increasingly establishing themselves as fundamental pillars of the blue bioeconomy, offering versatile solutions ranging from sustainable agriculture and carbon sequestration to energy production and compounds of high value for human health [9]. In Europe, this potential is being driven by initiatives that promote active collaboration between academia, industry, and the public sector, aiming to transform underutilized marine biomass and industrial waste into innovative new bioproducts through circular economy models [9]. Despite these advances, many macroalgal species remain understudied, due to their unexplored biochemical profiles. One example is *Cryptopleura ramosa* (*C. ramosa*), a thin membranous red seaweed within family Delesseriaceae, which commonly grows on intertidal and subtidal rocky substrates in costal environments of the NE Atlantic [10]. Although ecological and taxonomic studies describe *C. ramosa* as an abundant biomass [11], its commercial potential is yet to be acknowledged. This underutilization is likely a consequence of the scarce information on scientific literature concerning the chemical composition of this seaweed. A search using Scopus database (accessed in January 2026) revealed only 14 scientific publications addressing *C. ramosa*, most of them on taxonomy and ecology, with no data having been reported to date on its chemical or biochemical profile [7–20]. This pronounced knowledge gap highlights the need for a detailed chemical characterization, which is essential to identify bioactive compounds and support a targeted extraction, which consequently will help rationalize more disruptive valorization strategies.

In solid-liquid extraction processes, solvent selection plays a crucial role in determining extraction efficiency and selectivity, influenced by the chemical affinity between the solvent and the solute, which is determined by their polarity and solubility profiles [21]. To achieve a comprehensive chemical analysis, it is paramount to employ a multi-solvent approach that scopes the polarity spectrum of the biomass being screened; nevertheless, this approach is normally disregarded. Conventional solvents such as water and ethanol are typically used [21,22]. Water, being highly polar, effectively targets hydrophilic compounds such as carbohydrates, phycobiliproteins, and some peptides [23]. At the same time, ethanol is a versatile solvent that extracts both polar and less polar compounds, such as carotenoids, chlorophylls, and terpenoids [22]. However, to maximize compound recovery and enable broader biomass valorization, increasing attention has been directed toward alternative solvents, including ionic liquids (ILs), (deep) eutectic solvents (DESs), and more recently, bio-based solvents [23–25]. Bio-based solvents, also referred to as bio-solvents, are defined as solvents derived from renewable biological feedstocks, such as biomass, agricultural residues, or microbial fermentation, as opposed to petrochemical-derived conventional organic solvents. They are characterized by reduced environmental impact, improved biodegradability, and lower toxicity profiles, and encompass categories like terpene-derived solvents, and organic acid-based systems [24–26]. These bio-based systems offer improved extraction efficiency while aligning with sustainability principles [26,27]. Among them, ammonium lactate is considered a bio-based solvent [28], and its classification is supported by green chemistry criteria that distinguish it from the other alternative solvents. Lactate-derived solvent systems have garnered growing interest due to their renewable origin, predominantly produced via microbial fermentation of carbohydrate feedstocks, including agro-industrial residues and lignocellulose biomass, and their ability to solubilize complex classes of compounds derived from biomass [29,30]. Moreover, lactic acid, the precursor of ammonium lactate, holds Generally Recognized As

Safe (GRAS) status granted by the US Food and Drug Administration [31]. Unlike conventional ILs, which are typically derived from petrochemical precursors and may raise ecotoxicity concerns [23,24] or DESs, which require stoichiometric formulation of at least two components [24], ammonium lactate is commercially available as a ready-to-use aqueous solution with an established safety profile in cosmetic and pharmaceutical formulations [32,33]. These green chemistry characteristics, such as renewability and low toxicity [34], position ammonium lactate as a bio-based solvent of industrial relevance [25,30], yet it remains underexplored for the extraction of bioactive compounds from marine biomass. To date, no comprehensive study has investigated solvent-dependent chemical profiling of *C. ramosa* using this multi-solvent approach. Only one scientific publication has reported its polysaccharide fraction, detailing the isolation of a low-molecular-weight sulphated galactan derived from the cold-water extraction of tetrasporic *C. ramosa*, yielding 2.8% [35]. This polysaccharide consisted mainly of galactose (84.6%), with minor amounts of 6-O-methylgalactose (7.9%), xylose (4.6%), 3,6-anhydrogalactose (1.6%), and glucose (1.2%), and contained exclusively bound sulfate esters (35.2%) [35]. Although this study identified potential antiviral activity of *C. ramosa*'s sulphated polysaccharides against the herpes virus, a notable lack of information on other compound classes remains to be addressed, as well as the influence of different extraction solvents on the overall chemical profile of *C. ramosa*.

By recognizing this species as a potentially valuable, yet underexplored, marine bioresource, the present study aims to provide the first comprehensive study into its chemical composition using a solvent-dependent extraction approach. Three solvents (water, ethanol, and ammonium lactate) were selected to target different classes of compounds based on their polarity and solubility. The resulting liquid extracts were analyzed by UV–Vis spectrophotometry and Ultra-High-Performance Liquid Chromatography coupled with Diode Array Detection and Mass Spectrometry (UHPLC–DAD/MS) to identify major metabolites. In parallel, the monosaccharide composition of the biomass was determined to clarify the presence and composition of structural polysaccharides.

2. Materials and methods

2.1. Biomass collection and pre-treatment

Biomass of *C. ramosa* was sampled from a pilot integrated multi-trophic aquaculture (IMTA) system operated at ECOMARE, a research infrastructure of the University of Aveiro located in Gafanha da Nazaré, Portugal, in February 2025. Briefly, this red macroalga was produced in a 10.000-L recirculated aquaculture system (RAS) housing gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*) as fed species; the red seaweed *C. ramosa* was used as an extractive species to remove dissolved inorganic nutrients (namely nitrogen and phosphorous) available in the effluent water being supplied by gravity from the fish tanks to the seaweed tanks before effluent water was filtered and pumped back to the tanks housing the fish; salinity was kept stable at 35 ± 1 and temperature varied between 18 (min) and 23 (max) °C. Natural sunlight and photoperiod were used to produce the macroalgae, as the IMTA-RAS system was housed inside a greenhouse. The biomass collected was a composite sample of *C. ramosa* from three 250-L tanks strongly aerated and stocked with this red seaweed in the IMTA-RAS system described above. After sampling, all *C. ramosa* biomass was washed with distilled water, flash-frozen with liquid nitrogen, and grounded in a coffee grinder to disrupt the cell walls, to obtain smaller particles of biomass. The resulting powder was subsequently frozen at -20 °C until further use.

2.2. Chemicals

Ethanol (C₂H₅OH, 99.8%, CAS 64–17-5) and 1-methylimidazole

($C_4H_6N_2$, 99%, CAS 616–47-7) were purchased at Thermo Scientific. Ammonium lactate ($C_3H_9O_3N$, 20% w/w in water, CAS 515–98-0) and 2-deoxyglucose ($C_6H_{12}O_5$, $\geq 99\%$, CAS 154–17-6) were acquired from Sigma-Aldrich. Distilled water was obtained by double distillation, passed by a reverse osmosis system. Formic acid, acetonitrile and methanol were used for UHPLC-DAD-ESI/MS analysis. Trifluoroacetic acid (CF_3CO_2H , 99%, CAS 76–05-1) was purchased at AlfaAesar. 4-methyl-morpholineborane ($C_5H_{14}BNO$, 95%, CAS 15648–16-5) was acquired from AstaTech. Acetic acid glacial (CH_3COOH , $>99.5\%$, CAS 64–19-7) and acetic anhydride ($(CH_3CO)_2O$, CAS 108–24-7) were purchased from Carlo Erba. Dichloromethane (CH_2Cl_2 , $\geq 99.9\%$, CAS 75–09-2) was acquired from Honeywell.

For the ABTS assay, potassium persulfate ($K_2S_2O_8$, 99%, CAS 7727-21-1) was obtained from Thermo Scientific, ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), CAS 30931–67-0) was purchased from Panreac, Trolox (97%, CAS 53188–07-1) was acquired from Sigma-Aldrich.

2.3. Solid-liquid extraction

The solid-liquid extraction step was performed using a methodology adapted from Martins et al. (2021) [36]. The extraction process utilized three specific solvents [pure ethanol, distilled water, and ammonium lactate (20% w/w water)] which were selected regarding their distinct chemical characteristics and affinities. This approach was used to investigate the range of compounds that can be effectively extracted. The fresh biomass was homogenized with the solvent in a Rotator Multi Bio RS-24 under constant vertical rotation at 80 rpm and protected from light. To obtain extracts at a concentration suitable for spectrophotometric and direct chromatographic characterization without the need for pre-concentration steps, the solid-to-liquid ratio considered for this work was higher than the methodology proposed by [36]. The extraction was performed using a solid-liquid ratio on $0.1 \text{ g}_{\text{fresh biomass}} \cdot \text{mL}_{\text{solvent}}^{-1}$ for 30 min at room temperature (25–30 °C) in the dark [36]. The samples were centrifuged in a MIKRO 200 centrifuge (15,000 rpm) for 30 min at room temperature. The supernatant (initial extract) was collected and further analyzed.

2.3.1. Characterization of liquid extracts

2.3.1.1. UV–Vis spectroscopy analysis. The UV–Vis absorption spectra of the liquid supernatants obtained from the solid-liquid extraction step were determined using a SpectraMax® iD3 microplate reader, covering a wavelength range of 230 to 700 nm. The blanks used for reference were the pure solvents involved in the extraction process, namely water, ethanol, and ammonium lactate. Both ethanol and ammonium lactate extracts were diluted to 10 times their original concentration, whereas the water extract was diluted to 2 times to perform more adequately

UV–Vis readings (Fig. 1).

2.3.1.2. UHPLC-DAD-ESI/MS analysis. For the ultra-high-performance chromatography coupled to photodiode-array detection and electrospray ionization/ion trap mass spectrometry (UHPLC-DAD-ESI/MS) the sample solutions were filtered through a 0.2 mL Nylon membrane (Whatman). The analysis was performed using a Thermo Scientific Ultimate 3000RSLC (Dionex) equipped with a Dionex UltiMate 3000 RS diode array detector and coupled to a mass spectrometer. The column used was a Thermo Scientific Hypersil Gold C18 column (1000 × 20 mm) with a particle size of 1.9 μm and its temperature was maintained at 32 °C. The mobile phase was composed of (A) 0.1% formic acid in water (v/v) and (B) acetonitrile/methanol (70,30 – v/v), both degassed and filtered before use. The flow rate was 0.2 mL/min. The solvent gradient started with 85% of solvent B over 3.9 min, followed by a 6.1 min upgrade until 100% of solvent B and maintained during 25.3 min, then in 31 min lower to 85% solvent B, which was maintained during 38 min. The injection volume was 2 μL . UV–Vis spectral data were gathered in a range of 250–680 nm and the chromatographic profiles were documented at 260, 305, 450, 650 nm. The mass spectrometer used was an LTQ XL linear ion trap 2D equipped with an orthogonal electrospray ion source (ESI). The equipment was operated in positive-ion mode with electrospray ionization source of 5.00 kV and ESI capillary temperature of 275 °C. The full scan covered a mass range of 50–2000 m/z . Compounds were putatively identified by comparing the observed $[M + H]^+$ (m/z) values with theoretical masses and literature data.

The Relative Abundance (RA (%)) of each compound identified was calculated through Eq. 1.

$$RA(\%) = \frac{\text{Area of the peak}}{\text{total area of all peaks in the chromatogram}} \times 100 \quad (1)$$

2.3.1.3. Apparent inter-extract selectivity and enrichment factor. Since the UHPLC-DAD-ESI/MS data were expressed as relative abundance (RA, %) rather than absolute concentrations, true extraction efficiencies could not be calculated. Therefore, the solvent performance was evaluated using two semi-quantitative parameters: apparent inter-extract selectivity and enrichment factor.

For compounds detected in more than one extract, the apparent inter-extract selectivity of each solvent was calculated according to Eq. 2:

$$\text{Selectivity (Si)}(\%) = \frac{RA_{i,s}}{\sum RA_{i,\text{all extracts}}} \times 100\% \quad (2)$$

where $RA_{i,s}$ is the relative abundance of compound i in a given extract, and $\sum RA_{i,\text{all extracts}}$ is the sum of the relative abundance values of the same compound across all extracts.

The enrichment factor (EF) – Eq. 3 – was calculated for compounds

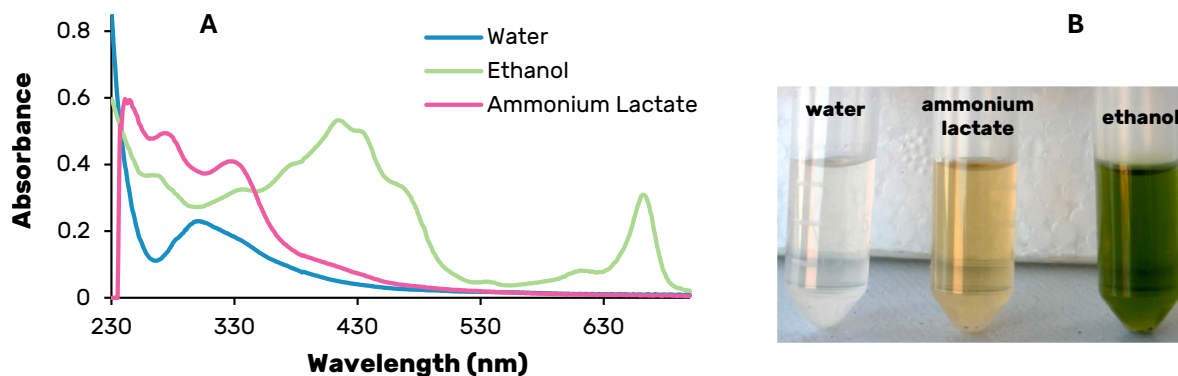


Fig. 1. (A) UV–Vis spectra of different crude liquid extracts obtained from *C. ramosa* by using different solvents; (B) Non-diluted liquid extracts obtained from the solid-liquid extraction process with each one of the three solvents selected.

detected in more than one extract as:

$$EF = \frac{RA_{i,s1}}{RA_{i,s2}} \quad (3)$$

where $RA_{i,s1}$ and $RA_{i,s2}$ correspond to the relative abundance of compound i in two different extracts.

2.3.1.4. Antioxidant activity through ABTS methodology. The ABTS (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)) assay measures the capacity of antioxidants to inhibit the absorbance of the radical cation 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonate) ($ABTS^{\bullet+}$). This radical cation exhibits a characteristic long-wavelength absorption spectrum, with a primary absorption maximum at 415 nm and secondary maxima at 660, 734, and 820 nm [37]. The ABTS scavenging assay was conducted in 300-well microplates, following the methodology described by Gonçalves et al. (2009) with some adjustments [38]. This method quantifies the direct production of the diammonium salt radical cation ($ABTS^{\bullet+}$) with a blue-green color, resulting from the reaction between ABTS and potassium persulfate ($K_2S_2O_8$). Following a 16-h incubation of the mixture at room temperature in the dark, a freshly prepared $ABTS^{\bullet+}$ + working solution is diluted with a solvent (water/ethanol/methanol) to achieve an absorbance of $0.70 (\pm 0.02)$ at 734 nm. Subsequently, 270 μ L of $ABTS^{\bullet+}$ + is added to 30 μ L of the sample, Trolox, or solvent (as a blank). The mixture is then incubated for 5 min at 30 °C, after which the absorbance at 734 nm is measured using a UV-Vis microplate reader, SpectraMax® iD3.

The scavenging activity was quantified as the percentage reduction in absorbance, measured against the control sample, where $A_{control}$ and A_{sample} represent the absorbances at 734 nm of the control and sample, respectively - Eq. (4).

$$ABTS \text{ scavenging } (\%) = (A_{control} - A_{sample}) \times \frac{100}{A_{control}} \quad (4)$$

Regression equations between ABTS scavenging and Trolox concentration were calculated, and the results expressed using the standard curve for each experiment. The results are expressed in $\mu M_{Trolox} \text{ mL}_{sample}^{-1}$.

2.3.2. Characterization of biomass (*C. ramosa*)

2.3.2.1. Carbohydrates composition analysis. For sugar analysis, the pretreated initial fresh algae and the biomass after the solid-liquid extraction were frozen at -80°C for approximately 24 h and then freeze-dried in a Lyoquest -85 plus® freeze dryer. Considering that *C. ramosa* is a red alga, sugar composition was determined following a reductive hydrolysis followed by acetylation, using borane-4-methylmorpholine complex (MMB) as a reductive agent to preserve 3,6-anhydrogalactose residues [39]. The freeze-dried samples of *C. ramosa* (1 mg) were dissolved in 200 μ L of trifluoroacetic acid (TFA) and 50 μ L of MMB (80 $\text{mg}\cdot\text{mL}^{-1}$). The MMB solution is prepared in distilled water at around 50°C , as it crystallizes at 20°C . The samples were submitted to 80°C for 5 min, furthermore 50 μ L of MMB were added and the solution was placed at 120°C for 1 h. After that, 100 μ L of MMB were added as well as 100 μ L of 2-deoxyglucose (1 $\text{mg}\cdot\text{mL}^{-1}$), used as an internal standard. TFA was then evaporated in a rotative evaporator, and the resulting residue was dissolved in 100 μ L of acetic acid glacial. In an ice bath, the samples were acetylated with 450 μ L of 1-methylimidazole, used as a catalyst [40], and 3 mL of acetic anhydride. The samples were placed at 30°C for 30 min and afterwards liquid-liquid extractions were performed using distilled water (3 mL) and dichloromethane (2.5 mL) twice, and distilled water (3 mL) other two times. After evaporating the organic phase, the alditol acetate derivatives were solubilized with anhydrous acetone and then analyzed by gas chromatography coupled with a flame ionization detector (GC-FID, Perkin Elmer Clarus 400, USA), following the chromatographic

conditions reported by Lopes et al. [41].

3. Results and discussion

3.1. Preliminary screening of solvents extracting bioactive compounds

Initially, a screening was conducted using the selected solvents, which included two conventional (water and ethanol) and one bio-based solvent (ammonium lactate). UV-Vis analysis was performed over a wavelength range of 230 to 700 nm (more details in Section 2.3.1.1.). This preliminary study aimed to identify the major chemical classes preferentially extracted by each solvent, providing valuable insights into the biochemical composition of the biomass and the polarity-dependent migration of bioactive compounds from *C. ramosa*.

Although universal heuristic rules for selecting bio-solvents are still not fully established, solvent polarity, hydrogen-bonding capacity, and ionic character, are key physicochemical parameters that should be considered, while recognizing that matrix-derived synergistic effects may modulate extraction performance in complex biomasses. As shown in Fig. 1A, the UV-Vis spectra of the three crude extracts revealed distinct spectral profiles, suggesting that solvents with different physicochemical properties promoted the recovery of different classes of bioactive compounds. It should be noted that these spectra were used as a qualitative and preliminary assessment of the extracts, based mainly on the position and shape of the absorption bands, rather than on direct comparison of absolute absorbance values, since different dilution factors were applied to the extracts. The aqueous extract, represented by the blue line in Fig. 1A, showed low absorbance in the visible range and a shoulder or peak in the UV-B/C region, around 250–280 nm. This spectral feature may be associated with the presence of moderately polar compounds, such as phenolic compounds or proteins [42,43]. Ethanol extract, represented by the green line in Fig. 1A, displayed a pronounced absorption band around 430 nm and a shoulder near 470 nm. This pattern is consistent with the presence of carotenoids, such as lutein and β -carotene, as well as other lipophilic compounds [44]. In addition, the absorption band observed around 660–670 nm is commonly associated with chlorophylls, which is also consistent with the green coloration of the extract (Fig. 1B). These spectral features suggest the affinity of these pigment classes for less polar organic solvents [22]. In contrast, the ammonium lactate extract, represented by the pink line in Fig. 1A, displayed a distinctive absorption maximum centered around 330 nm. This region is characteristic of MAAs, which are UV-absorbing compounds frequently reported in seaweeds [45]. This spectral profile suggests that the ionic nature of the renewable solvent may favor the selective recovery of these UV-protective compounds. Ammonium lactate containing 20% (w/w) of water may outperform water in the extraction of MAAs by providing an ionic and highly polar environment that better matches the zwitterionic nature of these compounds. Its ionic strength may also contribute to disrupting intracellular binding interactions and enhancing solvation, thereby improving mass transfer and selectivity toward MAAs. In conclusion, Fig. 1A indicates that the different solvents generated extracts with distinct UV-Vis spectral fingerprints, suggesting differences in the classes of compounds preferentially recovered from *C. ramosa*. However, due to the different dilution factors applied, the spectra should not be interpreted as a direct quantitative comparison of extraction efficiency or compound concentration. To precisely identify the metabolites responsible for the observed UV-Vis features and to obtain a comprehensive chemical profile of the algal extracts, detailed metabolite profiling was subsequently performed by UHPLC-DAD-ESI/MS.

3.2. Metabolic profile of *C. ramosa* assessed by UHPLC-DAD-ESI/MS

Following the initial spectroscopic screening, UHPLC-DAD-ESI/MS was performed to separate and identify the metabolites present in the three extracts obtained using ethanol, water, and ammonium lactate as

solvents (Section 2.3.1.2.). The UHPLC-DAD-ESI/MS full chromatograms of the extracts are shown in Figs. 2–5.

3.2.1. Bioactive compounds in the aqueous extract

The profile of the aqueous extract (Fig. 2), which compound identified are the detailed in Table 1, strongly supports the targeted recovery of highly polar chemical compounds, primarily flavonoids. The mass spectrometry data are essential for addressing the limited bibliographic support concerning seaweed flavonoids [42,46], as the m/z values provide objective experimental evidence to differentiate these compounds from structural isomers and degradation products. As such, the present results emphasize a combination of primary and specialized metabolites, with relative peak areas providing valuable quantitative information.

The compound identified as the most abundant in this extract is syringetin-3-*O*-galactoside ($R_t = 12.46$ min), accounting for 16.97% of the total integrated area [47]. This compound is a glycosylated derivative of syringetin, a 3',5'-dimethoxylated analog of the flavonol myricetin [48]. While this metabolite has been reported in terrestrial sources, such as red grapes *Lysimachia nummularia* and *Anthyllis henoniana* [47,48] its identification in red algae has not been reported in the literature so far. However, previous studies have referred the presence of flavonoid C-glycosides in green seaweeds, such as *Nitella hookeri* [46,49]. From a structural perspective, the presence of the galactose fraction at the C-3 position is critical, since glycosylation significantly increases the water solubility and stability of flavonoids compared to their aglycone form [50]. This modification explains its effective recovery in the water extract alongside other polar primary metabolites. Furthermore, flavonoids with multiple hydroxyl groups, such as the syringetin derivative, exhibit numerous hydrogen-bonding sites [50]. These sites not only enhance solubility but are also linked to important biological activities, including anti-inflammatory effects, reduction of free radicals during inflammation, and potential adsorption of environmental pollutants [50]. The presence of this methylated flavonoid is particularly encouraging, as syringetin derivatives are known to exhibit greater metabolic stability and diverse pharmacological properties, including antitumor, hepatoprotective, and antidiabetic activities. [51]

Additionally, the presence of other glycosides, such as chrysoeriol 8-*C*-glucoside malonylated (4.31%) and a methoxy derivative of luteolin found in Korean Naeng-i (*Capsella bursa-pastoris*), [52] further underscores the presence of highly polar bioactive C-glycosides in *C. ramosa* [53]. Chrysoeriol derivatives are recognized for their stability, favorable pharmacokinetic behavior, and anti-inflammatory activity, which adds a therapeutic dimension to the aqueous extract of *C. ramosa* [53]. Furthermore, the second major metabolite identified in the extract was glycyl-L-leucine (8.39%), a dipeptide formed from glycine and L-leucine linked by a peptide bond [54]. This compound contributes to the co-extraction of highly soluble nitrogenous substances in the extract. Additionally, the unexpected presence of the lipophilic ketocarotenoid canthaxanthin (5.51%) explains the yellowish color observed in the extract shown in Fig. 1(B) [55]. Canthaxanthin is primarily extracted using organic solvents due to its higher affinity and lipophilic nature [55–57]. Its detection in the aqueous extract may be explained by the co-extraction of [3-[2-aminoethoxy(hydroxy)phosphoryl]oxy-2-hydroxypropyl] palmitate, a lysophosphatidylethanolamine-type membrane phospholipid identified in this extract. Lysophospholipids are amphiphilic molecules able to self-assemble in aqueous media once their critical aggregation/micelle concentration (CAC/CMC) is reached. Although a specific CAC value for this exact LPE 16:0 species was not found, Stafford et al. [58] reported micromolar CMC values for structurally related lysophospholipids. These organized systems may create hydrophobic microdomains capable of incorporating and stabilizing lipophilic compounds such as canthaxanthin in the aqueous extract. This is indeed consistent with the known lipid composition of red macroalgae [59], which are rich in amphiphilic compounds that can act as natural surfactants during the extraction process, thereby favoring its apparent solubilization in the aqueous phase.

Some studies have reported the occurrence of canthaxanthin in microalgae, particularly in *Chlorella* sp., as well as in certain bacteria and fungi, although its presence is more uncommon in seaweeds. [55,56] Beyond its role as a coloring agent, canthaxanthin is known for its excellent antioxidant properties, making it desirable in feed, food, cosmetic, and pharmaceutical industries [56]. Extracting this pigment without using organic solvents offers a more environmentally friendly

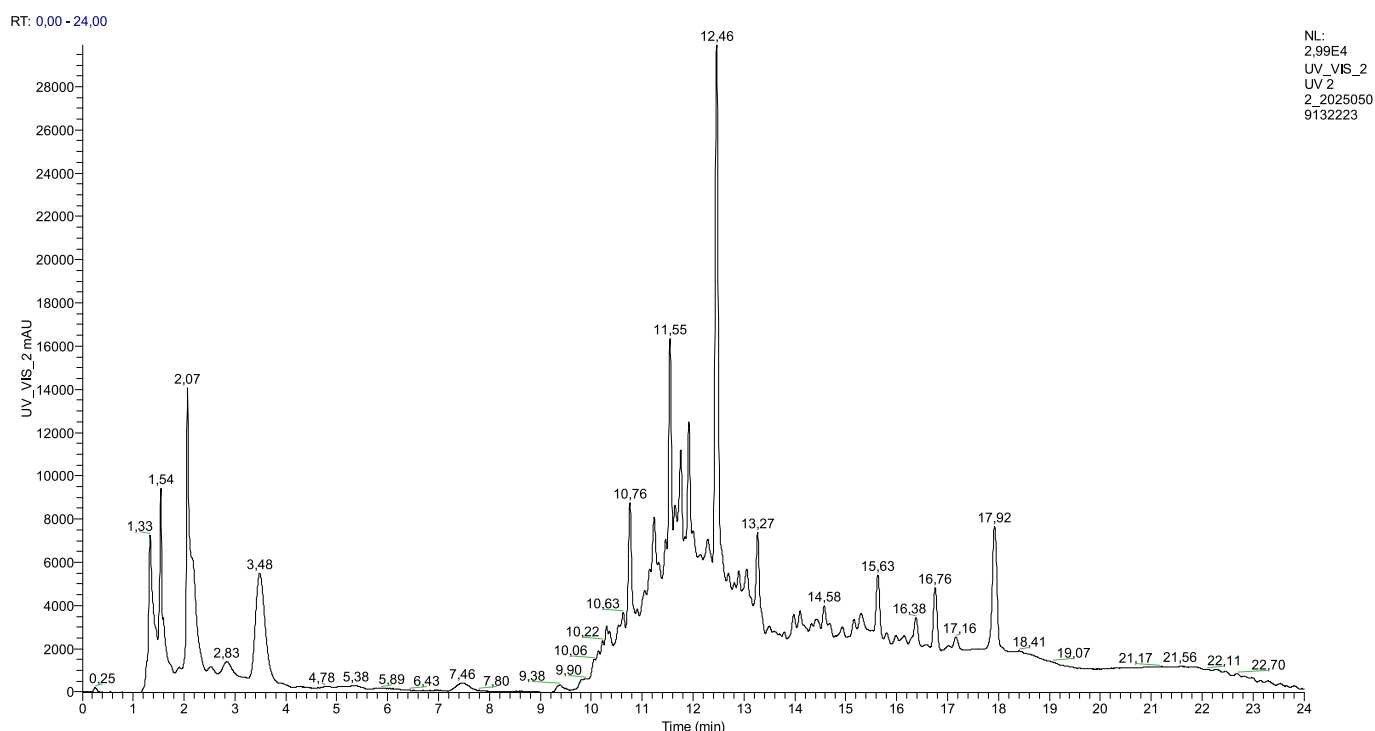


Fig. 2. UHPLC-DAD-ESI/MS chromatogram of *C. ramosa* the aqueous extract recorded at 305 nm.

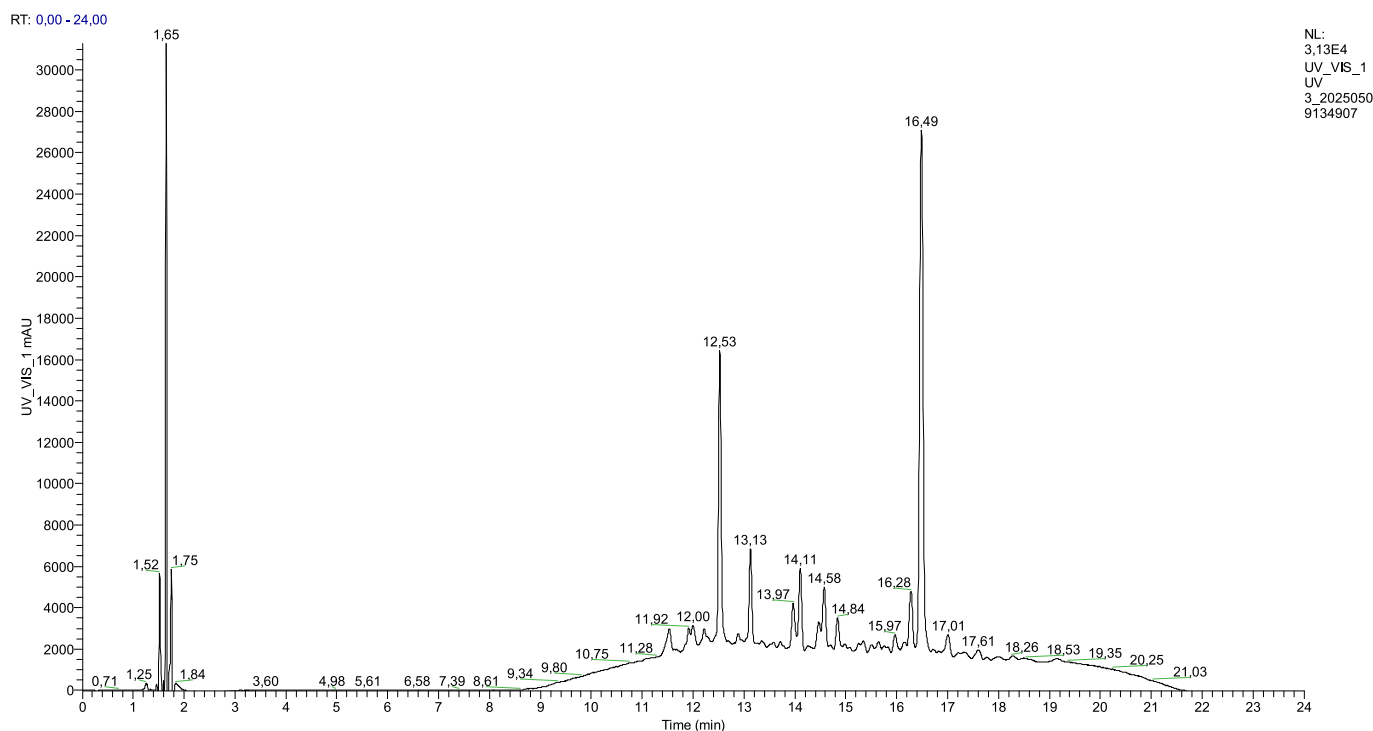


Fig. 3. UHPLC–DAD-ESI/MS chromatogram of *C. ramosa* ethanol extract recorded at 260 nm.

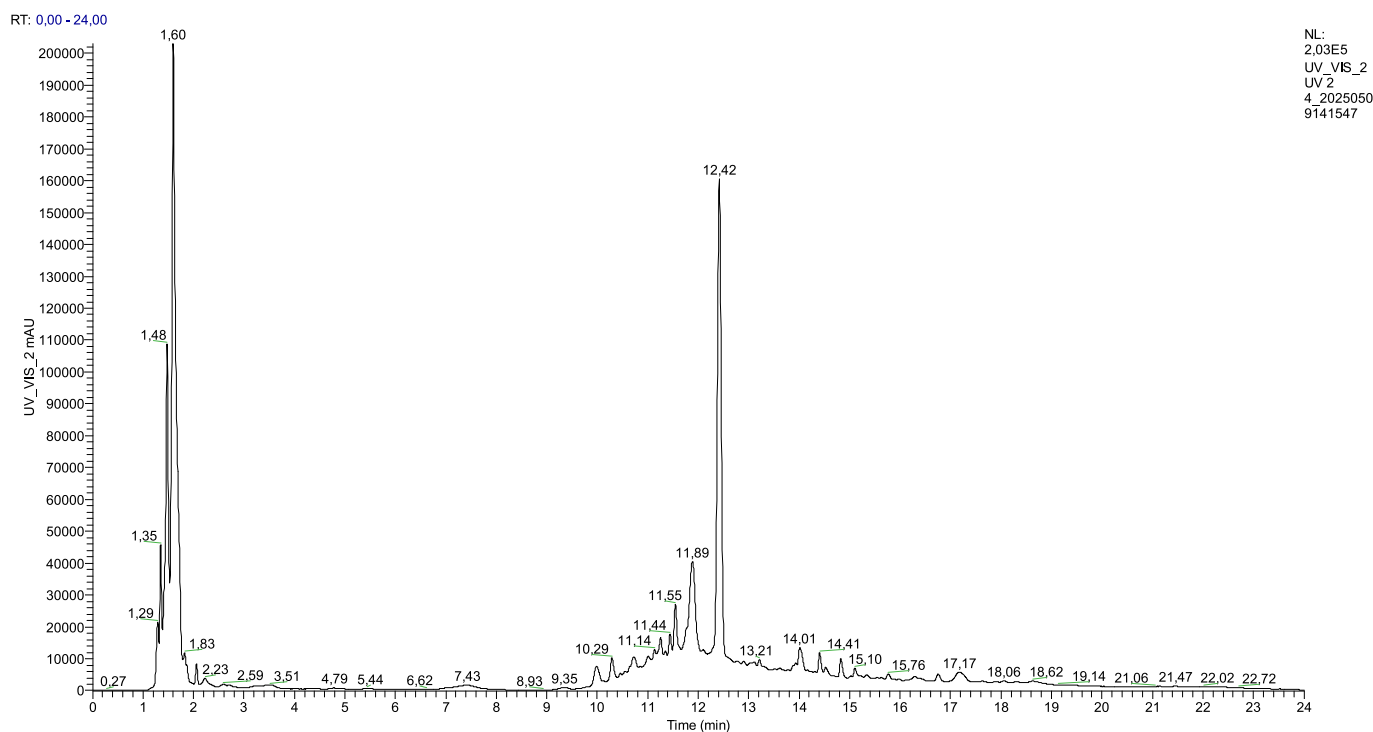


Fig. 4. UHPLC–DAD-ESI/MS chromatogram from ammonium lactate-based extract of *C. ramosa*, recorded at 305 nm.

approach when processing seaweed biomass.

3.2.2. Bio-active compounds in the ethanolic extract

In contrast to the solid-liquid extraction using water as solvent, the use of ethanol revealed a distinct set of bioactive compounds characterized by higher retention times and lower polarity (Fig. 3). Table 2 summarizes the compounds tentatively identified in the ethanol extract.

As illustrated in Table 2, the metabolic profile of the ethanol extract provides clear evidence of solvent selectivity, favoring the extraction of lipophilic molecules, including methoxylated flavonoids and lipid-derived metabolites. The major metabolite identified in this fraction was casticin, accounting for nearly 38% of the relative abundance in ethanolic extract. Unlike the glycosylated flavonoids identified in the water extract (e.g., syringetin-3-*O*-galactoside), casticin is a

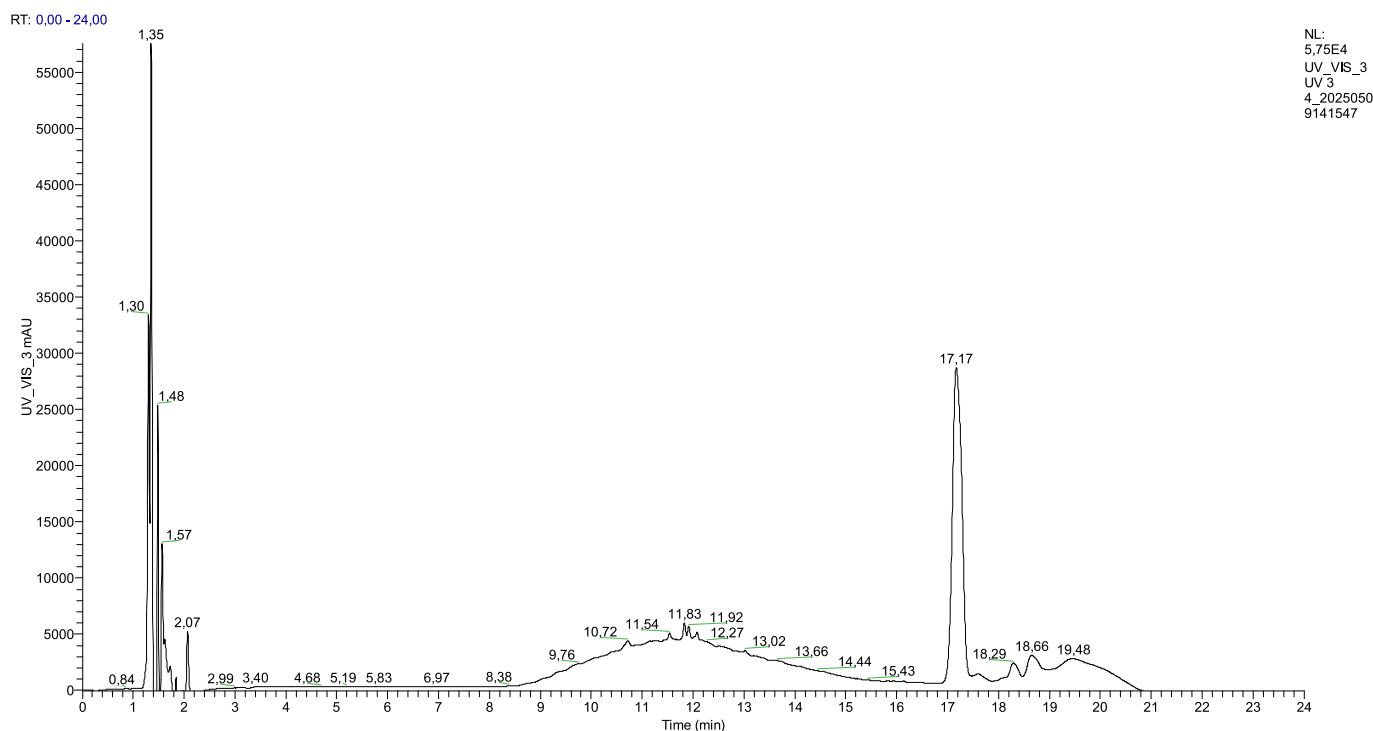


Fig. 5. UHPLC-DAD-ESI/MS chromatogram from ammonium lactate-based extract of *C. ramosa*, recorded at 450 nm.

Table 1

Bioactive compounds identified by UHPLC-DAD-ESI/MS from the aqueous extract of *C. ramosa* with Rt (min) corresponding to retention time, $[M + H]^+$ is pseudomolecular mass, λ (nm) is the UV-Vis wavelength, and RA (%) corresponding to Relative Abundance (%).

Rt (min)	λ (nm)	$[M + H]^+$ (m/z)	Compound	RA (%)
3.48	264	189	Glycyl-L-leucine	8.39
10.76	291	389	3,12-Dioxo-5 α -cholan-24-oic acid	3.02
11.55	472	566	Canthaxanthine	5.51
11.92	217, 268	550	Chrysoeriol 8-C-glucoside malonylated	4.31
12.46	218, 281	509	Syringetin-3-O-galactoside	16.97
13.27	218	454	[3-[2-aminoethoxy(hydroxy) phosphoryl]oxy-2-hydroxypropyl] palmitate ^(b)	2.92
17.92	221	268	^(c)	4.48

^(b) This compound appears to be of the type [3-[2-aminoethoxy(hydroxy) phosphoryl]oxy-2-hydroxypropyl] palmitate, it may have undergone degradation, either by reduction of the carbonyl group (15.63 min; 2-aminoethyl (2-hydroxy-3-((1-hydroxyhexadecyl)oxy)propyl) hydrogen phosphate; $[M + H]^+$ 456 m/z) or loss of the amino group (16.76 min; 3-((ethoxy(hydroxy)phosphoryl)oxy)-2-hydroxypropyl palmitate; $[M + H]^+$ 439 m/z);

^(c) it may be a derivative of cinnamic acid, but with some interference.

Table 2

Bioactive compounds identified by UHPLC-DAD-ESI/MS from the ethanol extract of *C. ramosa* with Rt (min) corresponding to retention time, $[M + H]^+$ is pseudomolecular mass, λ (nm) is the UV-Vis wavelength, and RA (%) corresponding to Relative Abundance (%).

Rt (min)	λ (nm)	$[M + H]^+$ (m/z)	Compound	RA (%)
1.65	267	286	Pentadecanoyl ethanolamide	18.99
12.53	217, 331	459	Daidzein derivative	16.32
13.13	218, 359	679	Luteone derivative	5.94
16.49	220, 328	375	Casticin	37.61

polymethoxylated flavone characterized by the absence of sugar parts, which distinguishes it from the flavonoids present in the aqueous extract. The presence of multiple methoxy groups significantly increases its lipophilicity, explaining its preferential extraction by ethanol and the selective recovery of less polar methylated flavonoid forms [60]. While casticin is commonly identified in terrestrial plants, particularly species of the genus *Vitex* [61], reports of polymethoxylated flavones in marine algae remain limited. However, casticin has been previously reported to exhibit antioxidant, anti-inflammatory, and anti-apoptotic activities, suggesting its potential relevance for the prevention or mitigation of oxidative stress-related disorders [60]. Thus, this finding highlights the biological potential of the ethanol extract [45]. The second most abundant compound was tentatively identified as pentadecanoyl ethanolamide (18.99%). This molecule belongs to the class of *N*-acylethanolamines, lipid-derived bioactive compounds structurally related to the endocannabinoid anandamide and known to act as signaling mediators in diverse organisms [62]. Additionally, through solid-liquid extraction with ethanol, it is also possible to extract isoflavonoid derivatives, namely daidzein (16.32%) and luteone (5.94%) derivatives. Daidzein is a well-known phytoestrogen with therapeutic potential, predominantly reported in leguminous plants [63,64], particularly in soybean. Nevertheless, the tentative identification of its derivatives is further supported by previous literature reporting the extraction of this isoflavonoid from marine algae using supercritical carbon dioxide modified with methanol-based co-solvents [65]. In those studies, daidzein was successfully recovered from red and brown macroalgae, respectively *Chondrus* sp. and *Sargassum* sp., as well as from cyanobacteria (*Nostoc* N17) [65], indicating that solvents of intermediate polarity, such as ethanol, are required to access these metabolites from algae. On the other hand, luteone is also an isoflavonoid previously isolated from terrestrial plant species, particularly members of the Fabaceae family, and has been reported to exhibit antimicrobial activity against a wide range of microorganisms [66]. Although reports of luteone in marine organisms are limited, its detection in the ethanol extract may reflect the selective recovery of low abundance isoflavonoid metabolites or structurally related compounds under extraction conditions favoring moderately lipophilic phenolics.

3.2.3. Bioactive compounds identified in the ammonium lactate-based extract

To achieve a sustainable and selective extraction approach, ammonium lactate (20% w/w in water) was chosen as a bio-based solvent, serving as an alternative to traditional organic solvents. The UHPLC–DAD–ESI/MS analysis confirmed that this solvent exhibited unique selectivity, allowing a very distinct profile of bioactive compounds in the extract (Figs. 4 and 5). The principal metabolites identified in the ammonium lactate-based extract are presented in Table 3 and have revealed a marked enrichment in MAAs and glycosylated flavonoids, clearly distinguishing this extract from those obtained using conventional solvents.

The metabolic profile obtained with ammonium lactate (20% w/w in water), an ammonium salt of lactic acid [67], provides experimental validation of its versatility as a solvent. The results demonstrate a bridging effect, allowing the solvent to interact with both hydrophilic and lipophilic chemical molecules. The ionic and pH environment characteristic of ammonium lactate plays a central role in its extraction selectivity, operating through complementary mechanisms.

Using ammonium lactate (20% w/w in water), porphyrin-334 could be identified, representing one MAA. MAAs are zwitterionic small-sized compounds, containing both positive and negative charges, characterized by their high-water solubility, and notable for their photoprotection and antioxidant properties [68]. MAAs retain their zwitterionic form and their electrostatic interactions with the negatively charged sulfated galactans cell wall matrix of *C. ramosa* [35] are effectively disrupted by the ionic strength of the dissociated NH_4^+ and lactate ions, promoting their release into solution. Consequently, the successful recovery of porphyrin-334 aligns with the characteristic absorption maximum observed at 330–340 nm in the initial screening (Fig. 1 (A)) and with the fact that it is present in different red seaweeds, such as *Palmaria palmata* and *Porphyra umbilicalis* [45]. Furthermore, this finding suggests that the ionic environment characteristic of ammonium lactate could interact more favorably with zwitterionic molecules such as MAAs. This ionic selectivity is further supported by the pH environment of ammonium lactate, which typically falls in the weakly acidic to neutral range, results from the neutralization of lactic acid ($\text{pK}_a = 3.86$) by ammonia ($\text{pK}_a = 9.25$) [32]. Additionally, the extract was primarily composed of syringetin-3-*O*-galactoside, which accounted approximately 58% of the total integrated area in the phenolic window. This represents a 3.4-fold enrichment compared to the aqueous extract (16.97%), as shown in Table 1. The significant efficiency of ammonium lactate in recovering syringetin-3-*O*-galactoside can be attributed to its hydrotropic properties. As demonstrated by Silva et al., [34] bio-based lactate-derived solvents can enhance the aqueous solubility of hydrophobic phenolic cores, such as the syringic nucleus, through water-mediated molecular aggregation. Similarly, the lactate anion of ammonium lactate acts as a

hydrotropic agent, enhancing the aqueous solubility of moderately hydrophobic aromatic cores ammonium lactate may increase the solubility of the hydrophobic methylated aglycone (syringetin) in the solvent system (with 20% w/w water) by promoting molecular aggregation, thereby shielding hydrophobic regions from the aqueous phase while allowing hydrogen-bond interactions with the galactosyl moiety. This mechanism effectively minimizes water and solute contact, explaining the 3.4-fold increase in recovery compared to distilled water as solvent and positioning this biobased solvent as a more efficient medium for the extraction of less water-soluble marine flavonoids, specifically the methylated ones. Moreover, the substantial recovery of chrysoeriol 8-*C*-glucoside malonylated (22.44%) is chemically significant for two reasons. Firstly, unlike *O*-glycosides, the sugar portion is attached via a carbon-carbon bond at position 8, which makes it exceptionally resistant to acid and enzymatic hydrolysis, suggesting that this metabolite may have superior metabolic stability and potential bioavailability in vivo [52]. The preservation of this C-glycosidic bond is consistent with the mild near-neutral pH of the ammonium lactate system, which avoids the acidic catalyzed hydrolysis that would occur at lower pH values [52], further supporting the structural compatibility of this solvent with labile glycoconjugates. This method effectively maintains the structural integrity of complex conjugates. Unlike the typical limitations of polar solvents, ammonium lactate effectively recovered the xanthophyll pigment violaxanthin (76.31%) (Fig. 4). This highlights the amphiphilic nature of the solvent system and its ability to recover pigments, again likely due to its hydrotropic nature provided by lactate anion.

Summing up, the ammonium lactate serves as a “hybrid” solvent, effectively bridging the gap between polar and less polar fractions. This unique solvent strength offers a highly efficient route for concentrating a diverse range of high-value targets, particularly the UV-protective MAAs and antioxidant flavonoids, which are currently in high demand in cosmetic and skincare industries [5]. By enabling the recovery of these valuable bioactive molecules in a biocompatible, biorenewable solvent, ammonium lactate opens new pathways for the development of sustainable cosmeceutical formulations with even greater bioactivity [32]. This comprehensive recovery process offers a significant advantage, allowing streamlined, single-step extraction methods, thereby establishing *C. ramosa* as a strategic feedstock for valorization.

3.3. Comparison of the S_i and EF_i

The UHPLC–DAD–ESI/MS data were further used to estimate the apparent inter-extract selectivity (S_i) and enrichment factor (EF_i) of the different solvent systems, shown in Table 4. Since the results are expressed as relative abundance (RA, %), the calculated values should be interpreted as semi-quantitative indicators of solvent selectivity rather than as absolute extraction efficiencies.

For compounds detected exclusively in one extract, an apparent S_i of 100% was assigned to the corresponding solvent and 0% to the remaining solvents. However, these values should be interpreted as exclusive detection under the applied analytical conditions, rather than absolute absence from the other extracts. In these cases, enrichment factors were not calculated because the compound was not detected in the other extracts, which would lead to undefined ratios. These compounds were therefore considered solvent-specific markers. In general, the selectivity analysis confirmed that each solvent promoted the recovery of a distinct metabolite profile with most compounds detected exclusively in one extract, resulting in an apparent selectivity (S_i) of 100% for the corresponding solvent and 0% for the remaining extraction media. These compounds were therefore considered solvent-specific markers under the applied analytical conditions. However, enrichment factors could not be calculated for these exclusive compounds, since their absence from the other extracts would lead to undefined ratios. In the aqueous extract, glycyl-L-leucine, 3,12-dioxo-5 α -cholan-24-oic acid, canthaxanthin, [3-[2-aminoethoxy(hydroxy)phosphoryl]oxy-2-hydroxypropyl] palmitate, and compound *(c) were detected exclusively,

Table 3

Bioactive compounds identified by UHPLC–DAD–ESI/MS from the ammonium lactate-based extract of *C. ramosa* with R_t (min) corresponding to retention time, $[M + H]^+$ is pseudomolecular mass, λ (nm) is the UV–Vis wavelength, and RA (%) corresponding to Relative Abundance (%).

R_t (min)	λ (nm)	$[M + H]^+$ (m/z)	Compound	RA (%)
1.60	332	347 ^(a)	Porphyra-334	— ^(a)
11.89	220, 275	550	Chrysoeriol 8- <i>C</i> -glucoside malonylated	22.44
12.42	219, 283	509	Syringetin-3- <i>O</i> -galactoside	57.51
17.17	220, 451	601	Violaxanthin	76.31 ^(b)

^a This compound is eluted simultaneously with solvents between 1 and 2 min. Its concentration is higher at 1.60 min; however, it is not possible to obtain its RA; ^(b)Relative abundance calculated within the detection method optimized for carotenoids at 450 nm.

Table 4

Apparent inter-extract selectivity (S_i) and enrichment factors (EF_i) calculated from the relative abundance (RA, %) of bioactive compounds identified by UHPLC–DAD–ESI/MS in the aqueous, ethanol, and ammonium lactate (AL)-based extracts of *C. ramosa*. For compounds detected exclusively in one extract, selectivity was assigned as 100% for that solvent and 0% for the remaining extracts. Enrichment factors were calculated only for compounds detected in more than one extract. ND: not detected; NC: not calculated.

Compound	Water RA (%)	Ethanol RA (%)	Ammonium lactate RA (%)	S_i	EF_i
Chrysoeriol 8-C-glucoside malonylated	4.31	ND	22.44	AL: 83.9%; water: 16.1%	AL/ water = 5.21
Syringetin-3-O-galactoside	16.97	ND	57.51	AL: 77.2%; water: 22.8%	AL/ water = 3.39
Canthaxanthin	5.51	ND	ND	Water: 100%	NC
Casticin	ND	37.61	ND	Ethanol: 100%	NC
Porphyra-334	ND	ND	Detected	AL: 100%	NC
Violaxanthin	ND	ND	76.31	AL: 100%	NC

indicating complete apparent selectivity (S_i) for water. The unpredicted detection of canthaxanthin, a typically considered highly hydrophobic, in the aqueous extract may be related to micellar solubilization mediated by the co-extracted amphiphilic lysophospholipid-like compound * (b), as discussed in detail in Section 3.2.1. The ethanol extract also exhibited a set of exclusively detected compounds, including pentadecanoyl ethanolamide, the daidzein derivative, the luteone derivative, and casticin, consistent with the intermediate polarity of ethanol and its affinity for less polar flavonoid-type and lipid-related metabolites. In the ammonium lactate extract, porphyra-334 and violaxanthin were exclusively detected ($S_i = 100\%$), reflecting the ionic and amphiphilic solvation capacity of this system, as detailed in Section 3.2.3. As noted in Table 3 (footnote b), the RA value of violaxanthin was obtained at 450 nm and should not be directly compared with values calculated at 305 nm. For compounds detected in more than one extract, the combined use of S_i and EF_i allowed a more detailed comparison of solvent performance. Malonylated chrysoeriol 8-C-glucoside and syringetin-3-O-galactoside were detected in both the aqueous and ammonium lactate extracts, but with markedly higher RA values. Malonylated chrysoeriol 8-C-glucoside showed an apparent selectivity of 83.9% for ammonium lactate ($S_i = 16.1\%$ for water), corresponding to a 5.21-fold enrichment relative to the aqueous extract. Syringetin-3-O-galactoside showed $S_i = 77.2\%$ for ammonium lactate and 22.8% for water, with a 3.39-fold

enrichment, confirming that ammonium lactate not only preferentially recovered these compounds but also markedly promoted their relative accumulation compared with the aqueous system.

3.4. Antioxidant activity (ABTS) and functional potential of the extracts of *C. ramosa*

To assess the functional potential of the three extracts obtained in this work and to understand the effect of their different compositions, their antioxidant capacity was evaluated using the ABTS radical cation scavenging assay (details in Section 2.3.1.4). Results were expressed both as percentage of radical scavenging (Fig. 6) and as Trolox equivalent antioxidant capacity (TEAC).

As shown in Fig. 6, all extracts, independently of the solvent, demonstrated antioxidant activity. The ethanol extract showed a high value of ABTS radical scavenging capacity, corresponding to $379.42 \mu\text{M}_{\text{TEAC}} \cdot \text{mL}_{\text{sample}}^{-1}$. The superior antioxidant activity of the ethanol extract correlates with its chemical profile, as UHPLC–DAD/MS analysis revealed a higher abundance of less polar metabolites, particularly phenolic compounds, which are widely known for their antioxidant activity [46]. On the other hand, the aqueous extract exhibited lower ABTS radical scavenging capacity, approximately 70%, with a value of $334.79 \mu\text{M}_{\text{TEAC}} \cdot \text{mL}_{\text{sample}}^{-1}$. This lower antioxidant activity of the aqueous extract comparatively to other extracts is predictable, given its higher composition of polar glycosides, specifically Syringetin-3-O-galactoside and C-glycosyl flavonoids, generally associated to lower intrinsic antioxidant activity [50,52]. Finally, the ammonium lactate extract demonstrated a total ABTS scavenging capacity around 95%, corresponding to $449.20 \mu\text{M}_{\text{TEAC}} \cdot \text{mL}_{\text{sample}}^{-1}$. However, an analysis of the controls is essential, since the pure ammonium lactate solvent exhibited an intrinsic activity of 30%, which may be attributed to pH changes related to ammonium and lactate intrinsic properties, overestimating the ABTS scavenging capacity of ammonium lactate extract. After adjusting for solvent interference, the “real” antioxidant activity of the extract of ammonium lactate is estimated to be around 65%. While this adjusted activity is lower than what the gross value suggests and is comparable to the aqueous fraction, rather than distinctly superior, it underscores the effectiveness of the bio-based solvent in selectively recovering a very specific bioactive fraction.

3.5. Carbohydrate content and composition of *C. ramosa* biomass

Following the comprehensive profiling of specialized metabolites, this work extended to the carbohydrate characterization of *C. ramosa*. To provide a more complete study of the biochemical potential of this biomass beyond this specific fraction, the sugar composition profile of the freeze-dried biomass was determined (details in Section 2.3.3). This

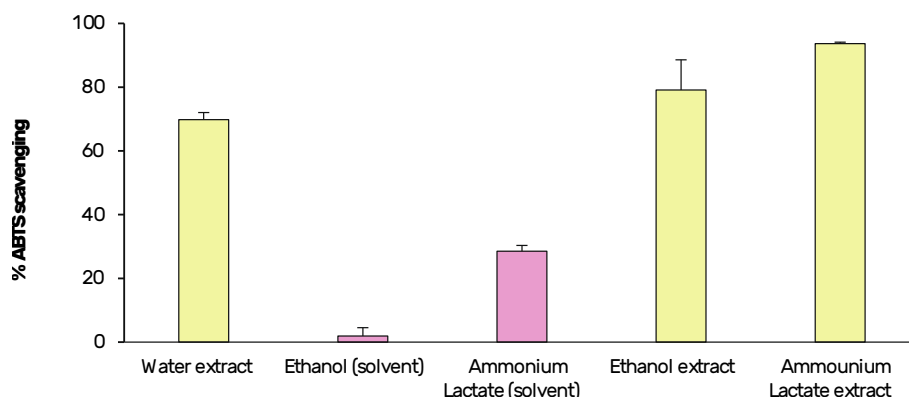


Fig. 6. Antioxidant activity by ABTS scavenging (%) of *C. ramosa* extracts (water, ethanol, and ammonium lactate-based) (yellow bars) and pure solvents (pink bars), ethanol and ammonium lactate. Each measurement was performed in triplicate, and the results are means $\pm$ standard deviation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

analysis revealed that carbohydrates constitute $22.6 \pm 2.1\%$ (w/w) of the dry biomass. The monosaccharide profile (Fig. 7) indicates a composition containing mainly galactose (Gal) ($11.3 \pm 2.0\%$ (w/w)) and glucose (Glc) ($7.9 \pm 0.6\%$ (w/w)). Small quantities of 3,6-anhydrogalactose (AnGal), xylose (Xyl), and mannose (Man) were also identified ($< 2\%$).

The dominance of Gal corroborates the taxonomic classification of *C. ramosa* as a Rhodophyta, where the cell wall is fundamentally composed of sulfated galactans, carrageenans and agars [54,55]. This aligns with the previous findings, which identified Gal as the major constituent of a purified cold-water fraction [35]. The results obtained for *C. ramosa* biomass reveal a significant presence of glucose, which could be attributed to cellulose, a structural polysaccharide. Furthermore, the low content of AnGal, which is $1.0 \pm 0.2\%$ w/w, is consistent with the reported profile of 1.6% w/w [35]. In red algal galactans, AnGal contributes to gelling properties, as indicated in previous studies [54,55]. Its low abundance suggests that *C. ramosa* produces either λ -type galactans, which are non-gelling, or highly sulfated precursor forms. By examining the total biomass instead of focusing on a specific fraction, these findings demonstrate that *C. ramosa* serves as a carbohydrate feedstock, providing sulfated galactans that have been described to have several biological properties [35], which expands its potential applications.

4. Conclusions

The present study presents the first comprehensive chemical profile of the underexplored red macroalga *C. ramosa*, successfully addressing a significant gap in the scientific literature. By implementing a targeted, solvent-dependent extraction strategy, this work demonstrated that *C. ramosa* holds a rich and chemically diverse metabolic profile that extends far beyond the previously reported fractions. The UHPLC–DAD–ESI/MS profiling revealed a clear and consistent solvent selectivity, providing a robust basis for the future valorization of this biomass. While water preferentially extracted highly polar glycosylated compounds, such as syringetin-3-O-galactoside, ethanol proved to be more effective in concentrating bioactive lipophilic metabolites, including polymethoxylated flavonoids, like casticin, and isoflavonoid derivatives. Notably, the use of the biorenewable solvent ammonium lactate emerged as an efficient and sustainable strategy for the selective recovery of zwitterionic MAAs, such as porphyra-334, together with polar flavonoid glycosides. At structural level, carbohydrate analysis confirmed that the cell wall matrix of *C. ramosa* is predominantly composed of sulfated galactans and cellulose, as evidenced by the dominance of galactose (9–13%) and glucose (7–8%), reinforcing its potential as a source of functional polysaccharides. Notably, the distinct chemical profiles yielded significant biological activity. The ethanol

extract exhibited the highest ABTS radical scavenging capacity, consistent with its enrichment in polymethoxylated flavonoids and lipophilic phenolics. In contrast, the ammonium lactate extract exhibited antioxidant activity comparable to the aqueous extract but lower than the ethanol extract, highlighting the complementary functional potential of the recovered compounds. Collectively, these findings shift *C. ramosa* from an overlooked red seaweed to a promising feedstock. The simultaneous presence of a structural polysaccharide matrix and a diverse suite of specialized antioxidant and UV-protective metabolites strongly support a cascade valorization model, particularly aligned with the development of sustainable and highly functional cosmetic ingredients. From an engineering perspective, the solvent-dependent extraction framework described here provides a sound foundation for the design of a cascade biorefinery process for *C. ramosa* biomass. The three solvents (water, ethanol, and ammonium lactate) employed exhibit complementary selectivity profiles that could be exploited sequentially, enabling the stepwise recovery of distinct compound classes from the same biomass batch and maximizing resource utilization in a near-zero-waste model [7]. In such a cascade approach, the aqueous fraction, enriched in polar flavonoid glycosides and sulfated galactans, serves as the primary extraction step, effectively preserving the most water-sensitive compounds. The subsequent ethanolic step is designed to recover lipophilic polymethoxylated flavonoids and carotenoids. Finally, the ammonium lactate step would selectively concentrate MAAs, polar glycosylated metabolites, and violaxanthin (the most abundant compound identified in this extract. Critically, the compatibility of all three solvents with downstream processing represents a key advantage. The ethanolic fraction can be directly subjected to liquid–liquid partitioning or solid-phase extraction for the further fractionation of carotenoids and flavonoid aglycones [69]. For the ammonium lactate fraction, aqueous two-phase systems, a biocompatible liquid–liquid technology scalable under mild conditions [70], represent a promising strategy for the selective purification of MAAs from co-extracted flavonoids, exploiting their differential partition coefficients between polymer-rich and salt-rich phases. To ensure the economic and environmental sustainability of this cascade process, downstream solvent recovery strategies, such as the evaporation of ethanol and water, or the use of solid-phase extraction and anti-solvents to isolate metabolites and recycle the ammonium lactate, should be integrated into the process design.

In summary, these considerations support the viability of integrating the described bio-based extraction strategy into a scalable, circular biorefinery model for the valorization of *C. ramosa* and related underexplored red macroalgae. In addition, a systematic optimization of the solid-liquid extraction parameters, including solid-to-liquid ratio, extraction time, and temperature, using response surface methodology is envisioned as a next step, building upon the chemical screening framework established in the present study. Future research should focus as well on isolating the identified compounds and evaluating their biological and formulation potential, including their photoprotective, anti-inflammatory, and antimicrobial properties, to further unlock the industrial value of this marine bioresource.

CRedit authorship contribution statement

Joana Pinto Oliveira: Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Ricardo Calado:** Writing – review & editing, Resources, Project administration, Funding acquisition. **Diana C.G.A. Pinto:** Writing – review & editing, Validation, Methodology, Formal analysis. **Inês Martins:** Writing – original draft, Validation, Methodology, Data curation. **Cláudia Nunes:** Writing – review & editing, Validation, Methodology, Formal analysis. **Sónia P.M. Ventura:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

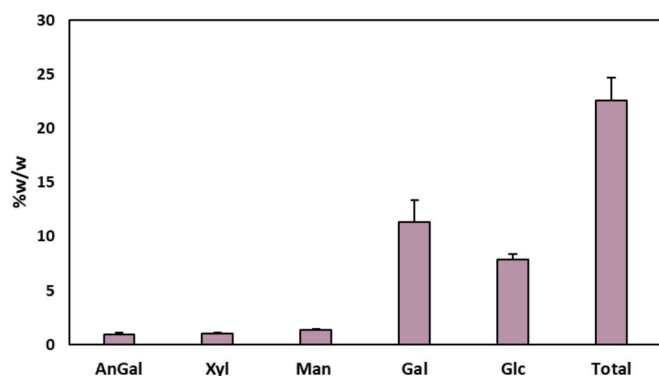


Fig. 7. Monosaccharide profile of *C. ramosa*. Each measurement was performed in triplicate, and the results are means $\pm$ standard deviation. Total and monosaccharide content of *C. ramosa* biomass. Each measurement was performed in triplicate, and the results are means $\pm$ standard deviation.

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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